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MERIT ABSTRACT AWARD POSTERS

The posters with 1000-level designations are ISSCR Merit Abstract Award winning posters. Each of these posters will remain on display throughout the three days of poster sessions. The presenter will be present at the assigned hour to discuss their work.

WEDNESDAY, JUNE 26, 2019

POSTER I - ODD
18:30 – 19:30

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-1001

CDX2 REPURPOSING IN THE ESTABLISHED MURINE TROPHOBLAST LINEAGE MAINTAINS STEM-CELL IDENTITY BY GATEKEEPING AGAINST A DEFAULT DIFFERENTIATION PATHWAY

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The transcription factor Cdx2 is required for the formation of several developmental lineages in the mouse embryo, and is thought to be able to play different roles within a lineage at different developmental stages. The role of Cdx2 in trophoblast fate establishment has been extensively studied. Yet, although its continued expression in established trophoblast cells is thought to be related to self-renewal and differentiation, the mechanisms that underpin this remain unknown. To address this, we generated ATACseq and RNAseq libraries from wild-type and Cdx2 knockdown (KD) trophoblast stem cells (TSCs) in vitro. ATACseq experiments show, as expected, that loss of Cdx2 causes decreased accessibility at Cdx2 consensus binding sites. However, of the regions showing differential chromatin accessibility in Cdx2 KD cells, two thirds have increased accessibility. These sites are enriched for the Tfap2c consensus motif and footprint: Tfap2 is a TSC marker that plays a part in trophoblast differentiation by driving genome-wide increases in accessibility. Gene ontology analysis of sites with increased accessibility in Cdx2 KD cells suggest that these regulatory regions are associated with trophoblast giant cell (TGC) differentiation. Consistent with this observation, continued Cdx2 KD drives homogenous differentiation into TGCs within days. Similarly, homozygous Cdx2 knock-out TSCs are unstable and spontaneously differentiate into TGCs. TSC differentiation can also be initiated in vitro by growth factor withdrawal, albeit in a heterogeneous manner. However, the sites of increased chromatin accessibility observed under these circumstances differ from those observed in Cdx2 KD cells. Moreover, although RNAseq analysis shows that 87% of genes whose expression changes in Cdx2 KD cells are also mis-regulated during conventional differentiation, 31% of these transcripts change in opposite directions. Our work suggests that Cdx2 normally maintains 'stemness' in TSCs by preventing them from differentiating directly and homogeneously into TGCs. We find it can do this even in cells overexpressing Hand1 and Tfap2c, both of which are upregulated in Cdx2 KD cells. This gatekeeping role of Cdx2 differs from its earlier function and reveals how Cdx2 can be repurposed to play different roles within lineages.

CARDIAC TISSUE AND DISEASE

W-1003

MODELING CONGENITAL HEART DISEASE-ASSOCIATED VARIANTS IN GATA6 USING CRISPR/CAS9 GENOME EDITING AND HUMAN IPSC-CARDIOMYOCYTES

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Congenital heart disease (CHD) represents a major cause of mortality in newborns and often requires surgical intervention upon birth. However, the molecular mechanisms by which CHD manifests in utero are not well-understood. Genetic variants thought to be causative for CHD would benefit from an in-vitro platform by which they can be validated in a high-throughput fashion. To address this challenge, we first conducted whole exome sequencing for a cohort of CHD individuals and identified patients who harbored loss-of-function (LOF) or missense variants in GATA6. This gene is a developmental transcription factor previously implicated in causing developmental outflow tract defects in animal models. We next introduced these patient-specific GATA6 variants into human induced pluripotent stem cells (hiPSCs) using CRISPR/Cas9 genome editing and subsequently differentiated these cells into cardiomyocytes (hiPSC-CMs) as an in-vitro analog for cardiovascular development. We characterized these GATA6 mutant hiPSC-CMs for alterations in gene expression and chromatin accessibility at multiple time points in the hiPSC-CM differentiation process to model the misregulated cardiac developmental mechanisms exhibited by individuals harboring GATA6 variants. GATA6 LOF and R456G missense hiPSCs exhibited an impaired capacity to differentiate into hiPSC-CMs. Differentiation capacity was quantitatively evaluated by re-engineering the GATA6 variants into a custom TNNT2-GFP reporter hiPSC-CM line. RNA-sequencing revealed that the GATA6 LOF and R456G missense hiPSC-CMs exhibited reduced expression of genes such as HAND2 that are involved in the development of the second heart field, which ultimately gives rise to the cardiac outflow tract. Chromosome conformation capture and Assay for Transposase-Accessible Chromatin using sequencing (ATAC-Seq) also revealed reduced chromatin accessibility in second heart field-related genes such as HAND2. This study demonstrates the capacity of CRISPR/Cas9 genome-edited hiPSC-CMs to rapidly and mechanistically validate genetic variants associated with CHD. We believe that this platform serves as a high-throughput system for evaluating the severity of CHD phenotypes and lends further credence to the role of hiPSC-CMs in cardiovascular precision medicine.

Funding Source: Funding support for this study was provided in part by the Fondation Leducq, the Engineering Research Centers Program of the National Science Foundation, the National Institutes of Health, and the Howard Hughes Medical Institute.

HEMATOPOIESIS/IMMUNOLOGY

W-1005

MAPPING THE EMERGENCE AND MIGRATION OF HEMATOPOIETIC STEM CELLS AND PROGENITORS DURING HUMAN DEVELOPMENT AT SINGLE CELL RESOLUTION

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Hematopoiesis is established during development through multiple waves of blood cell production, starting with lineage-primed progenitors required for the embryos needs, and culminating in the generation of self-renewing hematopoietic stem cells (HSCs) for life-long hematopoiesis. Although hematopoietic ontogeny has been studied extensively in mice, we lack knowledge of the anatomical, temporal and molecular map for hematopoietic development in human. Prior studies suggest that HSCs emerge from hemogenic endothelium in the aorta-gonad-mesonephros (AGM) region between 4-6 weeks of human gestation. Extraembryonic sites including the placenta, umbilical and vitelline arteries, and the yolk sac, have been proposed to generate HSCs in the mouse. However, whether the same sites generate HSCs in human is unclear, mainly due to the limited access to developmental tissues and lack of reliable methods to identify developing human HSCs. We created a single-cell transcriptome map of hemato-vascular cells (CD34+ and/or CD31+) from human hematopoietic tissues at 1st and

2nd trimester. Using a molecular signature of self-renewing HSCs defined in our previous molecular and functional studies, we could identify CD34+Thy1+RUNX1+HOXA7+MLLT3+HLF+ cells as HSCs throughout development. Analyses of 5-wk AGM revealed a distinct population of newly emerged HSCs that vanished by 7 wks. HSCs colonized the fetal liver by 6 wks, where they expanded and differentiated beyond 15 wks. Small but distinct population expressing HSC molecular markers was reproducibly detected in 5 wk placentas. At this time, the heart, umbilical cord and fetal liver lacked clear HSC populations, implying minimal spreading through circulating blood. Interestingly, preceding HSC colonization, the 5 wk fetal liver already harbored CD34+Thy1-RUNX1+HOXA7-MLLT3-HLF- progenitors that co-expressed markers associated with erythro-myeloid and lympho-myeloid potential. Comparable populations were abundant in the yolk sac, suggestive of their origin. This data-set provides an unprecedented resource to dissect the dynamics and molecular pathways governing the emergence and progression of distinct waves of hematopoietic cells during human development, and serves as a reference map for the generation of HSCs in vitro for therapeutic purposes.

Funding Source: UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Innovation Award

EPITHELIAL TISSUES

W-1007

STEM CELL ORIGINS AND DYNAMICS OF A SKIN-LIKE METAPLASIA THAT MEDIATES INTESTINAL WOUND HEALING IN MICE

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Metaplasia is a key, early lesion in the sequential progression of normal tissue into cancer. The origins of metaplasia are controversial but likely involve injury-induced reprogramming of tissue stem cells to alternate differentiation pathways to form ectopic epithelium. It is not known if metaplasia is the first sign of malignancy and should be aggressively targeted, or if it represents a beneficial adaptive change to chronic injury. We have resolved these unknowns in a mouse model of a skin-like metaplasia occurring in the distal colon (rectum) after inflammatory bowel disease. Here, we show that the emergence of this squamous metaplasia of mouse colon (SMMC) is an essential part of colonic wound healing. Moreover, SMMC restricts the initiation of colorectal tumors. Relying on the imaging and 3D reconstruction of chemically cleared specimens, we demonstrate that SMMC derives from a specialized exterior anal zone of skin-like Sox2+, Krt14- stem cells that migrate into the interior colon and proliferate to form intestinal crypt-like rete peg structures in the ulcer region. Mathematical modeling of empirical clonal fate data shows that the long-term stability of SMMC is mediated by two distinct populations of tissue-resident stem cells located at the periphery of the rete pegs; their homeostatic regeneration pattern exhibits both squamous and intestinal dynamics. At the molecular level, colonic restitutive signals, such as epidermal growth factor and prostaglandin signaling, within an intestinal organoid medium enables the growth of SMMC rete units in vitro. However, Notch signaling restricts the over-expansion of metaplasia. Thus, metaplasia formation via the mobilization of stem cells from neighboring tissue can be a programmed and self-limiting response to severe injury near organ junctions in the body.

Funding Source: NIH R01DK108648

STEM CELL NICHES

W-1009

THE METABOLIC SIGNATURE OF HEMATOPOIETIC STEM CELLS IS DIFFERENTIALLY REGULATED BY THEIR NICHE CELLS IN MOUSE BONE MARROW

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Hematopoietic stem cells (HSCs) confer a distinct metabolic signature from downstream lineages. Physiological variations in metabolite levels in HSCs influence their self-renewal and hematopoiesis. HSC self-renewal is also regulated extrinsically by their niche cells, such as endothelial cells and Lepr+ perivascular cells. An important question concerns whether niche cells regulate the metabolic state of HSCs. In our study, we developed a metabolomics method for the analysis of rare HSCs. We confirmed that each hematopoietic cell type had a distinct metabolic signature. Using HSC reporter mice, including α-catulin-GFP, Fgd5-Zsgreen and Hoxb5-BFP, we showed that long-term and short-term HSCs confer distinct metabolic levels. Using the H2B-GFP/rtTA label-retention mice, we showed that fast and slowly HSCs confer distinct metabolic levels. Notably, genetic alteration in Lepr+ perivascular cells, Cdh5+ endothelial cells and Osterix+ osteoblasts differentially influenced the metabolic level and frequency of HSCs in adult bone marrow. Taken together, our work revealed the metabolic heterogeneity of HSCs and highlighted the importance of niche cells in regulating the metabolic levels of HSCs.

NEURAL DEVELOPMENT AND REGENERATION

W-1011

MTOR SIGNALING REGULATES RADIAL GLIAL DEVELOPMENT IN THE HUMAN CORTEX

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The cerebral cortex, the folded exterior of the brain, is expanded in humans and is required for cognitive function. Abnormal cortical development is a leading cause of epilepsy and developmental delay and can lead to malformations where the brain does not fold or grow appropriately as in Lissencephaly, Microcephaly, and Megalencephaly. The cortex expands by directing appropriate proliferation of its resident neural stem cells, radial glia cells. There are multiple types of radial glia, and the outer radial glia (oRG) population is dramatically expanded in humans, suggesting its contribution to human cortical expansion. oRG cells have unique morphology, their basal process provides a scaffold for neuronal migration and they display a characteristic "jumping" migratory behavior, called mitotic somal translocation (MST), prior to division. However, we currently have limited understanding of the processes that regulate oRG development. Using single cell RNA sequencing our lab discovered that there is an increase in expression of mTOR genes in human oRG cells during gestation. We then assessed the functional role of mTOR signaling in oRG generation using two human-specific models of cortical development: ex vivo culture of developing human cortical tissue and in vitro culture of iPSC-derived cortical organoids. We observed that manipulation of mTOR signaling results in a decrease in the number of oRG cells in these cultures, as well as a reduction of the basal process length in the remaining oRGs, resulting in significant disruption to the glial scaffold. While oRGs retain their MST division behavior, the distance that cells translocate after mTOR inhibition is dramatically reduced, suggesting a fundamental change to both cell morphology and behavior. These studies provide insight into the mechanisms guiding the appropriate development of a human-enriched neural stem cell population and may inform therapeutic approaches toward cortical diseases resulting from mTOR dysregulation, such as pediatric epilepsy and Autism.

W-1013

MOUSE CORTEX-SPECIFIC DELETION OF DYRK1A CAUSES DIFFERENTIATION DEFECTS IN DEVELOPING NEURONS VIA MODULATION OF CALCIUM/CAN/NFAT SIGNALING

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During embryonic cortical development, extrinsic and intrinsic signals are integrated in neural progenitor cells (NPCs) to guide their differentiation into neurons and build functional circuits. In addition to genetic programs that regulate the adoption of specific neuronal fates, electrical activity is known to regulate cellular processes involved in the generation and maturation of neurons. The precise mechanisms by which electrical signals are transduced into transcriptional changes during NPC differentiation remain poorly understood. Calcium (Ca2+) is the primary intracellular mediator coupling electrical signals at the membrane to the regulation of transcription. Mutations in genes encoding voltage-gated Ca2+ channels, as well as members of Ca2+-activated signaling pathways, have been reproducibly associated with psychiatric disorders of developmental origin, including autism spectrum disorders and intellectual disability. We have identified a dual role for DYRK1A — a kinase associated with neurodevelopmental disorders that opposes the Calcineurin (CaN)/NFAT Ca2+ signaling pathway — in both NPC maintenance and neuronal differentiation. Loss of one or both copies of Dyrk1a in the developing cortex results in dose-

dependent microcephaly, depletion of radial glia, and a shift in excitatory neuron subtype abundance. Similar changes in the abundance of excitatory neuron populations have been observed in several genetically-defined neuropsychiatric syndromes and channelopathies, suggesting that Ca2+-regulated mechanisms driving neuronal differentiation are a point of convergence for psychiatric disease. In response to membrane depolarization and Ca2+ influx, activation of the CaN phosphatase complex triggers nuclear translocation of the NFATc1-4 transcription factors, whereas DYRK1A activity exports the NFATcs out of the nucleus. Using a series of gain- and loss-of-function approaches targeting the CaN/NFAT pathway, we also uncovered that modulating CaN/NFAT signaling in vivo alters cortical projection neuron specification in a similar fashion to Dyrk1a deletion. Our findings suggest that disruption of the Ca2+/CaN/NFAT signaling axis during embryonic neurogenesis may represent a core molecular substrate for various psychiatric disorders.

Funding Source: UCSF Sandler Fellows Program (awarded to GP), LLHF Postdoctoral Fellowship (awarded to RP)

POSTER I - EVEN
19:30 – 20:30

MUSCULOSKELETAL TISSUE

W-1002

ONCOGENIC AMPLIFICATION OF ZYGOTIC DUX FACTORS IN REGENERATING P53-DEFICIENT MUSCLE STEM CELLS DEFINES A MOLECULAR CANCER SUBTYPE

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Rhabdomyosarcoma (RMS) is a rare and aggressive childhood cancer and the most common soft-tissue sarcoma in children and adolescents. Rhabdomyosarcomas are generally thought of as skeletal muscle tumors, in large part because they typically arise in or near muscle beds and show features of myogenic differentiation. However, as for many cancer types, a long standing open question is what the cellular origin of RMS is and what the driven genes. The hypothesis has been fueled that muscle progenitors or muscle stem cells could be a cellular origin of RMS. The idea has been put forward that a somatic mutation in a physiologically healthy stem cell would give rise to a tumor propagating cell that essentially would be the source of a respective tumor. Nevertheless, purification of tumor stem cell also helps to identify novel driven genes. Employing lineage tracing and skeletal muscle regeneration as a paradigm, here we show that regeneration-based loss of muscle stem cell quiescence is necessary to elicit spontaneous acquisition of oncogenic copy number amplifications in p53 deficient stem cells resulting in 100% penetrance of rhabdomyosarcoma formation. Through genomic analyses of purified, lineage-traced tumor cells we discovered discrete oncogenomic amplifications driving tumorigenesis including, but not limited to, the homeobox transcription factor Duxbl. We show that Dux transcription factors driving embryonic/zygotic gene signatures define a molecular subtype of a broad range of human cancers. We found that Duxbl initiates tumorigenesis by enforcing a mesenchymal-to-epithelial like transition and demonstrate that targeted inactivation of Duxbl specifically in Duxbl expressing tumor cells abolishes tumor expansion. These findings suggest that a subtype of RMS is driven by Dux transcription factors.

CARDIAC TISSUE AND DISEASE

W-1004

AMINO ACID PRIMING OF MTOR IS ESSENTIAL FOR HEART REGENERATION

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The adult mammalian heart has a limited regenerative capacity after injury; however, there is a brief period of cardiac regeneration in mammalian neonates that is conserved throughout the lifetime of other vertebrates such as zebrafish. Previous studies have demonstrated that the damaged tissue is replaced through de-differentiation and division of ventricular cardiomyocytes (CMs), however the underlying mechanisms that prime these tissues for cell-cycle re-entry are unknown. We have demonstrated that the regenerative capacity of neonatal mouse and zebrafish CMs is dependent on metabolic state and amino acid profile. Using a zebrafish chemically induced ventricular CM ablation model, we have shown that mTOR activation drives CM proliferation in an amino acid-dependent manner. High levels of intracellular glutamine are present in the uninjured heart, priming the CMs for amino-acid dependent mTORC1 activation. These high glutamine levels are conserved in neonatal, regenerative mouse hearts, but lost in the adult mouse. Looking upstream of this mTOR activation, we further demonstrated that ventricular CM ablation in zebrafish results in Wnt/β-catenin signaling from the endocardium, resulting CM upregulation of Lin28 and c-Myc. Upregulation of c-Myc led to an increase in expression of electron transport chain Complex I as well as an increase in expression of oxidative phosphorylation associated genes. To further assess cell fate decisions after injury, we performed single cell RNA-sequencing analysis during the first week of cardiac regeneration after ventricular ablation. Using monocle based pseudotime and unbiased clustering analysis, we identified the quiescent and activated, pro-regenerative states of the major cardiac cell types, including cardiomyocytes, epicardial cells, endocardial cells, and bulbus arteriosis cells. Together, these findings demonstrate for the first time the transcriptional and metabolic mechanisms underlying early zebrafish heart regeneration.

PANCREAS, LIVER, KIDNEY

W-1006

A SMALL MOLECULE PROMOTES THE PROLIFERATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED PANCREATIC PROGENITOR CELLS

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Pancreas and islet transplantation can achieve insulin independence in diabetes patients but are limited by the scarcity of donor organs. Pancreatic progenitor cells (PPCs) derived from pluripotent stem cells have been investigated as an unlimited source of cells for transplantation. PPCs expressing pancreatic and duodenal homeobox 1 (PDX1) can give rise to all cell types found in the adult pancreas including the insulin-producing β cells, in vitro or when transplanted. These cells hold great therapeutic potential to treat diabetes, and hence there is tremendous demand in discovering new ways to stably expand progenitor cells in vitro. We have screened a panel of compound library composed of kinase inhibitors, and found a compound, AT7867, which promotes the proliferation of PDX1-expressing PPCs differentiated from several lines of human embryonic and induced pluripotent stem cells. The compound treatment increases the number of PPCs approximately five-fold within six days and results in a higher proportion of Ki67+ and pHH3+ cells. Contrary to this, the number of cycling cells declined rapidly in the control. We observed that the compound does not appear to exert effect on other cell types. Moreover, the compound-treated cells differentiate into β-like cells in vitro suggesting that they retain developmental potential into pancreatic cells. However, the compound's function as a dual inhibitor of AKT and p70S6K suggests that inhibiting this pathway results in cell apoptosis, cell cycle arrest and reduced protein synthesis. Therefore, we hypothesized that there is another molecular target and an affected downstream signaling that provides a critical cue for cell maintenance and proliferation. To elucidate the molecular mechanism of the compound-stimulated cell proliferation, global gene expression profiles between untreated and treated cells were compared. Gene set enrichment analysis showed that treated cells were upregulated in several KEGG pathway gene sets related to cell proliferation such as DNA replication, basal cell carcinoma, p53 and a number of other signal transduction pathways. Our finding may contribute to the elucidation of proliferation mechanism in these cells, in addition to providing a regenerative medicine approach to treat diabetes.

EPITHELIAL TISSUES

W-1008

MOLECULAR FINGERPRINT OF HUMAN EPIDERMAL STEM CELLS AIMED TO GENE-THERAPY APPLICATIONS

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Autologous cultures of human primary keratinocytes are used in clinics for treatment of limbal stem-cell deficiency or ex-vivo gene therapy approaches for severe genetic skin diseases, such as Epidermolysis Bullosa. In vivo epithelia are continuously renewed through the activation of Keratinocytes stem cells (KSCs) and a correct balance between multiplication and differentiation of Transient Amplifying progenitors (TACs) originated from the KSC. During in vitro cultivation, KSC gives rise to Holoclones (H), instead TACS give rise to Meroclones (M) or Paraclones (P). Recently our group demonstrated that regeneration of a long-lasting epidermis is sustained only by Holoclones. Nowadays, H M and P could be identified only by clonal analysis and no further molecular characterization of these different clonal

type has been performed. In order to molecularly characterize these three clonal types, we isolate H, M and P from different strains of human primary keratinocytes by clonal analysis and the corresponding transcriptomic profile has been analysed. We found that pathways like cell cycle progression or DNA repair result upregulated in Holoclones, whereas cell necrosis or apoptosis result downregulated, reflecting some characteristic identified in quiescent stem cells in different types of tissue such as hematopoietic system. Moreover, we found a novel function of the transcription factor FOXM1. High amount of FOXM1 is observed in Holoclones respect to Meroclones and the complete disappearance of FOXM1 during clonal conversion follows the same trend of known stem cells markers such as p63, BIRC5. Through gain and loss of function studies, we demonstrate that FOXM1 is a master gene for the maintenance of the stem cell identity acting downstream of YAP/TAZ pathway.

CANCERS

W-1010

DOWN SYNDROME-RELATED TRANSIENT ABNORMAL MYELOPOIESIS IS DERIVED FROM A NEWLY IDENTIFIED HEMATOPOIETIC SUBPOPULATION

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Down syndrome-related transient abnormal myelopoiesis (TAM) is a temporal preleukemic state. TAM is caused by a somatic GATA1 mutation, but which specific cellular subpopulations during hematopoietic differentiation are responsible for the initiation of TAM have not been precisely determined. In this study, using trisomy 21 isogenic pluripotent stem cell pairs, we identified a specific hematopoietic progenitor cells (HPCs) subpopulation that is responsible for the skewed differentiation associated with the GATA1 mutation. We successfully recapitulated the TAM phenotype with abnormal hematopoiesis in GATA1-mutant clones. Interestingly, although we could find only slight differences in the morphology or cell surface marker phenotype prior to the initiation of lineage specific culture, correlation analyses between each HPCs subpopulation at the beginning of lineage specific differentiation and the cell number of each lineages after a week of culture indicated that the frequency of the CD34+CD43+CD235-CD11b-CD71+CD41+ subpopulation was significantly associated with the number of erythroid cells in the control clones and the number of immature megakaryocytic cells in the GATA1-mutant clones. Furthermore, RNA expression profiling analyses of this subpopulation revealed a significant enrichment of gene sets related to myeloid-biased differentiation and enhanced proliferation in GATA1-mutant clones at the early stage of differentiation. Finally, progenitor assays started with this subpopulation of GATA1-mutant clones induced extreme myeloid skewing compared with control clones. Taken together, our data strongly suggest that blastogenesis of TAM is dependent on the CD34+CD43+CD235-CD11b-CD71+CD41+ subpopulation. This subpopulation may act as a potential therapeutic target to treat TAM and prevent TAM from converting into life-threatening leukemia.

NEURAL DEVELOPMENT AND REGENERATION

W-1012

AIMING PTEN, SEEKING PULSE, FINDING DOX : A TARGETED, TRANSIENT IN VIVO APPROACH TO FACILITATE FUNCTIONAL REPAIR IN MICE SPINAL CORD INJURY

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In Spinal cord injury (SCI), the primary injury/assault is only the tip of the iceberg; the real threat being the secondary injury associated with a cascade of molecular events that follow - inflammation, macrophage type switch, cytokine outburst, ROS, apoptotic signals, infiltration of inflammatory cells, glial scarring, demyelination, axonal dieback, and fibrosis to name a few. Unattended, it progresses to an irreversible chronic phase and ultimately to paraplegia - an economic burden to and social death of the individual. Although many targets have been previously implied in SCI, due to the complexity in pathophysiology and therapeutic interventions required, it remains a "Molecular Disease," where standard clinical, cell and rehabilitation therapy has had minimal impact on augmenting motor function. Over the past decade, PTEN deletion showed some potential, but long term tumorigenicity, toxicity, and immunogenicity employing shRNA/viral vectors, the fate of demyelinated axons and the inhibitory glial scar/lesion are greater challenges to be addressed. Combinatorial therapies introduce more variations in mode of delivery, optimization of dosage and period of intervention/assessment – thus arises the quest for a "magic bullet" in SCI. We have developed a novel, targeted, inducible, virus-free, localized yet safe approach by electroporating engineered DOX-inducible miR-E constructs carrying a GFP reporter into the injured spinal cord to modulate PTEN in vivo in mice models during the therapeutic window period. Among the randomly divided Dox+/Dox- groups, our results show that DOX+ PTEN modulated mice were constantly associated with a marked increase in spinal cord tissue sparing, reduced cavity/lesion size and glial scarring, improved BMS scores displaying functional motor recovery, alleviated astrogliosis in lesions, increased re-myelination of the spared axons, and significant motor evoked potentials when compared to DOX- where evoked potentials were absent altogether in hindlimb. Our findings collectively suggest that targeted therapy by transient expression of sh-PTEN-miR during the therapeutic window period is a promising therapeutic strategy to augment functional repair in spinal cord injury.

Funding Source: We acknowledge Indian Council of Medical Research for JRF/SRF (20393) to Franklin Herbert and Department of Biotechnology, Govt of India for Ramalingaswami Fellowship and research Grant # BT/PR8527/MED/31/234/2013 to Sanjay Kumar

POSTER II - ODD
18:00 – 19:00

NEURAL DISEASE AND DEGENERATION

T-1015

SINGLE NUCLEOTIDE VARIANCE (SNV) OF CRITICAL GENE INDUCE TUMORIGENICITY OF IPS CELL-DERIVED NEURAL STEM CELLS (IPSC-NSCS) AFTER TRANSPLANTATION FOR SPINAL CORD INJURY

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A clinical trial is prepared at our institution investigating the efficacy of iPS cell-derived neural stem cells (iPSC-NSCs) transplantation for patients with spinal cord injury. However, as demonstrated previously, there still remains tumorigenic issues in some cell lines. The purpose of this study is to investigate the cause of tumorigenicity in iPSC-NSCs. Two integration-free iPSCs lines were prepared (836B3-iPSCs, 414C2-iPSCs), and were induced to iPSC-NSCs (tumorigenic cell line; 836B3-NSCs, safe cell line; 414C2-NSCs). ES cells were also used for analyses as a target for comparison of iPSCs. Analyses of gene expression, single nucleotide variance (SNV), copy number variation (CNV), and DNA methylation were performed using HumanHT-12, Hiseq2500, Ion AmpliSeq, Omni Express-24 and Methylation450 for finding tumorigenic driver. The gene expressions of undifferentiated markers were not significantly different between the two iPS cell lines, but genome stabilizing genes such as USP28 and DPPA3 were highly expressed in ES cells and 414C2-iPSCs compared with 836B3-iPSCs. In SNV analysis, we found missense mutation of SRGAP3's GAP domain in 836B3-iPSCs and 836B3-NSCs, which is important for neural development and antitumor effect. 836B3-NSCs revealed genome and epigenome instability in DNA methylation and CNV analyses compared to 414C2-NSCs and ES-NSCs. We induced mutation allele of SRGAP3 to 414C2-NSCs using lentivirus and transplanted to the spinal cords of NOD-SCID mice (n=10). Tumors were formed in all cases, confirmed by bio-imaging, histological and motor functional evaluation. It is well known that some iPS cell lines reveal a few SNVs. However, the biological effects and the impact of such mutations have not been clear. In this study, we revealed that some SNVs could

be the driver mutations for tumorigenicity. Because genome instability generates such detrimental SNVs, it is substantially important for safe cell lines to express higher levels of genome stabilizing genes.

T-1017

ESTABLISHING AN IN VITRO AGEING MODEL IN HUMAN PLURIPOTENT STEM CELLS AND NEURAL DERIVATIVES TO MODEL NEURONAL AGING

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Aging is an inevitable phenomenon in every living cell. With aging, comes deterioration in cellular processes like cellular senescence, telomere attrition, genomic instability, metabolic dysfunction and epigenetic alterations among others. Since aging is the largest risk factor of neurodegenerative diseases, we theorize that these aging hallmarks contribute to neuronal degradation and death. The exact causes of neurodegenerative diseases that are mainly sporadic like Amyotrophic Lateral Sclerosis and Frontotemporal Dementia are still unknown. Physiological aging has been evidenced to be contributed by several genes like telomerase and sirtuins. Current animal aging models do not explain the pathology of neurodegenerative diseases.The use of human pluripotent stem cells (hPSCs) have been widely used in disease modelling. Hence, we aim to use hPSCs with specific mutations of the aging genes to generate cells with the aged hallmarks and discover specific molecular pathways in which aging-related processes directly cause neurodegeneration. Thus far, our preliminary data demonstrates that TERT knockout neural derivatives indicated stem cell exhaustion and have some disease phenotypes like ER stress while SIRT3 knockout neurons have mitochondrial deficits. Therefore, with our accelerated aging system, it will accelerate aging of neurons and will allow us to study the molecular contributions of aging to neurodegeneration.

ORGANOIDS

T-1019

BUILDING AN ORGANOID-BASED MODEL FOR OVARIAN CANCER

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Over the last few years several studies have led to a significant change in the field of ovarian cancer and it is currently believed that the fallopian tube and not the ovary surface epithelium (OSE) is the main origin of high-grade serous ovarian cancer (HG-SOC). Nevertheless, due to the lack of unique markers and adequate model systems the relative contribution of each tissue is not yet clear and the notion that OSE has a role in HG-SOC development was not cast aside altogether. In this work we have established novel organoid systems derived from both mouse OSE and oviduct (Ovi, the equivalent of human fallopian tube). These systems recapitulate their tissue of origin and demonstrate differences in medium requirements as well as gene expression. To establish comparable tumor progression models for both OSE and Ovi we used CRISPR-Cas9 technique and targeted commonly mutated genes in ovarian cancer (Trp53, Brca1, Nf1 and Pten). Thus, we were able to establish clones with different combinations of mutations. Histological, metaphase spread and gene expression analysis of the mutated organoid clones from both OSE and Ovi demonstrated different degrees of deviation from their wild type counterpart. This deviation became more evident as the amount of introduced mutations increased. Transplantation experiments showed that both Ovi and OSE triple mutants (Trp53, Brca1 and Pten or Trp53, Brca1 and Nf1) are able to give rise to tumors, however, tumors derived from Ovi origin tend to be more aggressive and grow faster than respective OSE mutants. Further analysis will reveal what are the differences of ovarian tumors derived from the two distinct origins. Taken together, in this study we present the first comparable Ovi/OSE research platform that enables addressing questions related to origin and early stages of HG-SOC development.

TISSUE ENGINEERING

T-1021

THE USE OF RECELLULARIZED HEPATIC SCAFFOLD IN A RAT HETEROTOPIC AUXILIARY LIVER TRANSPLANTATION MODEL

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Severe hepatic failure is the result of long-term liver injury. Liver transplantation is the only efficient treatment, but is currently limited by organ shortage. In this context, the creation of a bio-artificial liver might solve this clinical problem. The aim of this work was to optimize a surgical heterotopic auxiliary liver transplantation (HALT) technique to transplant recellularized liver scaffolds. Donor Wistar rats were heparinized, anesthetized and then submitted to transverse abdominal incision. The portal vein (PV) was separated and cannulated, Teflon tube was attached to the inferior vena cava (IVC) and fixed, then the superior vena cava (SVC) was clamped. For decellularization, the livers were transferred to be perfused through portal vein using an infusion

pump at 3 ml/min with water for 2 hours followed by 1% Triton X-100 for 2 hours and SDS 1% for 24h. For recellularization, approximately 109 endothelial cells (HUVEC) were injected through PV and allowed to attach for 2 hours at 37oC. Subsequently, 109 human hepatocyte carcinoma (HepG2) cells were continuous perfused with medium and FBS 10% using an infusion pump at 4 ml/min for 7 days at 37oC. Cells presence in the recellularized extracellular matrix (rECM) were confirmed by DAPI staining and secretion of albumin was detected by ELISA. To transplant rECM, heparinized and anesthetized rats were submitted to transverse abdominal incision to clamp the left arterial and renal vein to remove the left-side kidney. The PV and IVC of the recellularized liver scaffold were anastomosed to the recipient rats' left arterial and renal vein respectively, in an end-to-end anastomosis. After 7 days, DAPI staining showed cells nuclei in rECM. Also, the cells were secreting albumin. Active blood flow within the recellularized liver scaffold was observed indicating that the PV and IVC of the scaffolds were able to sustain the arterial blood pressure when the circulation was re-established. Here, we performed a HALT surgical technique to transplant rECM. Also, the decellularized ECM protocol preserved the structural characteristics of the native microvascular network allowing the recellularization for 7 days. In conclusion, our method is a promising approach of transplanting an engineered liver tissue for application in the hepatic regenerative medicine.

Funding Source: CNPq, Capes, FAPERJ, INCT-REGENERA, Ministério da Saúde

T-1023

IPSC-BASED CELL THERAPY IN TENDON INJURY MODEL

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Tendons are remarkably tough fibrous connective tissues that help to transmit the mechanical force of muscular contraction to the bone. It is widely known that tendons are one of the soft tissues having remarkably high tensile strength, and have the power, essential for withstanding the mechanical stress, which is attributed to the parallel orientation and hierarchical structure of tendon fibers. Tenocytes are embryologically developed from several origins but somite, a family of paraxial mesoderm, is considered to give rise to tenocytes in the trunk part of our body. We have been studying human somite development with human induced pluripotent stem cells (hiPSCs) and recently demonstrated a novel method for hiPSC differentiation into tenocytes in xeno-free/chemically defined conditions for the first time. Using the hiPSCs-tenocytes, we are currently working on cell therapy in a tendon injury model. First, we established a rat Achilles tendon rupture model and then evaluated the effect of hiPSCs-tenocytes injected into the injured area. Consequently, the kinematic function (AFI: Achilles functional index, heel height, an angle of the ankle) in the transplantation group seems likely to be recovered steadily compared to the non-treated group at 2 and 4 weeks after operation. Therefore, hiPSCs-tenocytes might provide a novel promising therapeutic option in Achilles tendon injury. We are currently testing the biomechanical function (ultimate failure load) and histological assessment to evaluate the effects of transplantation in more detail. We also aim to attempt transplantation with other cell types such as bone marrow mesenchymal stem cells and hiPSC-derived somite cells as controls.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-1025

THE HISTONE DEMETHYLASE KDM4A IS CRUCIAL FOR MATERNAL-TO-ZYGOTIC TRANSITION IN MAMMALS

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Manaf, Adeel - *Rikshospitalet, University of Oslo, Norway*
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Gonzalez, Javier Martin - *Transgenics Core Facility (Danstem), University of Copenhagen, Denmark*
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The impact of germ line inherited chromatin landscapes on early mammalian development is only beginning to be understood. In this context, the majority of lysine 9 trimethylation on histone H3 (H3K9me3) is restricted to transcriptionally quiescent heterochromatin, while the comparatively modest amounts of H3K9me3 in euchromatin regulate cell-type specific gene expression. Here, we have explored in oocytes the role of maternally inherited KDM4A, the only known demethylase for H3K9me3 expressed in mouse and human oocytes. Using a KDM4A knockout mouse model, we show that it's loss in oocytes and resultant embryos lead to compromised pre-implantation development with hallmarks of genomic instability. Furthermore, oocytes ablated for KDM4A harbour a global increase in maternal H3K9me3. Using low input Chip-Seq we found this increase to surprisingly co-occur in a bivalent fashion of previously unseen scale with broad domain H3K4me3 (trimethylated lysine 4

on histone H3) that is well preserved over large regions of normally open chromatin. Through single cell transcriptomics, we demonstrate how aberrantly inherited H3K9me3 from the maternal oocyte epigenome negatively impacts gene expression activation early on during pre-implantation development at the maternal-to-zygotic transition. This coincides with reduced expression of large families of repetitive elements and transposons that are usually active in the early preimplantation embryo. At the meeting, we will also present on our efforts to rescue the developmental fate of maternal zygotic Kdm4a mutant embryos through mRNA supplementation in an enzyme dependent manner. Our results demonstrate a critical role for KDM4A in preserving the germ line epigenomic landscape by preventing establishment of H3K4me3-H3K9me3 bivalency that is likely critical for pre-implantation gene expression activation that is essential for proper early development.

T-1027

DECOMISSIONING AND RECOMISSIONING ENHANCERS ENABLES HUMAN GERMLINE CELL SPECIFICATION

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Primordial germ cells (PGCs) are the embryonic precursors that establish the germ cell lineage. How human PGCs (hPGCs) are specified during embryogenesis is largely unknown. To understand mechanisms in hPGC formation, we differentiated hPGC-like cells (hPGCLCs) from human embryonic stem cells (hESCs) and applied assay for transposase-accessible chromatin using sequencing (ATAC-seq) to systematically characterize regions of open chromatin in hPGCLCs relative to undifferentiated hESCs as well as bona fide hPGCs isolated from human embryonic ovaries and testes. Through this analysis we discovered that the transcriptome of hPGCLCs and hPGCs resembles naïve ground state pluripotency, with a large fraction of TFAP2C-bound naïve-specific enhancers becoming decommissioned in primed pluripotency before regaining accessibility in hPGCLCs and hPGCs. Using CRISPR/Cas9, we found that deleting the TFAP2C bound naïve enhancer at the OCT4 locus (also called POU5F1) impairs hPGCLC specification, and we are currently performing single cell RNA-Seq analysis to determine the precursor cell where specification goes awry. Taken together, we propose that specification of hPGCs involves the decommissioning and recommisioning of the naive OCT4 enhancer, and that this mechanism is driven by the pioneering transcription factor TFAP2C.

Funding Source: BSCRC training program

POSTER II - EVEN
19:00 – 20:00

NEURAL DEVELOPMENT AND REGENERATION

T-1014

SINGLE-CELL TRANSCRIPTOMICS IDENTIFIES DEVELOPMENTAL TRAJECTORIES CHARACTERIZED IN THE HUMAN NEUROEPITHELIUM TO RADIAL GLIA TRANSITION

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The human cerebral cortex consists of billions of cells that are primarily generated during developmental stages. During neural development, the neuroepithelium gives rise to radial glia, which is the canonical neural stem cell. Radial glia then asymmetrically divide into transit amplifying intermediate progenitors, which differentiate into excitatory neurons. These steps of neurogenesis have been well characterized. However, there have been very few studies dedicated to understanding the molecular identity of neuroepithelia, exploring the transition from neuroepithelia to radial glia, and teasing apart their contribution to the neocortex. Here we leverage single-cell RNA sequencing from first trimester human cortical samples to reveal several gene candidates that are enriched in progenitors during early first trimester development. C1ORF61, which encodes the transcription factor, Croc-4, is enriched immediately after the switch from neuroepithelium to radial glia. Lineage trajectory analysis using RNA velocity demonstrates a clear lineage

trajectory from the neuroepithelial clusters to radial glial clusters. When we enriched for genes that influence RNA velocity the most, C1ORF61 was amongst the top genes influencing the cell fate switch. Therefore, we hypothesize that C1ORF61 is an early marker for radial glia, and is important during the transition from neuroepithelium to radial glia. Interestingly, mRNA expression of C1ORF61 in the germinal zone of the developing cortex was previously validated via in situ hybridization, suggesting that C1ORF61 may play a role in the determination and maintenance of early radial glia generation. To test our hypothesis, we performed genetic modulations of C1ORF61 in radial glia cells, and found that modulation of C1ORF61 leads to altered fate specification of radial glia in the developing human cortex.

NEURAL DISEASE AND DEGENERATION

T-1016

IPSC-BASED DISEASE MODELING FOR LATE ONSET NEURODEGENERATIVE DISEASES USING A CHEMICAL COMPOUND ACCELERATING SENESCENCE

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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 inhibitor library and found that a compound accelerated differentiation and maturation of iPSC-derived neurons. We confirmed that dopaminergic neurons (DAN) derived from iPSCs treated with this compound exhibited aging phonotypes including increased number of SA-Gal positive senescent cells accompanied with abnormal nuclear membrane structure by day 14 as well as longer culture (36 days) without this compound. In addition, this compound accelerates the phenotype of PARK4-iPSCs derived DAN, which a late-onset Parkinson's disease. By adding this compound, we observed α-synuclein accumulation significantly earlier (14 days) compared to the conventional method (~30~50 days). This compound can induce accelerate neuronal differentiation and maturation easily without any gene transfection and is useful to reproduce pathological condition specific to neurodegenerative diseases in a shorter period than conventional cultivation.

ORGANOIDS

T-1018

A HUMAN CELL CULTURE MODEL FOR EML1-INDUCED SUBCORTICAL BAND HETEROTOPIA

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During development the human brain has to form a complex neuronal network, which requires a precise choreography of neurogenesis, neuronal migration and synaptogenesis. Human malformations associated with defects in cortical development can result in cortical dis-organization with severe consequences including epilepsy and intellectual disability. Various mouse models have been used to study human MCD, but they are limited by structural differences between the murine and the human brain. With the advent of efficient gene editing technologies in human cells in combination with the ability to generate human induced pluripotent stem cells (iPSC) and organotypic PSC-derived cerebral organoids we are now technologically equipped to decipher the molecular changes associated with the dysfunction of single genes leading to MCD. Here,

we used forebrain-type organoids derived from patients and genome edited iPSCs to address pathophysiological changes associated with subcortical band heterotopia (SBH) caused by mutations in the EML1-gene. We found that EML1-patient and KO-derived organoids indeed reflect a SBH phenotype with ectopic proliferating progenitor cells accumulating at the basal side of the cortical structures and neurons organized into two bands, above and below the ectopic cells. When investigating the cellular identity of the ectopic progenitors we identified that the majority of the cells express markers for radial glial cells (RGC) and / or outer RGCs while intermediate progenitors could only very occasionally be detected. To further investigate the origin of the ectopic localized progenitors we assessed apical RGC cell delamination. We found that primary cilia which anchoring the RGC to the apical surface are reduced in number and length and show perturbed structures in EML1-patient and KO derived cortical cultures. In addition, we observed significant changes in the cleavage angle of apical RGC towards oblique and/or horizontal angels without apparent neurogenesis. By that our data hint to the possibility that perturbed delamination of a proportion of cells from the VZ might be the primary cause of the heterotopia phenotype and suggest that organoid-based systems serve as promising models to study early human cortical development and associated disorders.

Funding Source: The authors acknowledge the generous support of the Hector Stiftung II.

T-1020

A NOVEL HUMAN IPSC-DERIVED DIFFERENTIATION PLATFORM FOR THE GENERATION OF MESENCHYME-FREE, REGIONALLY PATTERNED INTESTINAL ORGANOIDS

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Generation of induced pluripotent stem cell (iPSC)-derived human intestinal organoids (HIOs) has opened new opportunities for in vitro modeling for a variety of diseases affecting the gastrointestinal tract, ranging from inflammatory bowel disease to Cystic Fibrosis and colorectal cancer. Currently published protocols rely on serum-based differentiation media supplemented with strong Wnt and FGF4 signaling agonists, leading to the generation of mesenchyme-supported HIOs. Here we describe a novel robust intestinal differentiation protocol that generates proximal small intestinal epithelial organoids free of mesenchyme. Following hiPSC differentiation into definitive endoderm, cells are exposed to a short dual inhibition of TGFß² and BMP that generates cells competent to form lineages throughout the developing gut tube. Cells were sorted using either a positive selection for CDX2 or negative selection against the anterior marker NKX2-1/CD47 at day 15, and were plated in several culture conditions upon re-seeding in 3D Matrigel. We found that addition of Wnt and FGF7 signaling combined with R-spondin, noggin and EGF leads to robust specification of proximal intestinal epithelium and the emergence of 3D intestinal organoids free of mesenchyme. In order to track kinetics of CDX2 expression as well as optimizing yield and purity throughout the differentiation process, we herein report a novel CDX2-eGFP iPSC “knock-in” reporter line that confirmed that CDX2 expression was primarily driven by Wnt signaling. Our protocol enables the regional patterning of mesenchyme-free intestinal organoids towards either proximal small intestinal or distal colonic phenotypes. Finally, using patient-specific Cystic Fibrosis iPSC-derived HIOs, we were able to robustly model intestinal CFTR function in the dish. In summary, we report a novel directed differentiation protocol for the generation of mesenchyme-free HIOs that can be primed towards more colonic or proximal intestinal lineages, furthering our ability to study both development and diseases of the epithelium of the gastrointestinal tract.

TISSUE ENGINEERING

T-1022

GENETICALLY ENGINEERED HEMATOPOIETIC PROGENITOR CELLS ENABLE THE ROBUST PRODUCTION OF NEUTROPHILS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Chemotherapies for cancers induce granulocytopenia and additively increase the risk of infection. Infections in patients with granulocytopenia, especially neutropenia, have remained as a major problem. Though granulocyte transfusion therapy(GTX)is a therapeutic option against neutropenic infections refractory to supportive therapies, GTX has not been in wide use mainly due to its physical burden on donors. To overcome the problem, leading to donor-free GTX, we established a robust production system of neutrophils using genetically engineered hematopoietic progenitors priming neutrophils(NeuP-HPCs)from human induced pluripotent stem cells(iPSCs). To obtain NeuP-HPCs, c-MYC and BMI1 were transduced with doxycycline-inducible lentiviral vectors in CD34+ and CD43+ HPCs derived from human iPSCs. Obtained NeuP-HPCs, immunophenotypically defined as CD10-, CD11b-, CD13+, CD14-, CD16b-, CD33dim, CD34- CD45+, CD64+, and CD66b-, were differentiated into the similar number of neutrophils in four days, more than 90% of which were band or segmented neutrophils. NeuP-HPCs were able to expand for 12 weeks, doubling time of which ranged from 35.5 hr to 63.6 hr, indicating that one NeuP-HPC was estimated to achieve 5.0 x 1010 cells, clinically meaningful scale, in 10 weeks. After the differentiation, flow cytometry showed the expression of neutrophil-specific markers, CD16b and CD66b, in the neutrophils from NeuP-HPCs. The neutrophils formed toxic granules with lipopolysaccharides and produced reactive oxygen species with phorbol-12-myristate-13 acetates, suggesting that the NeuP-HPCs generated functional neutrophils. To investigate genes enhancing the function of NeuP-HPCs, we performed single-cell analysis of focused 83 genes in HPCs and identified the population committing neutrophils in early phase, which enriched the expression of BCL6, CEBPB, CEBPD, and LITAF. Of four genes, CEBPB prolonged the survival of NeuP-HPCs and LITAF enhanced the formation of toxic granules. In conclusion, we achieved the robust production of functional human neutrophils from NeuP-HPCs. NeuP-HPCs are potential sources of neutrophils for donor-free GTX.

Funding Source: This work was supported by Center of Innovation Program, Japan Science and Technology Agency (JST), Japan Agency for Medical Research and Development, the collaboration and the research funding of Kyowa Hakko Kirin Co. Ltd.

T-1024

DEVELOPMENT AND IN VITRO CHARACTERIZATION OF AN IPS-DERIVED FUNCTIONAL LIVER ACCESSORY VASCULAR SHUNT

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The human liver is responsible for important metabolic functions. Patients chronically exposed to hepatotoxic agents can develop a long-term tissue inflammation, resulting in the accumulation of non-functional fibrotic tissue within liver parenchymal (i. e. cirrhosis). In most cases, due increased vascular perfusion resistance, the portal system gets hyper-pressurized. Here we report a method to produce a hepatic-vascular accessory liver combining decellularization technology and differentiation of hiPS cells towards liver cellular components (i. e. hepatocytes, endothelial cells and mesenchymal cells). Rat thoracic aorta sections were harvested and decellularized in rotating wall bioreactor at constant pressure of 120 mmHg, and interval perfused with dH2O and Triton X-100 1% for 48h. Residual DNA was below 50 ug of DNA/mg tissue. hiPS differentiation was confirmed by flow cytometry for hepatic markers (ALB/UGT1A1), endothelial markers (CD31/VECAD) and MSC markers (CD90/CD73). hiPS-derived cells were harvested and intraluminal seeded on decellularized aortic tissue and rotated for 24 hours (1 rpm). Cells were washed and tissue was cultured for 18 days in perfusion culture (5 mL/min, 80 mmHg at 5 rpm). The characterization of the generated tissue shows expression of hepatic markers such as ALB, CK18 and A1AT. RT-qPCR shows expression of important metabolic enzymes of phase I, II and III. Furthermore, secretion of albumin, alpha1-anti-trypsin, APOB100 and alfa-fetoprotein confirmed hepatic function of the neo hepatic tissue. Animal testing will be performed next. In conclusion, engineered vascular-liver accessory tissue could be used as an alternative to reduce associated comorbidities of portal-systemic shunts.

Funding Source: FAPESP (2015/14821-1), CNPQ and CAPES.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-1026

DIFFERENTIATION OF PRIMATE PRIMORDIAL GERM CELL-LIKE CELLS USING MOUSE XENOGENEIC RECONSTITUTED TESTIS

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Xenotransplantation and homologous transplantation of rhesus macaque primordial germ cell-like cells (rPGCLCs), generated from induced pluripotent stem cells (iPSCs), into the adult testicular niche leads to the in vivo advancement of rPGCLC differentiation. Recently, human oogonia-like cells were generated in vitro using hPGCLCs when reconstituted with female somatic cells from dissociated embryonic mouse ovaries (xrOvaries) suggesting that an embryonic niche may be required to support coordinated PGCLC differentiation. To address this, we evaluated rhesus and human PGCLC differentiation in xrTestis self-assembled from single cell suspensions of E12.5 embryonic mouse testicular cells. These single cell suspensions were aggregated as floating cultures in low adhesion 96-well plates before transferring to transwell membranes to create self-assembling rTestes. Using immunofluorescence (IF) staining, we found Sox9-positive (+) sertoli cells cluster and polarize in the rTestes, forming tubule-like structures, as early as day (D) 14 after transfer to the membrane. By D21 of transfer, these tubule-like structures become more numerous and morphologically complex. At both D14 and D21 we discovered that the extracellular matrix protein Laminin formed a basement membrane enclosing the sertoli cells as epithelial tubes. To evaluate whether this self-assembling embryonic testicular niche supports rPGCLC differentiation, we combined FACS-sorted GFP+/EpCAM+/ITAG6+ D4 PGCLCs (human or rhesus) with SSEA1 MACS-depleted embryonic mouse testicular cells (lacking endogenous mouse PGCs) to generate rhesus-mouse and human-mouse xenogeneic reconstituted testis (xrTestis). Using this approach, we observed donor-specific incorporation and survival of GFP+ PGCLCs in developing xrTestis. Taken together our findings suggest that the xrTestis may be a powerful model to study testicular niche development and physiology in vitro, as well as provide a new research tool for studying human prenatal germ cell differentiation towards in vitro gametogenesis.

POSTER III - ODD
18:00 – 19:00

PLURIPOTENCY

F-1029

CALCINEURIN-NFAT SIGNALING IS REQUIRED FOR PROPER DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Calcineurin-NFAT signaling is associated with various biological processes and diseases. Our previous study showed that this pathway plays a critical role in mouse embryonic stem cell (ESC) differentiation. However, its function in human ESCs remains unclear. Here, we report that expression of PPP3CC, the gene encoding the catalytic subunit of calcineurin, increases during human ESC differentiation and its knockdown (KD) enhances the self-renewal ability of human ESCs with a simultaneous reduction in the expression of differentiation-associated markers regardless of culture conditions. Moreover, we find that NFATC3 translocates from the cytoplasm to the nucleus when human ESCs exit from a self-renewal state. NFATC3 KD inhibits the expression of differentiation-associated genes. These results indicate that calcineurin-NFAT signaling is activated and required during human ESC differentiation. Mechanistically, NFATC3 interacts with JUN in hESCs and co-expression of exogenous NFATC3 and JUN upregulates lineage markers remarkably under a self-renewal condition. Our further studies identify a potential target of NFATC3, named sushi repeat containing protein X-linked 2 (SRPX2). KD of SRPX2 can phenocopy the reduced expression of lineage markers observed in NFATC3 KD cells. Importantly, NFATC3 binds directly to the SRPX2 promoter region to promote its expression together with JUN. Additionally, inhibition of this cascade represses MAPK signaling rapidly, including ERK1/2, JNK and P38. Taken together, this study delineates the importance of the calcineurin-NFATC3/JUN-SRPX2 signaling cascade for the pluripotency of human ESCs.

PLURIPOTENT STEM CELL DIFFERENTIATION

F-1031

ASSESSMENT OF BIPOTENCY OF EXPANDED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NKX2.1+ LUNG PROGENITOR CELLS TO BE DIFFERENTIATED INTO BOTH AIRWAY AND ALVEOLAR EPITHELIAL CELLS

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The lung is an organ with great expectation for regenerative medicine due to the serious problem of donor shortage for lung transplantation, but lung regeneration research has been delayed as compared with other organs because access to human lung tissue is limited and culture of lung epithelial cells is difficult. In addition, under normal conditions, cell turnover in the lung is relatively low compared to other tissues such as the intestine and skin. Human induced pluripotent stem cells (hiPSCs) were reported to be differentiated into various organ lineage cells and would be useful for regenerative medicine. Previously, we reported a method for the efficient generation of alveolar organoids derived from hiPSCs simulating lung development. We developed the stepwise differentiation from hiPSCs into endoderm, anterior foregut and ventral anterior foregut cells. Then we used carboxypeptidase M (CPM), a surface antigen, to isolate NKX2.1+ cells from the ventral anterior foregut cells. Using these purified cells, airway and alveolar epithelial cells could be induced in three-dimensional culture, respectively. In the present study, we found that NKX2.1+ cells could be expanded, and these cells were demonstrated to be differentiated into alveolar epithelial cells. In addition, we also found that expanded NKX2.1+ cells could be differentiated into airway epithelial cells, depending on the culture condition, indicating that expanded NKX2.1+ cells were bipotent cells for both airway and alveolar epithelial cells. Finally, we attempted to engraft these cells into immunodeficient mice and human NKX2.1+ cells were shown to survive in these mice. These findings suggest that respiratory epithelial cells derived from hiPSCs in our induction methods might be useful for future studies on lung regeneration.

PLURIPOTENT STEM CELL: DISEASE MODELING

F-1035

SINGLE-CELL RNA-SEQ AND PATIENT-SPECIFIC IPSC REVEAL ENDOCARDIAL AND ENDOTHELIAL ABNORMALITY IN HYPOPLASTIC LEFT HEART SYNDROME

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Hypoplastic left heart syndrome (HLHS) is a “single ventricle” malformation which results in severe underdevelopment of the left ventricle, mitral valve, aortic valve, and ascending aorta. A previous study showed that fetal hypoplastic left hearts had a reduced endothelial cell (EC) population and lower capillary density compared with normal hearts. Several genetic variants that are robust determinants of endocardial/endothelial development and function, are also implicated in HLHS. However, the nature of the EC insufficiency as an underlying mechanism causing HLHS is not fully understood. Our overarching goal is to identify the underlying transcriptomic abnormalities attributed to various EC subpopulations, which cause abnormal EC phenotypes associated with HLHS. Thus, we generated induced pluripotent stem cells derived ECs (iPSC-EC) from three HLHS patients and three age-matched controls. Single Cell RNA-Seq profiling revealed a significant reduction of endocardial subpopulation (NPR3+, CDH5+) with defects in NOTCH signaling, extracellular matrix organization, focal adhesion, and innate immune system in HLHS iPSC-ECs compared with controls. The coronary endothelial subpopulation (APLN+/CDH5+) from HLHS iPSC-ECs showed dysregulated cell cycle genes and abnormal glucose metabolism. To further confirm endocardial and endothelial abnormalities in HLHS, we carried out functional analysis and found that HLHS iPSC-ECs showed impaired angiogenic capacity, increased cell apoptosis under stress, and reduced proliferation and adhesion compared with healthy controls, all of which are indicative of impaired vascular function and deranged EC regenerative capacity. Genes involved in endothelial to mesenchymal transition were also down-regulated in HLHS iPSC-ECs compared with controls under treatment of TGFβ2, which may account for the underdevelopment of the valve. This is the first study provided evidence that developmentally impaired differentiation of endocardial cell could lead to the ventricular and valvular hypoplasia in HLHS at single cell resolution using patient-specific iPSC-ECs. Identifying the basis of the endocardial/endothelial defect may hold the clue to reversing the pathogenesis of HLHS.

Funding Source: The research is supported by single ventricle gift fund

F-1037

HUMAN IPSC-BASED DRUG SCREENING FOR PARK9, A FAMILIAL PARKINSON'S DISEASE WITH IMPARED AUTOPHAGY

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Parkinson's disease (PD) is a neurodegenerative disorder with the degeneration of midbrain dopaminergic neurons. However, few disease modifying drugs for PD patients have been identified so far. To explore the pathogenesis and to perform a drug screening of PARK9, one of familial PD with impaired autophagy, we have generated iPSCs from the T cells of a PARK9 patient with a mutation in ATP13A2 gene that encodes a lysosomal type 5 P-type ATPase. First, we confirmed impaired autophagy in PARK9 iPSC-derived dopaminergic neurons by looking at LC3B, a particular autophagosome marker, accumulation caused by impaired lysosomal functions. Then, we succeeded in quantifying LC3B accumulation in cell bodies of PARK9 dopaminergic neurons by using IN Cell Analyzer. We first screened a compound library including 320 chemical compounds by looking at decreased LC3B accumulation in PARK9 dopaminergic neurons as an indicator. We narrowed down candidates to 70 chemicals by this first screening and then performed second screening to exclude the chemicals that reduce LC3B production. We report detailed method and results of this two-step and 96 well-based high-throughput screening to identify disease modifying drugs that improve impaired lysosomal dysfunction in PD-dopaminergic neurons.

F-1039

RORA REGULATION OF HUMAN PANCREAS DEVELOPMENT REVEALED BY A DISEASE MODIFYING SNP IN GATA6 MUTANT PATIENT IPSCS

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GATA6 is a critical regulator of pancreas development, with heterozygous mutations in this gene being the major cause of pancreatic agenesis. Interestingly, patients with GATA6 heterozygous mutations display a large variability in phenotypes. These phenotypes range from pancreatic agenesis, to adult-onset diabetes, to absence of diabetes even in adulthood. We hypothesized that a variant in a non-coding regulatory region of GATA6 may act in conjunction with a coding region mutation resulting in a stronger phenotype. We found that six patients with pancreatic agenesis caused by GATA6 coding mutations all carry the minor allele variant (A) of a non-coding SNP rs12953985. This SNP lies approximately 8kb downstream of the 3â€™ end of the GATA6 gene. Using CRISPR-CAS9 genome editing, we analyzed the effect of this variant on GATA6 expression during in vitro pancreas differentiation from multiple genetically matched hPSC lines. We found that the minor allele variant of rs12953985, in conjunction with a GATA6 heterozygous mutation, led to the largest reduction in GATA6 protein expression specifically during pancreas specification and a more severe defect in generating pancreatic progenitors. We also determined that the minor allele variant of this SNP impairs retinoic acid receptor-related orphan receptor Î± (RORÎ±) binding by chromatin immune-precipitation (ChIP) assay. By using an inverse agonist of RORÎ± to inhibit its function, we found that it decreased GATA6 expression and inhibited pancreas development. This effect was observed only in lines with an intact RORÎ± binding site supplied by the major allele variant of the SNP. Finally, we modified the minor allele variant of the SNP to a consensus RORÎ± binding site in the GATA6 patient iPSC line. We found that the iPSC line where both the coding mutation was corrected and the RORÎ± binding site was introduced had the highest levels of GATA6 expression and most efficient pancreas differentiation capacity. Our investigation provides insight into RORÎ± as a regulator of pancreas development in humans and also highlights the benefits of using hPSCs to study the effects of disease modifying non-coding variants during development. As we get closer to using hPSC derived Î² cells for therapeutic use, a deeper understanding of human pancreas development becomes vital.

POSTER III - EVEN
19:00 – 20:00

CHROMATIN AND EPIGENETICS

F-1028

XIST-RNA AS THE ARCHITECT FOR CHROMATIN AND TRANSCRIPTIONAL CHANGES ALONG THE INACTIVATING X-CHROMOSOME

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During stem cell differentiation, transcriptional and chromatin modification changes co-occur but the order and causality of events often remain unclear. We explore the interrelationship of these processes by using X-chromosome inactivation (XCI) as a paradigm of facultative heterochromatin formation. Using female mouse embryonic stem cells, we initiate XCI by inducing Xist and then monitor the temporal changes in transcription and chromatin by allele-specific profiling. An unprecedented temporal resolution allowed for the identification of histone deacetylation and H2AK119 ubiquitination as the earliest chromatin alterations during XCI. We show that HDAC3 is pre-bound on the X chromosome and that, upon Xist coating, its activity is required for efficient gene silencing. This is most likely achieved through SPEN, which we show to be vital for in vivo XCI. Of the repressive histone marks, PRC1-associated H2AK119Ub accumulation precedes that of PRC2-associated H3K27me3. This primary Polycomb (PcG) accumulation occurs initially at large intergenic domains that can then spread into genes. By mapping chromatin and transcriptional states in cells expressing two mutant Xist RNAs, unable to silence genes or recruit PcG machinery, we uncouple gene silencing from H2AK119Ub deposition. Indeed, H2AK119Ub spreads into genes only in the context of histone deacetylation and gene silencing. On the other hand nearly normal silencing can ensue even without PcG recruitment. All in all, our results reveal the hierarchy of chromatin events during the initiation of XCI and identify key roles for chromatin in the early steps of transcriptional silencing.

Funding Source: This work was funded by ERC advanced investigator awards (ERC-ADG-2014 671027 to E.H. and ERC-ADG-2015 693023 to P.C.), and Sir Henry Wellcome Postdoctoral Fellowship (201369/Z/16/Z; to J.J.Z.)

PLURIPOTENT STEM CELL DIFFERENTIATION

F-1030

ADGRL2 IS AN ESSENTIAL MARKER FOR CARDIAC LINEAGE SPECIFICATION AND EMBRYONIC HEART DEVELOPMENT

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Specific surface markers that enable monitoring of cell subsets would be valuable for establishing the conditions under which PSCs differentiate into cardiac progenitor cells (CPCs) and cardiomyocytes (CMCs). To develop broadly applicable strategies for enriching PSC-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). GPCRs are well-known on the functional nature of these receptors during development. To verify whether a specific marker is expressed during heart development, we assessed its expression using the CLARITY technique. After immersion in a solution with a refractive index matching that of the CLARITY hybrid, the mouse embryo became transparent. After immunostaining the cleared embryo sample, Adgrl2 was exclusively observed in cardiac cells expressing α-SA at embryonic day E9.5 and E10.5. Our clarified 3D images and movies show that four chambers of the heart are fully developed at E10.5 but not at E9.5. At E9.5, Adgrl2 is observed at the ventricle and atrium, while Adgrl2 is present in all chambers of the heart at E10.5. Next, we performed LacZ (β-Gal) staining in heterozygous Adgrl2 KO embryos to evaluate Adgrl2 expression. As a result, LacZ staining showed that Adgrl2 was predominantly expressed in

the heart during the embryonic developmental stage. Adgrl2 knockout in mice was embryonically lethal because of severe heart, but not vascular, defects. To examine the use of Adgrl2 as a bona fide CPC marker during heart development, we tracked Adgrl2 expression during early embryonic development. The heart of Adgrl2–/– embryos at E10.5 exhibited occlusion of the RV, and the expression levels of Gata4 and Nkx2.5 were not as high as those in wild-type and Adgrl2+/– embryos. Interestingly, the heart of Adgrl2–/– embryos, unlike those of wild-type and Adgrl2+/– embryos between E13.5 and E15.5 had a single ventricle revealing a ventricular septal defect. The specific expression pattern of Adgrl2 in PSC-derived cardiac lineage cells as well as in embryonic heart, adult mice, and human heart tissues suggests that this receptor plays a pivotal and functional role across all strata of the cardiomyogenic lineage, as early as the precursor stage of heart development.

Funding Source: This study was supported by grants from the Korea Health Technology R&D Project "Strategic Center of Cell and Bio Therapy" (HI17C2085) funded by the Ministry of Health and Welfare, and the Republic of Korea.

F-1032

GENERATION OF FUNCTIONAL SALIVARY GLAND ORGANOID FROM MOUSE EMBRYONIC STEM CELLS BY SOX9 AND FOXC1 TRANSDUCTION

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The salivary glands arise as a thickening of the primitive oral epithelium and continually develop by branching morphogenesis. However, little is known about the exact molecular mechanism during the earliest stages of salivary gland formation. We generated gene expression profiles of embryonic salivary gland rudiment and oral epithelium separated using a laser micro dissection. Sox9 and Foxc1 were identified as specific transcription factors in embryonic salivary gland rudiment. To identify genes regulated directly by Sox9, Sox9 ChIP-seq was performed in embryonic submandibular gland. Several genes related to salivary gland development were identified as Sox9 putative target genes. However Foxc1 was not detected as putative Sox9 target genes. Next, to determine whether Sox9 and Foxc1 are necessary for salivary gland organogenesis, we suppressed Sox9 and Foxc1 expression in the organ cultures of E13.5 submandibular gland using siRNA. Sox9 and Foxc1 knockdown inhibited branching formation, respectively. Moreover, the combination of Sox9 and Foxc1 promoted salivary gland differentiation from mouse embryonic stem cell-derived oral epithelium in an organoid culture system. The salivary gland organoid consisted of acinar cells, ductal cells, and myoepithelial cells, and morphologically resembled to the embryonic salivary glands. Through hierarchical clustering of RNA-seq data, the salivary gland organoid gene expression profiles were found to be relatively similar to those observed at E15.5 and E18.5. The induced salivary gland organoid developed in vivo and had physiological functions, following orthotopic transplantation into mice whose salivary glands have been removed. The Engrafted salivary gland organoid secreted the saliva by gustatory stimulation. Here we show that the transduction of Sox9 and Foxc1 genes represents a promising tool for the generation of functional salivary gland organoid from mouse embryonic stem cells.

F-1034

PDX1-/NKX6.1+ PROGENITORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS AS A NOVEL SOURCE OF INSULIN-SECRETING CELLS

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Human pluripotent stem cell (hPSC)-derived insulin secreting cells are a promising alternative for diabetes treatment. Generation of functional pancreatic beta cells from hPSCs have presented certain challenges; however, clinical trial with hPSC-derived pancreatic progenitors is underway. We previously demonstrated the generation of a novel population of pancreatic progenitors using hPSCs that expressed the transcription factor NKX6.1, which is expressed exclusively in human beta cells and is critical for their functionality, independently of the master regulator of pancreatic development, PDX1 (PDX1-/NKX6.1+). Herein, we present that this novel population is similar to pancreas-derived mesenchymal stem cells (MSCs), and can differentiate into insulin-secreting beta cells in vitro.

PDX1-/NKX6.1+ structures strongly co-expressed NESTIN, a pancreatic stem cell marker, along with NCAM-1 and NEUROD1, in the absence of the epithelial marker E-Cadherin. The expression of these markers along with NKX6.1 indicate that this population is representative of the pancreatic mesenchyme. RNA-Seq analysis of stage 4-derived PDX1+/NKX6.1+ and PDX1-/NKX6.1+ pancreatic progenitor populations revealed the downregulation of the main pancreatic developmental markers such as PDX1, FOXA1, FOXA2 and GATA6 as well as key tight junction proteins showing the loss of pancreatic epithelial identity in the novel progenitors. Additionally, it revealed upregulation of key neural crest and TGF-beta signaling mediated EMT-related markers, which is supportive of their mesenchymal nature. On further differentiation through endocrine stages, some PDX1-/NKX6.1+ structures detached as a whole from the adherent monolayer. Both self-detached and re-plated as well as the adherent PDX1-/NKX6.1+ population were able to differentiate into INSULIN and GLUCAGON expressing mono- and poly-hormonal cells, using previously published beta-cell protocols. These findings were reproduced in three different hPSC lines, including hESC-H1 and H9 as well as hiPSCs. Therefore, our novel PDX1-/NKX6.1+ pancreatic progenitors can be used as an alternative source of insulin-secreting cells for cell therapy for diabetes and studying human pancreatic beta cell development.

Funding Source: IGP ID 2016 001

PLURIPOTENT STEM CELL: DISEASE MODELING

F-1036

TRANSLATIONALLY CONTROLLED TUMOUR PROTEIN (TPT1) IS A NOVEL THERAPEUTIC TARGET FOR LYMPHANGIOLEIOMYOMATOSIS

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Lymphangioleiomyomatosis (LAM) is characterized by destruction of alveoli and pulmonary vasculature caused by the invasion of hyperproliferative smooth muscle-like cells (LAM-SMCs). This cancer-like behaviour of LAM-SMCs stems from dysregulated mTOR signaling. Unfortunately, current mTOR inhibitors are cytostatic rather than curative, highlighting the need for novel therapeutic targets. Translationally controlled tumor protein (Tpt1), an upstream positive regulator of the mTOR signaling cascade, is implicated in transformation of cancer cells. We have shown that Tpt1 is highly upregulated in LAM-SMCs relative to healthy SMCs; therefore, we posit that silencing of Tpt1 will decrease proliferation of LAM-SMCs. Patient-specific LAM-iSMCs were derived from iPSCs. Knockdown (KD) of Tpt1 expression was achieved by transduction with a selective siRNA sequence, compared to a non-specific scrambled control sequence. Tpt1 KD in LAM-iSMC was confirmed by both Q-PCR and WB (≥50%). Tpt1 KD resulted in cell cycle arrest at G0/G1 and a marked reduction in LAM-iSMC proliferation compared to controls as assessed by Edu incorporation flow cytometry. Since we have previously shown that LAM-iSMCs disrupted endothelial cells (EC) networks by paracrine mechanism, we investigated if Tpt1 KD reduced the destructive effect of its secretome. Annexin V/PI flow cytometry noted reduction in EC apoptosis upon culture with conditioned media (CM) from Tpt1 KD LAM-iSMCs compared to CM from scrambled LAM-iSMCs, while EC viability was increased in the former. Likewise, ECs exposed to exosomes (Exo) derived from Tpt1 KD LAM-iSMCs exhibited less pronounced time-dependent (24-72H) inhibition of EC gene and protein (eNOS, TIE2 and CD31) expression compared to ECs cultured with Exo from scrambled LAM-iSMCs. Finally, the inhibition of Exo production by LAM-iSMC with GW4869 produced an even greater improvement in EC associated gene/protein expression and cell viability, with a further reduction in apoptotic and dead ECs. Of note, Exo depletion further reduced the detrimental effects of Tpt1 KD LAM-iSMC CM on ECs. In conclusion, endogenous Tpt1 expression contributes to hyperproliferation of dysfunctional LAM-SMC and the release of EC-toxic Exos. Thus Tpt1 inhibition may provide a novel therapeutic target for treatment of LAM disease.

F-1038

UNRAVELLING THE GENETIC BASIS OF A SEVERE AND COMPLEX CONGENITAL HEART DEFECT

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Hypoplastic left heart syndrome (HLHS), one of the most severe forms of congenital heart defects, is predominantly characterized by underdevelopment of the left side of the heart. Although conventionally HLHS was considered to have hemodynamic origins, recent studies suggest complex genetic etiology. However, our current knowledge of the disease-causing pathways is very limited. To harness the molecular underpinnings of the disease, we have generated an in vitro model of HLHS using human induced pluripotent stem cells (hiPSCs). hiPSCs were generated from 10 unrelated HLHS

patients and their parents (trio design; 3 clones per individual; 87 hiPSC lines in total), thus providing controls that are as genetically similar to the patients as possible. To investigate differences during early stages of cardiovascular development, hiPSCs were differentiated into cardiac and vascular smooth muscle cells, and their cellular populations and gene expression were studied. Our gene expression analysis revealed no significant differences between vascular smooth muscle cells derived from HLHS-hiPSCs and their parents. In contrast, flow cytometry analysis performed on hiPSC cultures after directed cardiac differentiation at 5-day intervals (day 0-30) showed that ventricular cardiomyocyte differentiation in HLHS-hiPCSs was perturbed. Time course analysis using RNA sequencing on hiPSCs differentiated into cardiomyocytes from 5 HLHS families revealed that the greatest differences between patients and parents were at day 20 post-differentiation initiation, with down-regulation of cell cycle being the main driver. Moreover, transcriptome analysis suggested maturation defect in cardiac cells derived from HLH-hiPSCs. These findings were further confirmed using remaining 5 independent HLHS families. Cell phenotyping also indicated that beating cardiomyocytes derived from patients were more immature and their calcium flux properties were significantly different ($n>1000$; $P<0.001$). In summary, our findings thus far suggest that the progression of cardiogenesis in HLHS-hiPSCs is perturbed, which may be due to disruptions in cell cycle control and maturation. In conclusion, our data suggest a common pathogenic pathway underlying the early development of HLHS despite genetic heterogeneity of disease causation.

REPROGRAMMING

F-1040

CHEMICALS PLASTICIZE MOUSE FIBROBLASTS FOR MULTIPLE LINEAGE PRIMING AND PROGRESSIVE CELL LINEAGE SPECIFICATION

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With the treatment of a cocktail of small molecules, somatic cells can be reprogrammed to be pluripotent stem cells (CiPSCs) through an intermediate extraembryonic endoderm (XEN)-like state. However, since none of the chemicals is associated to a specific cell fate, it is completely unclear how the chemicals orchestrate the cell fate determination in chemical reprogramming. In this study, we analyze the molecular network dynamics in chemical reprogramming process from fibroblasts to XEN-like state, in a time-course manner and in single cell resolution. We find that the XEN-like cell generation undergo two major biological processes, stochastic gene activation (0-4 days) and XEN fate specialization (4-12 days). In the first process, the endogenous expression of multiple lineage related transcription factors is activated, such as Ascl1, Hopx, Gbx2, Hnf1b, Gsx2 and Sox17, heterogeneously dispersed in different single cells. In the second process, XEN master genes, such as Sall4, Gata4 and Foxa2, are progressively upregulated in Sox17 expressing cells. Only when the major transcription factors of XEN cells are highly co-expressed, cell fate transition is triggered around day 12, with significant downregulation of fibroblasts program and stabilization of a stable network of major XEN regulators. In addition, we find the core reprogramming chemicals CHIR99021, 616452 and Forskolin cooperate in both steps, while the small molecule boosters VPA and CH55 function separately in different steps. Interestingly, if the chemicals and culture medium for the second step are fine-tuned after cell plasticization, the reprogramming roadmap can be switched to approaching other cell types, such as hepatocyte-like cells, neurons and skeletal muscle cells. Taken together, our findings reveal the molecular dynamics in early chemical reprogramming, providing a new framework and a general strategy to develop chemical reprogramming methods for other cell types through a multi-lineage priming state.

Funding Source: This study was also supported by the Science and Technology Department of Sichuan Province (2018JZ0025)

WEDNESDAY, JUNE 26, 2019

POSTER I - ODD
18:30 – 19:30

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-2001

CREB1 IS A POTENT MASTER REGULATOR OF GLUTATHIONE LEVEL IN HUMAN MESENCHYMAL STROMAL/STEM CELL

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Continuous exposure to oxidative stress during the expansion of mesenchymal stromal/stem cells (MSCs) based on traditional culture techniques results in a progressive loss in proliferative and differentiation potential of MSCs. To prevent these reactive oxygen species (ROS) mediated damages, it is required to understand the regulation network of glutathione (GSH), a major anti-oxidant in living cells. Recently, we reported a new method to real-time monitor intracellular GSH level employing a newly synthesized fluorescent probe. Here, we fractionized human MSCs into GSH-high and -low cells based on FreSHtracer and compared their transcriptomes. As results, several genes related to DNA metabolism and repair were highly expressed in GSH-high MSCs. Furthermore, MSCs with high level of GSH showed increased expression and transcription activity of cyclic AMP-responsive element-binding protein 1 (CREB1). In this regard, CREB1 enhanced the recovery capacity of GSH after exposure to oxidative stress. Accordingly, CREB1 positively modulated core functions of MSCs including self-renewal, chemoattraction to growth factors, and angiogenesis activities. Taken together, these results demonstrate that CREB1 is a master regulator of GSH dynamics in MSCs, thus it will be a potent target to improve the therapeutic efficacy of MSCs

W-2003

LARGE-SCALE EXPANSION OF UMBILICAL CORD (UC)-DERIVED HUMAN MESENCHYMAL STROMAL CELLS (MSC) UNDER GMP CONDITIONS DOES NOT ALTER THEIR QUALITY

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Human mesenchymal stromal cells (hMSCs) are non-haematopoietic, multipotent cells with ability to differentiate into mesodermal, ectodermal and endodermal lineages. In the past few years, an extraordinary interest was dedicated to research of anti-inflammatory features of hMSCs and their promising applications in regenerative medicine. hMSCs can be isolated from fetal as well as adult tissues of mesenchymal origin including Wharton´s jelly of the umbilical cord (UC). The advantages of UC-hMSCs are a noninvasive procedure of derivation, fast self-renewal properties and minimal contact with the environment. The aim of this study was to establish a simple procedure for UC-hMSCs isolation, characterization and expansion under GMP conditions, followed by a detailed comparison of UC-hMSCs properties cultivated either on T-flasks or in a large scale bioreactor (Quantum Cell Expansion System; Terumo BCT, Denver, CO, USA). Four GMP quality culture media were tested for cell viability, proliferation and surface markers expression, and compared with laboratory standard media supplemented by fetal bovine serum (FBS). The most suitable medium (proportion of efficacy/costs) was used for large scale expansion and expanded cells were tested for trilineage differentiation, immunomodulatory properties, cellular senescence, genetic stability and in vitro tumorigenicity. We found that large-scale expansion of UC-hMSCs using Quantum Cell Expansion System under GMP conditions does not change identity and quality of cells. Therefore, this method of UC-hMSC expansion represents a suitable tool for introduction in clinical applications.

Funding Source: Supported by Technology Agency, Czech Republic, Program DELTA, project TF03000037, and Ministry of Education, Youth and Sports, Czech Republic, National Program of Sustainability II, project LQ1605.

W-2005

MODELING PREECLAMPSIA USING INDUCED PLURIPOTENT STEM CELLS

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Preeclampsia (PE) is a hypertensive disorder of pregnancy associated with abnormal trophoblast differentiation. PE placentas have a defective layer of multinucleated syncytiotrophoblast (STB) with increased apoptosis; in fact, GCM1, a transcription factor involved in STB formation, is reduced in PE placentas. However, there is a lack of good in vitro model systems for studying these defects; therefore, we attempted to model this disease using induced pluripotent stem cells (iPSCs). Five iPSC lines (three PE and two non-PE), whose

pluripotency was confirmed by Pluritest, were used. Cells were cultured in Essential-8-based media with 2uM IWP2 (Wnt inhibitor) and 10ng/ml Bone Morphogenetic Protein-4 (BMP4) for 4-5 days for differentiation into cytotrophoblast (CTB) stem cells (first-step). Subsequently, cells were replated in feeder-conditioned media + 10ng/ml BMP4 for terminal differentiation into STB (second-step). Differentiation was analyzed by flow cytometry and qPCR. STB formation was assessed morphologically by calculating the fusion index; apoptosis was assessed at the end of the second step by western blot for PARP. There were no differences in CTB marker expression by flow cytometry (EGFR) or qPCR (EGFR, CDX2, p63); however, GCM1 was down-regulated (3-74-fold, $p<0.05$) in PE-iPSC. At the end of the second-step, PE-iPSC-derived trophoblast showed reduction in STB-associated genes (ERVW1, PSG4) (2-20-fold, $p<0.05$) by qPCR. However, neither the fusion index nor the apoptosis assay showed any differences between PE and non-PE iPSC lines ($p=0.25$). Our results indicate that PE-iPSC-derived trophoblast show altered STB markers, without altered morphology or apoptosis in the mixed culture. Future studies will focus on evaluation of additional markers of STB maturation and assessment of apoptosis specifically in iPSC-derived multinucleated STB, to further dissect the placental defects of PE.

ADIPOSE AND CONNECTIVE TISSUE

W-2007

ACTIVIN E CONTROLS ENERGY HOMEOSTASIS IN BOTH BROWN AND WHITE ADIPOSE TISSUES AS A HEPATOKINE

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Brown adipocyte activation or beige adipocyte emergence in white adipose tissue (WAT) increases energy expenditure, leading to a reduction in body fat mass and improved glucose metabolism. We found that activin E functions as a hepatokine that enhances thermogenesis in response to cold exposure through beige adipocyte emergence in inguinal WAT (ingWAT). Hepatic activin E overexpression activated thermogenesis through Ucp1 upregulation in ingWAT and other adipose tissues including interscapular brown adipose tissue and mesenteric WAT. Hepatic activin E-transgenic mice exhibited improved insulin sensitivity. Inhibin βE gene silencing inhibited cold-induced Ucp1 induction in ingWAT. Furthermore, in vitro experiments suggested that activin E directly stimulated expression of Ucp1 and Fgf21, which was mediated by transforming growth factor-β or activin type I receptors. We uncovered a function of activin E to stimulate energy expenditure through brown and beige adipocyte activation, suggesting a possible preventive or therapeutic target for obesity.

Funding Source: JSPS KAKENHI Grant JP21580370 JP26450442 JP25640109

W-2009

HUMAN ADIPOSE STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS FROM OBESE PATIENTS SHOW SPECIFIC DIFFERENCES IN MITOCHONDRIAL FUNCTION

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Human adipose stem cell-derived hepatocyte-like cells (hASC-HLCs) hold considerable promise for future clinical individualized therapy of liver failure or dysfunction. However, increasing prevalence of obesity in adults, caution should be taken as their adipose tissue niche may affect the functional

properties of hASC-HLCs. Here, we focused on the properties of mitochondrial function of hASC-HLCs from obese individuals. hASCs from visceral adipose tissues from three female obese donors (BMI >35 kg/m2) and three female control donors (BMI <25 kg/m2) were separately cultured and differentiated to HLCs as our previously three-stage protocol. Differentially expressed genes profiling between obese and control individuals were acquired from RNA-sequence analysis. Results revealed that 49 down-regulated genes and 48 up-regulated genes were found in hASC-HLCs from obesity individuals (ob-hASC-HLCs). The down-regulated genes included the encode mitochondrial related protein such as mitochondrial elongation factor 1(MIEF1), elastin (ELN), and microtubule associated protein 1 light chain 3 beta (MAP1LC3B). Gene Set Enrichment Analysis revealed that genes associated with the biology process including mitochondrial electron transport (NADH to ubiquinone), protein targeting to mitochondrion, mitochondrial translation, mitochondrial respiratory chain complex I assembly, and oxidative phosphorylation paythway in ob-hASC-HLCs were down-regulated. However, reactive oxygen species (ROS) assay using 2',7'-dichlorofluorescin diacetate fluorogenic dye showed that the production of ROS in ob-hASC-HLCs was significantly higher than that in control-hASC-HLCs after treatment with 50 uM fluvastatin sodium. Mitochondrial respiration stress activity measured using a Seahorse XF-24 analyzer revealed that the oxygen consumption rate (OCR) of basal level, ATP-coupled, and maximal respiratory rates in ob-hASC-HLCs were significantly higher than that in control-hASC-HLCs. These data suggesting that the lower expression of mitochondrial content and dysfunctional oxidative activity in ob-hASC-HLCs may be associated with abnormal functional of mitochondrial OxPhos in hepatocytes in obese subjects, which probable further promotes the development of non-alcoholic fatty liver diseases.

Funding Source: This work was supported by National Natural Science Foundation of China (31171310; 81770616); Beijing Natural Science Foundation of China (5172009).

MUSCULOSKELETAL TISSUE

W-2013

REVISITING THE VASCULARITY AND RESIDENT STEM CELL POPULATION OF THE HUMAN MENISCAL WHITE-WHITE ZONE

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The meniscus is at the cornerstone of knee joint function, yet very vulnerable to injury and age-related degeneration. Meniscal tears are reported as the most common pathology of the knee with a mean annual incidence of 66 per 100,000. Knee osteoarthritis progresses rapidly in the absence of a functional meniscus. Historically, tears extending to the inner portion of the meniscus (a.k.a. white-white, WW zone), were considered as untreatable and were often resected, due to the lack of vascularity in the WW zone. Perfusion-based anatomical studies in the 1980s shaped the current dogma that human meniscus has poor regenerative capacity, partly due to limited blood supply. We hypothesized that the WW zone of the meniscus possesses regenerative capacity due to a resident stem/progenitor cell population and some vascularization that was not previously detected. Fifteen menisci from fresh healthy human knees (mean age: 21.53±6.53 years) were acquired from three tissue banks. Vasculature was assessed per zone by histology and CD31 immunofluorescent staining. Additionally, to map the vasculature in 3D, menisci were optically cleared using a modified uDISCO procedure, labeled with CD31, and imaged using light-sheet fluorescence laser microscopy. Cells from fresh menisci (up to 1 week post-harvest) were characterized immediately after isolation using CFU and flow cytometry with antibodies against MSC consensus surface markers (CD105, CD90, CD44 and CD29) and after propagation in culture. Isolated cells from all zones were also successfully induced in vitro toward the three MSC lineages (osteogenic, adipogenic and chondrogenic), suggesting that a proportion of the cultured meniscus cells are multilineage stem cells. Our results determine that CD31-expressing micro-vessels were present in all zones, including the WW zone, which was previously considered completely avascular. Additionally, stem/progenitor cells were shown to be present in all three zones of menisci, including the WW zone, showcasing its regenerative potential.

Funding Source: American Orthopaedic Society for Sports Medicine

W-2015

GANODERMA LUCIDIUM PROMOTES PROLIFERATION OF SATELLITE CELLS THROUGH TAK1-JNK-AP-1 SIGNAL PATHWAY BY ACTIVATION OF TLR2/4

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The volumetric and functional recovery for muscular atrophy are always been important issues in clinical medicine. Although the cause of muscle atrophy is various, current evidences indicate to the depletion or the impaired maturation (differentiation) of satellite cell (SC), since SC is known to play an important role during postnatal muscle regeneration. The Ganoderma Lucidum (GL), a traditional Chinese medicine which contains voluminous Sacchachitin has shown the positive effect for health promotion. Recently, these GL-based compounds have been applied clinically for neuromuscular disorders and showed indeed outlooking results. Although the GL-based drugs showed positive effect on muscular and/or neuromuscular diseases, the molecular mechanism behind the effect on SC, remains unclear. The purpose of this study is to investigate the efficacy and the molecular mechanism of Sacchachitin on SC. In this study, the SCs (Pax7+ve) isolated from TA muscle were co-cultured with GL-contained growth medium and a significant enhanced proliferation of SC (Pax7+veBrdU+ve, $p \leq 0.05$, $n=6$) was admitted. Our data revealed, the GL is able to activate TAK1-JNK-AP-1 signal pathway that mediated by TLR2/4. Intriguingly, JNK was solely activated but not p38 MAP kinase nor ERK was activated by GL. The results of this study elucidated the possible therapeutic mechanism of GL on muscular atrophy for the first time. Furthermore, these data indicated the pertinence of GL in clinical application for improving the skeletal muscle atrophy.

W-2017

DISSECTING STEM CELL HETEROGENEITY DURING ZEBRAFISH SKELETAL REGENERATION

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In most vertebrates, skeletal tissues have a modest capacity for repair. In humans, larger bone injuries will not heal without medical intervention, and cartilage has an even more limited ability to repair in adults. A better understanding of how to mobilize endogenous skeletal stem cells for repair would provide new opportunities to improve outcomes after major skeletal injuries. We have previously shown that the lower jawbone of adult zebrafish displays remarkable regenerative abilities, yet the nature of the skeletal stem cells that mediate such regeneration remain unknown. Here, I have developed two additional models of skeletal regeneration in zebrafish. In the first, I show that the ceratohyal (an endochondral bone) can regenerate to the same extent as the intramembranous lower jawbone. In the second, I have developed a genetic cartilage ablation model and find that cartilage can effectively regenerate in adult fish. Using a combination of single-cell RNA-sequencing and lineage tracing experiments, I will present my ongoing work dissecting the types of skeletal stem cells mediating regeneration of these three types of skeletal tissues (intramembranous bone, endochondral bone, cartilage). The long-term goal of my work is to identify distinct types of skeletal stem cells that can mediate distinct types of damaged skeletal tissue.

W-2019

THERAPEUTIC POTENTIAL OF PLURIPOTENT STEM CELL-DERIVED CHONDROCYTES IN A PORCINE MODEL OF ARTICULAR CARTILAGE INJURY

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The pathogenesis of OA often begins with an injury to articular cartilage, which establishes chronic, low-grade inflammation that eventually promotes matrix degradation leading to the destruction of cartilage. Currently, there are no agents that efficiently slow or inhibit this process. Pluripotent stem cell-derived chondrocytes (PSCDC) represent a promising new tool for cartilage repair, but specification of these cells in vivo remains unclear. We have recently shown that the transcriptional, epigenetic and functional signatures of human PSCDC closely mimic their primary counterparts, but PSCDC remain immature in vitro. We also demonstrated that human articular chondrocytes are heterogenous and form functionally and spatially distinct cells populations marked by integrin alpha 4 (ITGA4) and Bone Morphogenetic Protein Receptor 1B (BMPR1B). Single cell RNA-sequencing of bioreactor-produced PSCDC revealed robust generation of matrix-producing articular chondrocytes and no detectable residual PSCs. Implantation of human PSCDC formulated in a cryopreservable, lesion-customizable off-the-shelf membrane generated articular cartilage tissue in injured pig joints; Safranin O and IHC staining for the cartilage markers COL2 and SOX9 showed the presence of human cartilage tissue in pig defects minimally expressing COL1 and 10. In contrast, control animals repaired cartilage defects with a highly disorganized fibrotic tissue marked by COL1. Moreover, IHC for BMPR1B and ITGA4 showed correct zonal organization of human chondrocytes in pig cartilage lesions. No

signs of neoplastic growth were detected in any tissues of the transplanted pigs. Six-month follow up of transplanted pigs will be presented. Together, these data demonstrate the potential of allogenic, mass-produced pluripotent stem cell-derived chondrocytes as a therapeutic agent for articular cartilage repair.

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W-2021

MACROPHAGE-MEDIATED ACTIVE PROLIFERATION AND FUSION OF PHARYNGEAL SATELLITE CELLS UNDER BASAL CONDITION

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Satellite cells (SCs), also known as adult muscle specific stem cells, are critical for muscle regeneration upon muscle injury. Although limb muscle SCs are quiescent in sedantary muscles, craniofacial muscle SCs, such as extraocular (for eyeball movement) or pharyngeal (for swallowing) muscle SCs (PSCs), represent higher level of proliferation and differentiation/fusion without injury. However, the mechanism of activation of craniofacial muscle satellite cells remains unclear. In order to study the mechanism underlying craniofacial muscle satellite cells, we investigated to the relationship between PSCs and macrophage to explain the high level of regenerative capacity. First, we measured in vivo proliferation via flow cytometry using specific surface markers for SCs (CD31-/CD45-/Sca1-/Intergrin7α+). At 12 months of age, the percentage of proliferating BrdU+ SCs were significantly increased above 10-fold in PSCs compared with limb SCs. To track SC fusion in vivo, we used a genetically engineered mouse which express tdTomato under satellite cell specific promotor, Pax7 (Pax7 CreERT-tdtomato mouse). Utilizing inducible Cre-lox system, our mice will express tdTomato in satellite cells by Tamoxifen injection, which allows to trace SC fusion into myofiber during certain periods. After tamoxifen injection, we found that pharyngeal muscles showed significantly higher tdtomato fluorescent intensity in myofibers than limb muscles implying higher SC fusion in pharyngeal muscles. Second, to study the underlying mechanism of PSCs, we performed comparative analysis of transcripts by microarray that revealed up-regulated genes involved in regulation of TNF signaling, and interaction of cytokine-cytokine receptor in PSCs compared to limb SCs. In addition, pharyngeal muscles without injury showed a significantly higher population of CD206+ M2 macrophage compared to limb muscle by immunohistochemical data. The mRNA levels of macrophage-related cytokines (Lif, Ccl2 and Ccl7) in PSCs were also higher than those of limb SCs. Finally, we confirmed that PSCs attracted more macrophages than limb SCs after in vivo transplantation into limb muscles. Taken together, this study give new insights into why pharyngeal muscles have high level of regenerative capacities even under basal condition.

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W-2023

DEFINING HUMAN IN VIVO SKELETAL MUSCLE DEVELOPMENT FOR IN VITRO MUSCLE STEM/PROGENITOR CELL MATURATION

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Skeletal muscle wasting disorders including muscular dystrophies and sarcopenia result in inferior quality of life, loss of mobility or even early patient death. Skeletal muscle stem/progenitor cells (SMPCs) derived from human pluripotent stem cells (hPSCs) are a promising sources for cell-based therapy for muscle wasting diseases. However, currently available hPSC directed myogenic differentiation protocols result in highly heterogeneous cell populations with immature SMPCs that are unsuitable for clinical implementation. To better understand human skeletal

muscle development and guide hPSC-SMPC generation and maturation, we employed single cell RNA-sequencing (scRNA-seq) to profile human skeletal muscle tissues from embryonic, fetal to postnatal stages. In silico, we unbiasedly isolated the SMPCs away from other cell types present in the tissues at each individual stages and constructed a "roadmap" of human skeletal myogenesis across development. In a similar fashion, we also profiled the heterogeneous cell cultures generated from multiple hPSC myogenic differentiation protocols and observed variation among directed differentiation timepoints. In general, we found neuronal, Schwann cell, smooth muscle, cartilage and fibroblastic lineages as major populations other than the myogenic cells present in the cultures. Next, we computationally purified the in vitro hPSC-SMPCs and consistently mapped them using our in vivo myogenic "roadmap" to a developmental period corresponding to embryonic to fetal transition (7-9 weeks prenatal) across all differentiation protocols tested. Moreover, we developed a gene network analysis algorithm tailored for scRNA-seq data and discovered co-regulated gene groups present in distinct stages of human myogenesis and from hPSC-SMPCs. We further identified transcription factors (TFs) within the gene groups enriched in fetal and postnatal SMPCs, and are currently developing TF overexpression strategies to "mature" hPSC-SMPCs to fetal or postnatal stages. In summary, this work serves as a resource for advancing our knowledge of human myogenesis and to guide the generation of the most regenerative cells for translational applications in muscle diseases.

CARDIAC TISSUE AND DISEASE

W-2025

CHARACTERIZATION OF AGE-DEPENDENT CARDIAC PROLIFERATIVE POTENTIAL IN A PORCINE MODEL

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The mammalian heart was previously thought to be a terminally differentiated organ. However, studies in mice have shown cardiomyocyte proliferative potential within one week of birth. Given the paucity of data in large animals, we aim to establish a timeline for and elucidate patterns of age-dependent proliferative potential in a porcine model. Two sows proceeded with natural birth and their 22 offspring were randomly assigned to postnatal time points for necropsy (day 0, 1, 3, 7, 21, and 84). At each time point, 3 animals were injected with bromodeoxyuridine 24 hours prior to necropsy (except at day 0). Mid-right ventricle (RV) and mid-left ventricle (LV) free wall samples were collected. Reverse transcriptase polymerase chain reaction of cardiac gene panels and immunohistochemistry with Ki-67 were used to assess cardiac proliferation. ANOVA models compared differences for time points versus day 0 samples for gene expression and Ki-67 % positive nuclei. Over 84 days, the animals' cardiac mass grew by a factor of over 10 (11.1 ± 3.6 g to 115.6 ± 15.8 g) and overall body mass by a factor of almost 15 (1.6 ± 0.4 kg to 31.6 ± 3 kg). The RV did not significantly increase in thickness but the LV free wall thickness more than doubled (2.7±0.5 mm to 6.5±0.4 mm). A statistically significant drop in nuclear Ki-67 staining was noted between day 21 and 84 in both the RV (20.9% vs 2.7%, respectively; $p<0.01$) and LV (21.5% vs 3.7%, respectively; $p<0.01$). Bromodeoxyuridine/troponin T double staining was used to confirm which replicating cells were cardiomyocytes. Numerous predictive, progenitor, and fetal cardiac genes demonstrated increased expression compared to day 0 baselines at day 21 and even 84 (Table 1). Overall, it appears that the porcine heart has cellular proliferation capacity for at least 3 weeks after birth with a subsequent drop. Earlier significant increases in gene expression were seen in the RV (as early as day 3) versus the LV (usually at day 21). Cellular and subcellular pathways for assessment of cardiomyocyte proliferative potential can delineate methodology and timing for clinical trials for congenital cardiac disease novel therapies (e.g. intraoperative myocardial stem cell injection). Our observation appear to extend the mammalian window of cardiac proliferative potential to 3 weeks and beyond in porcine hearts.

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W-2027

FUNCTIONAL DYNAMICS OF CHROMATIN TOPOLOGY IN HUMAN CARDIOGENESIS AND DISEASE

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Functional changes in spatial genome organization during human development or disease are poorly understood. We have investigated these dynamics in two models: (1) the differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CM); (2) hPSC-CM from patients with cardiac laminopathy, a genetic dilated cardiomyopathy with severe conduction disease due to mutations in LMNA (which encodes for the nuclear intermediate filament proteins Lamin A/C). We combined omics methods to probe nuclear structure (Hi-C), chromatin accessibility (ATAC-seq), and gene expression (RNA-seq), and genetic perturbations by CRISPR/Cas9. We found that as hPSC differentiate the heterochromatin compacts and large cardiac genes transition from a repressive (B) to an active (A) compartment. We identified a network of such gene loci that increase their association inter-chromosomally, and are targets of the muscle-specific splicing factor RBM20. Genome editing studies showed that the TTN pre-mRNA, the main RBM20-regulated transcript in the heart, nucleates RBM20 foci that drive spatial proximity between the TTN locus and other RBM20 targets such as CACNA1C and CAMK2D. This mechanism promotes RBM20-dependent alternative splicing of the resulting transcripts, indicating the existence of a cardiac-specific trans-interacting chromatin domain (TID) functioning as a splicing factory. In the context of disease, we found that Lamin A/C haploinsufficient hPSC-CM have marked electrophysiological, contractile, and gene expression alterations. While large-scale changes in chromatin topology are evident, differences in chromatin compartmentalization are limited to a few hotspots. These regions normally transition from A to B during cardiogenesis, but remain in A in mutant hPSC-CM. Non-cardiac genes located within such aberrant domains are ectopically expressed, including the neuronal P/Q-type calcium channel CACNA1A. Importantly, pharmacological inhibition of the resulting currents partially mitigates elongation of field potential duration during the contraction of mutant hPSC-CM. Altogether, this work demonstrates the dynamic nature of genome organization during human cardiogenesis and in disease, and shows how these spatial relationships can regulate lineage-specific gene expression.

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W-2029

MODELLING LEFT VENTRICLE CARDIOMYOCYTE DIFFERENTIATION IN VITRO USING LESSONS FROM THE MOUSE HEART

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Cardiovascular disease is the leading cause of death in industrialized countries. Human embryonic stem cells (hESCs) offer the possibility to model some of these diseases in vitro, with the aim of finding drugs for better management of clinical outcomes. Current cardiomyocyte differentiation protocols often produce heterogenous cell cultures. In addition, in vitro cardiomyocytes are immature and do not have all the hallmarks of their in vivo counterparts. To circumvent this problem, a thorough understanding of how the various heart compartments are formed is needed. We have performed RNA-seq analysis of micro-dissected regions of the mouse heart across 4 time points of development, ranging from early tube bulge to the first stages of looping. Our temporal and region specific information has allowed us to identify signals and transcription factors which are uniquely enriched (or downregulated) in the left ventricle, the right ventricle and the atria. Most of these genes are region specific from the onset of tube formation and only a few are increasing in expression levels as the heart tube develops. We are now performing spatial transcriptomics of the mouse heart so we can build a 4D map of heart gene expression. Moreover, some of the identified candidate genes and pathways, which represent an array of biological functions, are now being modulated to optimise in vitro differentiation of hESCs into a homogenous left ventricle cardiomyocyte fate.

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W-2031

ROLE OF TELOMERE DYSFUNCTION IN DUCHENNE MUSCULAR DYSTROPHY CARDIOMYOPATHY

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Duchenne muscular dystrophy (DMD) is a devastating X-linked genetic disorder that affects 1 in 3500 males. Characterized by progressive muscle degeneration that culminates in respiratory failure and dilated cardiomyopathy, DMD is caused by a lack of dystrophin, a protein that provides structural support between the sarcomeric cytoskeleton and the extracellular matrix. Loss of dystrophin leads to a leaky plasma membrane, contractile stress, and disruption of cellular homeostasis. However, the molecular mechanism that eventually leads to cell death remains to be explored. Recently, the Blau lab discovered a pathogenic link between DMD cardiomyopathy and telomere dysfunction. While mdx mice that lack functional dystrophin do not exhibit dilated cardiomyopathy as in human patients, when crossed with mTR mice that lack the RNA component of telomerase (TERC), mdx mice with "humanized" telomere lengths fully manifested the severe muscle wasting and cardiac failure seen in patients. Notably, the longer telomeres, characteristic of mice, appear to be cardioprotective. Importantly, we observed telomere shortening in cardiomyocytes, but not other cell types, of DMD patients compared to age-matched controls. Preliminary data suggests that contractile stress due to the lack of dystrophin leads to a pathogenic feed-forward loop which ultimately culminates in cardiomyocyte cell death. Using human iPS cells derived from DMD patients, we have modeled telomere shortening and aspects of cardiomyocyte dysfunction characteristic of DMD, including aberrant calcium transients, mechanical stress, and arrhythmia. By comparing cardiomyocytes derived from DMD iPS cells with those from CRISPR-corrected isogenic controls on patterned bioengineered hydrogel platforms of varying stiffness, we can recapitulate 30 years of patient life in 30 days of culture, enabling elucidation of the role of telomere dysfunction in the premature demise of DMD cardiomyocytes. Pinpointing the early molecular events that trigger the pathogenic feed-forward loop will provide strategies for intervention to ameliorate DMD cardiomyopathy.

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W-2033

MULTI-PARAMETRIC ASSESSMENT OF COMPOUND-INDUCED PRO-ARRHYTHMIC EFFECTS IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Development of biologically relevant and predictive cell-based assays for compound screening and toxicity assessment is a major challenge in drug discovery. The focus of this study was to establish high-throughput compatible cardiotoxicity assays using human induced pluripotent stem cell (iPSC)-derived cardiomyocytes. To assess the utility of human iPSC-derived cardiomyocytes as an in vitro pro-arrhythmia model, we evaluated the concentration dependence and responses to 28 drugs linked to low, intermediate, and high torsades de pointes (TdP) risk categories. The impact of various compounds on the beating rates and patterns of cardiomyocyte spontaneous activity was monitored by changes in intracellular Ca2+ oscillations measured by fast kinetic fluorescence with calcium-sensitive dyes. Advanced image analysis methods were implemented to provide multi-parametric characterization of the Ca2+ oscillation patterns. In addition, we used high content imaging methods to characterize compound effects on viability, cytoskeletal re-arrangements, and mitochondria potential of cardiac cells. This phenotypic assay allows for the characterization of parameters such as beating frequency, amplitude, peak width, rise and decay times, as well as cell viability and morphological characteristics. The results demonstrate the utility of hiPSC cardiomyocytes to detect drug-induced pro-arrhythmic effects in vitro.

W-2035

THE HUMAN DISEASE GENE TFPA/HADHA IS REQUIRED FOR FATTY ACID BETA OXIDATION AND CARDIOLIPIN RE-MODELING IN HUMAN CARDIOMYOCYTES

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Mutations in hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase alpha subunit (HADHA) result in impairment of long chain fatty acid beta-oxidation (FAO) and severe, early-onset cardiomyopathy, including sudden infant death syndrome (SIDS). To better characterize the cardiac phenotype, we generated stem-cell derived cardiomyocytes (CMs) from HADHA-deficient induced pluripotent stem cells (iPSCs) as well as from patients containing a single (G1528C) point mutation in exon 15 of HADHA, resulting in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). To mature these iPSC-derived CMs from a fetal to a more adult-like state, we utilized a novel, HOPX-targeting MircoRNA Maturation Cocktail (MiMaC). When matured using MiMaC and challenged with fatty acid rich media, HADHA-deficient CMs displayed impaired FAO and accumulation of lipid intermediates characterized by lipidomic analysis. These cells also displayed abnormal calcium handling and beat rate abnormalities, suggesting an arrhythmic state responsible for SIDS. HADHA-deficient, fatty acid challenged CMs further displayed a loss in mitochondrial proton gradient and loss of defined cristae structure by electron microscopy. Using mass spectrometry, we demonstrated that both HADHA-deficient and LCHADD CMs display abnormalities in cardiolipin species, suggesting impaired cardiolipin re-modeling. Furthermore, we investigated the abundant cardiolipin species in wild type and HADHA-deficient stem cell derived CMs before and after maturation, and determined that maturation and HADHA-dependent re-modeling are essential for a cardiolipin profile that more closely resembles that of an adult CM. Based on these data, and sequence similarities between HADHA and monolysocardiolipin acyltransferase (MLCL-AT) we propose that HADHA has a dual role, performing both FAO and cardiolipin re-modeling and is essential for cardiomyocyte maturation and function.

W-2037

VINCULIN IS REQUIRED FOR CARDIAC NEURAL CREST TO MEDIATE OUTFLOW TRACT SEPTATION AND SEMILUNAR VALVE DEVELOPMENT IN MOUSE

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Vinculin (Vcl) is a key adaptor protein at the focal adhesion (FA) mediating various cellular processes and developmental events. In this project, we elucidated how Vcl regulates cardiac neural crest (CNC) development using a NC specific Vcl knockout (VclKO) mouse model. VclKO mutants died within few hours after birth, due to heart failures. VclKO mutants at E18.5 present with ventricular septal defect (VSD), interrupted aortic arches (IAA) and persistent truncus arteriosus (PTA). Formation of semilunar valves (SLV) was also found interrupted, in which the aortic and pulmonary valves of the mutants were much thicker than those of the control and of unequal size. The cardiac defects of the mutants are primarily caused by the failure of CNCs to properly populate the cardiac outflow tract (OFT) and differentiate into vascular smooth cell (VSMC). Delayed migration of CNCs was observed along the cardiac OFT and in the endocardial ridges. VclKO CNCs failed to fully populate the proximal aorta and to give rise to VSMC, resulting in the incomplete septation of aorta and pulmonary artery, leading to IAA and PTA. During the SLV development, CNCs induce the endothelial-to-mesenchymal transition to generate enough mesenchymal cells for the expansion of OFT endocardial cushions (EC); regulate the myocardialization to guide myocardial cells to invade ECs; and provide instructive signals to orchestrate apoptosis and extracellular matrix production for the valve remodelling. The reduced number of CNCs in the OFT of the mutants severely interrupted the myocardialization, in which the myocardial cells failed to invade the ECs to support the SLV development. Moreover, the valve remodelling process was found defective in VclKO, such that excessive mesenchymal cells were present, resulting in the thickened valves. Ventricular septum closure relies on the coordination between the ECs of the proximal OFT and the atrioventricular canal (AVC). The defective development of the OFT ECs in VclKO disturbed the movement and remodelling of the AVC ECs, and interrupted the formation of the membranous ventricular septum, leading to VSD. In sum, Vcl is playing the pivotal roles in the migration and differentiation of CNCs during the OFT development, SLV formation and ventricular septum closure.

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W-2039

PROFILING CARDIO-ACTIVE COMPOUND RESPONSE BETWEEN HIGH TRHOUGHPUT 2D MONOLAYERS, ANISOTROPIC AND 3-DIMENSIONAL HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) have become a prominent tool in safety pharmacology, toxicology studies and disease modeling. However, in traditional 2D monolayers hiPSC-CMs typically display an abnormal sub-cellular structural organization with reported features of cellular immaturity. Micro-engineered high-density screening platforms with mechanical cues to promote cardiomyocyte alignment generate anisotropic cultures with readily identifiable, correctly patterned myofibrils along the cell body, intercalated disc components targeted to the distal ends of the cells and more elongated nuclei. Directional contraction patterns of hiPSC-CM preparations were observed to become markedly uniform in this platform. Parallelly, complex 3D cultures of cardiomyocytes, fibroblasts and endothelial cells have been reported to promote more physiological contraction patterns, with positive increases in the force-frequency relationship in response to ionotropic compounds. Here we compared cardiomyocytes cultured as 2D monolayers, anisotropic cultures and 3D cardiomyocyte spheroids in high throughput screening formats for their ability to respond to cardioactive compounds. Interestingly, our observations indicate that the different systems can display marked differences their in response to compounds of interest. Examples of compounds with clear differential response include the potassium channel blocker Dofetilide, the beta-adrenergic agonist dobutamine and a cocktail of hypertrophic stimuli. Altogether the results obtained in this study highlight that specific hiPSC-CM based platforms present advantages and limitations which need to be weighed in to ensure suitability to their context of use.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

W-2043

HUMAN INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED NEUROECTODERMAL EPITHELIAL CELLS MISIDENTIFIED AS BLOOD-BRAIN BARRIER ENDOTHELIAL CELLS

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Brain microvascular endothelial cells (BMECs) have unique properties termed the blood-brain-barrier (BBB) that are crucial for immunological and homeostatic brain functions. Modulation of the BBB function is essential for treatment of neurological maladies and augmenting tumor targeting. Studies on the BBB have been hampered by lack of models, which cultivate BMECs that sustain their BBB-specific vascular cell fate. As an alternative approach, studies have reported differentiation of induced pluripotent stem cells (iPSC) into BMECs (iBMECs) with BBB-like properties developing a robust in vitro model for drug delivery and understanding mechanisms of neurological diseases. However, the molecular identity of these iBMECs remains unclear. Employing single-cell RNA-sequencing, we examined the molecular and functional properties of these putative iBMECs and found iBMECs that differentiated either in the absence or presence of retinoic acid lack expression of endothelial-lineage genes and ETS transcription factors (TFs) essential for the establishment/maintenance of an EC identity. iBMECs failed to respond to angiogenic stimuli and form lumenized vessels. Using a combination of bio-informatic analyses and immunofluorescence for pathways/TFs/surface markers, we demonstrate that human iBMECs are not BBB-ECs but rather misidentified EpCAM+ neuroectodermal epithelial cells (NE-EpiCs). NE-EpiCs-Epi form tight junctions that can resemble those present in BBB-forming BMECs in part due

to the lack of specificity of the Claudin-5 antibody. Moreover, overexpression of ETS TFs (ETV2, FLI1, and ERG) reprograms NE-EpiCs into cells resembling an authentic EC. Therefore, while direct differentiation of human iBMECs is an infrequent event and primarily give rise to epithelial cells, it might be remedied by overexpression of several ETS TFs to recreate a true vascular BBB model in vitro.

W-2045

SHEAR STRESS MEDITATED ENHANCEMENT OF VASCULAR PHENOTYPES IN HUMAN MESENCHYMAL STEM CELLS

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Peripheral arterial disease (PAD) is a chronic condition that impacts more than 8.5 million Americans over age 40. Current therapies for PAD, including percutaneous interventions and surgical revascularization, are often inadequate as long-term solutions due to continued progression of vascular disease and restenosis. Mesenchymal stem cell (MSC) therapy is a promising approach for the treatment of PAD but has not yet achieved consistent therapeutic benefits in clinical trials. Studies to differentiate MSCs into endothelial cells have had varying results between groups and contradictory findings. To address this issue, we used a novel high throughput device to apply shear stress to MSCs and examined the synergy between applied flow and biochemical signals in enhancing endothelial cell (EC) differentiation of MSCs. Shear stresses of 1 dyn/cm2, 5 dyn/cm2 or +1/-1 dyn/cm2 (oscillatory shear stress; OSS) in combination with substrate compliance and biochemical treatments were applied to MSCs to optimize their vascular differentiation. We first applied shear stress to the MSCs on substrates of varying compliance. A detailed analysis of the cell phenotypes using flow cytometry revealed increased endothelial differentiation and a reduction in vSMC phenotype on soft substrates (0.2 or 0.5 kPa) with shear stress. We next performed a high throughput screen for 80 compounds on cells on soft substrates with exposure to shear stress. This analysis revealed several inhibitors that led to increased endothelial differentiation of the cells synergistically with shear stress, while reducing the expression of the vSMC marker aSMA. We further confirmed the endothelial phenotype of the cells by repeating the treatments and performing immunostaining and flow cytometry. Cells that underwent shear stress treatments exhibited greater tube formation in Matrigel and showed higher vessel density in a rat subcutaneous implantation model. Together, our results support that the endothelial differentiation of MSCs can be enhanced under optimized conditions of substrate stiffness, shear stress and pharmacological compound treatment.

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W-2047

SEX DIFFERENCES IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELL FUNCTION

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Cardiovascular diseases (CVDs) continue to be the leading cause of death for both men and women, although women have lower risk of developing heart diseases until menopause. Following the decrease in the estrogen hormone levels post-menopause, the risk becomes comparable to men. This suggests that elucidating the underlying mechanisms of sex differences in cardiovascular function is crucial to improve the diagnosis and treatment of CVDs in both sexes. The objective of this study is to compare the angiogenic function of female and male induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) to identify basic signaling mechanisms by which estrogen and the estrogen receptors mediate the process. In initial experiments, three pairs of age and race matched primary endothelial cells from healthy patients were implanted subcutaneously into male SCID mice within Matrigel plugs for two weeks. Quantitative PCR analysis demonstrated that female primary endothelial cells had upregulated expression of fibroblast growth factor-2 and endothelial nitric oxide synthase genes which play roles in angiogenesis and endothelial function. Histological analysis of implanted plugs demonstrated a significant increase in murine capillary density when implanted with female cells than male cells, suggesting greater paracrine release of angiogenic factors by female cells. To validate these findings, we obtained iPSCs generated from blood plasma of male and female donors. When differentiated into IPSC-ECs, these cells differ from each other in growth rate, migration and their response to VEGF treatment. Ongoing studies intend to reveal the angiogenic potential of sex-specific iPSC-ECs in the presence of estrogen treatment to elucidate the role of estrogen and its receptors in male and female endothelial function.

HEMATOPOIESIS/IMMUNOLOGY

W-2049

RUNX1 AND TGF-B SIGNALING REGULATE EXPRESSION OF P15 TO CAUSE CELL CYCLE ARREST SO AS TO BLOCK THE EARLY HUMAN HEMATOPOIESIS

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RUNX1 plays a key role in regulatory function on human hematopoiesis and blood diseases, and is absolutely required for HSC formation and definitive hematopoiesis. Overexpression of RUNX1b (one of its isoform) in H1 hESC has been reported to block the hematopoiesis in our AGM-S3 co-culture system, which could be partially rescued by RepSox, the TGF-β signaling inhibitor against ALK5. D4 co-cultures were detected and cell cycle G1 arrest was found in DOX-induced RUNX1b/hESC co-culture and be partially reverted when RepSox was added. In order to elucidate the molecular mechanism of hematopoiesis blockage caused by RUNX1b, the cell cycle-related genes were screened by qRT-PCR so as to find the genes that were differentiately expressed between untreated and DOX-induced D4 co-culture of RUNX1b/hESC with or without RepSox. Among them P15 was up-regulated after DOX induction and reversed to original expression level when RepSox was added. The expression level of P15 at day 4 of co-culture was highly consistent with the level of RUNX1b when the RUNX1b/hESC co-culture was induced by different concentration of DOX. Above results indicated that up-regulation of P15 depend on both overexpression of RUNX1 and enhanced TGF-β signaling. The P15 inducible hESC line based on piggyBac inducible system was also established and the overexpression of P15 could obviously block the hematopoiesis and lead to cell cycle G1 arrest, which ought to be a key step to cause the blockage of RUNX1b to hematopoiesis, including CD34+CD43+ and CD34+CD43-. TGF-β1 could up-regulate TGF-β signaling and block hematopoiesis which is similar to the effect of RUNX1b or P15 on CD34+CD43+ and CD34+CD43- population. But RUNX1b could not block the generation of CD34lowCD43- population when TGF-β signaling was inhibited by RepSox, which indicated that RUNX1b itself or other downstream pathway controlled by RUNX1b might be regulate the expression of P15 with the indispensable help of enhanced TGF-β signaling. The severe blockage to hematopoiesis depends on up-regulation both of them. Our study ought to help to reveal the cellular/molecular mechanism of RUNX1 gene to control early hematopoiesis.

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W-2051

LONG-TERM EX VIVO EXPANSION OF FUNCTIONAL HEMATOPOIETIC STEM CELLS AFFORDS NON-CONDITIONED TRANSPLANTATION

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Utilizing multipotent and self-renewing capabilities, hematopoietic stem cells (HSCs) can maintain hematopoiesis throughout life. The mechanism of such striking abilities of HSCs remains unanswered despite many years of research, mainly because of the paucity of HSCs in the bone marrow. Ex vivo expansion has been a holy grail of HSC research for both basic research and clinical applications, yet, no such system currently exists. By taking a reductionist optimization approach, we have developed a simple culture platform that supports functional mouse HSCs ex vivo over 1-2 months. Limiting dilution transplantation analysis of day-28 HSC cultures estimates a ~900-fold expansion of functional HSCs (based on >1% multilineage engraftment at 16-weeks post-transplantation) with secondary transplantation analysis estimating >200-fold expansion of serially-engraftable long-term HSCs. Functional HSCs can also be expanded clonally using this system but display significant heterogeneity in expansion capacity, suggesting an important role for intrinsic regulation of HSC self-renewal. The large numbers of functional HSCs generated by this long-term ex vivo expansion system even enables non-myeloablative HSC transplantation, curative for immunodeficient recipients. Finally, this simple culture system also supports human HSC ex vivo, as determined by 16-week engraftment in NSG mice. Thus, the ex vivo expansion of HSCs provides a platform not only to interrogate HSC self-renewal and lineage commitment but also suggests a novel approach in clinical HSC transplantation.

W-2053

RECAPITULATION OF MURINE T CELL DIFFERENTIATION FROM HEMATOPOIETIC STEM AND PROGENITOR CELLS IN ARTIFICIAL THYMIC ORGANOIDS

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Due to the spatio-temporal complexity of the T cell differentiation process in the thymus, it has been challenging to recapitulate thymopoiesis from hematopoietic stem and progenitor cells (HSPCs) in vitro. The OP9-DL1 monolayer co-culture system has revolutionized the field allowing the commitment of HSPC to the T cell lineage in a dish. However, results with this serum-dependent monolayer system can be inconsistent. We have recently developed a powerful 3-D model of in vitro T cell differentiation from human HSPCs as well as human pluripotent stem cells that reproducibly produces mature naïve T cells in artificial thymic organoids (ATOs). Here we developed a similar approach using murine bone marrow (BM) HSPCs in serum-free conditions. Murine ATOs remarkably mimicked normal murine thymopoiesis with the production of all T cell subsets including double negative (DN1, DN2, DN3, DN4), immature single positive (ISP8), double positive (DP) and single positive (CD8SP and CD4SP) populations. The use of BM from a Notch reporter (TNR) mouse in ATOs showed highly active Notch signaling in early thymic progenitors (ETPs) that markedly subsided after cells committed to the T cell lineage, indicating that Notch signaling was induced in a manner similar to that of the thymus. Murine ATOs could recapitulate thymopoiesis from any subset of BM populations (Lineage negative c-Kit+ Sca-1+ (LSK), hematopoietic stem cells (HSC), lymphoid-primed multipotential progenitors (LMPP), Common Lymphoid Progenitors (CLP)) or ETPs. In addition, the system is remarkably efficient using isolated single cells (either HSC or LSK). Murine ATOs produced TCR-γδ+ cells, and also mature CD3+ TCR-β+ CD8 and CD4SP cells that expressed maturation markers such as CD62L, responded to TCR activation and exhibited a broad TCR Vβ repertoire consistent with positive selection. Strikingly, the use of Foxp3 reporter mouse BM demonstrated that a fraction of the CD4SP cells produced in ATOs displayed a Treg phenotype with the expression of Foxp3 and CD25. The murine ATO system is technically simple, reproducible and efficient using BM from at least 4 different murine strains and is thus a powerful platform to study murine T cell development and maturation from a large variety of murine models and hematopoietic progenitor populations.

W-2055

A TRANSIENT P53-MEDIATED DNA DAMAGE RESPONSE PRESERVES HEMATOPOIETIC STEM CELL FUNCTION FOLLOWING PRECISE GENE EDITING

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Precise genome editing in Hematopoietic Stem/Progenitor Cells (HSPC) holds therapeutic promise for several diseases. Yet, little is known about the cellular responses triggered by programmable nucleases in edited HSPC, which may negatively impact their engraftment and long-term repopulation capacity. Locus specific gene editing inevitably generates double strand breaks (DSB), highly toxic DNA lesions that trigger the DNA damage response (DDR). We hypothesize that the induction of DDR pathways during the editing procedure, if not specific and restricted in time, will most likely impair the repopulating potential of HSPC. We induced one or few DSB in cord blood-derived CD34+ cells with heterodimeric Zinc-Finger and CRISPR/Cas9 nucleases against loci of therapeutic interest. As controls we employed equimolar amounts of a single ZFN monomer or commercially available Cas9 unloaded or loaded with a computationally validated gRNA with no predicted activity against the human genome. We monitored DDR foci induction, cell cycle progression and transcriptional responses on progenitor and primitive subpopulations up to single cell level. We also investigated HSPC functionality in response to genome editing with AAV6 DNA template, both in vitro and in vivo. HSPC displayed accumulation of upstream DDR mediators at the targeted loci early upon nucleases-induced DSB. Activation of the p53-mediated DDR pathway was the predominant response to even single nuclease-induced DSB across all HSPC types/states, and excess DSB load and/or combination with DNA repair template delivered by AAV, caused cumulative activation of the p53 pathway constraining the proliferation and yield of edited HSPC. This proliferation impairment was reversible, at least when the DDR burden remained low, and could be overcome by temporary antagonism of the p53 trigger, leading to increase output of clonogenic and repopulating edited cells. Importantly, genome editing procedure did not detectably impact on chromosomal translocations, gross genomic instability and mutational burden of edited HSPC and transient p53 inhibition did not further aggravate the impact of the procedure. Our findings provide molecular evidence of the feasibility of seamless gene editing in HSPC giving confidence to its prospective translation in humans.

W-2057

SMALL RNAS INVOLVED IN THE DIFFERENTIATION OF HUMAN HEMATOPOIETIC STEM CELLS TO ERYTHROID LINEAGE

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Different classes of small non-coding RNAs that regulate gene expression have been reported in eukaryotic cells. Among these, microRNAs (miRNAs) have been extensively studied in hematopoiesis for their function in the maintenance of hematopoietic stem cells (HSCs) and in their differentiation to multiple lineages. The transcriptional regulatory circuitries involving miRNAs and lineage-specific transcription factors (TFs) have not been studied in hematopoietic differentiation. We performed experiments to identify the small RNAs that are involved in human erythropoiesis. We differentiated CD34+

hematopoietic stem and progenitor cells into erythroid cells, and small RNA Sequencing was performed from different time points of erythroid differentiation after flow cytometric analysis of upregulation of erythroid surface markers, CD71 and CD235a. We found 76 miRNAs with differential expression with ≥10 fold change. Interestingly, the number of downregulated miRNAs was 3 times higher than the upregulated miRNAs. In addition to the miRNAs that were previously shown to have a significant difference in their expression during human erythropoiesis, we identified an additional 51 differentially expressed miRNAs. There were 26 intragenic miRNAs with 13 of them in the protein-coding genes. Comparison of the expression of the miRNAs and their host genes showed that 50% of the miRNA-host gene pairs had the same expression kinetics and they were co-transcribed. ChIP-sequencing analysis showed that GATA1, KLF1 and TAL1 bind within 10kb upstream of the highest upregulated 8 miRNAs, suggesting that their erythroid stage-specific expression is regulated by these TFs. We found a miRNA cluster that was not previously reported in erythropoiesis, miR-183-182-96 cluster, which showed very high levels of upregulation during erythropoiesis. TF binding showed that its expression is regulated by the erythroid TFs. The results on the functional characterization of the most upregulated miRNAs, by gene editing using CRISPR/Cas9, will be presented. We also identified other small RNAs, piRNAs and snoRNAs, with significant differential expression. This is the first study carried out for the comprehensive analysis of small RNAs and their transcriptional circuitries in the maintenance of HSCs and erythropoiesis.

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W-2059

NOTCH LIGAND FUNCTIONALIZED MICROBEAD PLATFORM FOR THE GENERATION OF HUMAN PROGENITOR AND MATURE CONVENTIONAL T CELLS FROM MULTIPLE SOURCES OF STEM CELLS

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T cells are critical mediators of adaptive immunity and can be harnessed as therapeutic agents for regenerative medicine and in cancer immunotherapy. The generation of T cells in vitro from both hematopoietic stem/progenitor cells (HSPCs) and human induced pluripotent stem cells (iPSCs) offers the prospect of generating a self-renewing source of T cells that can be readily genetically modified. An unmet challenge in the field is the development of a clinically relevant system that could be easily scaled to generate large quantities of T-lineage cells. Here, we report a serum an-free, bead-based approach that supports the efficient in vitro development of both human progenitor T (proT) cells and naïve conventional T cells from CD34+ cells sourced from cord blood, GCSF-mobilized peripheral blood and iPSC-derived haemogenic endothelium. Our strategy uses an artificial Notch signaling system, wherein DL4-Fc is immobilized to microbeads (DL4-µbeads). DL4-µbeads, along with the requisite cytokines, induced an orderly sequence of commitment and differentiation of CD34+ cells to naïve CD3+CD8αβ+ and CD3+CD4+ conventional T cells with a diverse T cell receptor (TCR) repertoire. The adoptive transfer of CD34+CD7+ proT cells demonstrated efficient engraftment in the thymus of NOD-SCID IL2rγnull mice, restoring the thymic architecture and thereby facilitating subsequent thymic seeding by HSC-derived progenitors. Future work aims to investigate the mechanism by which proT cells regenerate the thymus at single-cell resolution and demonstrate the ability of these cells to serve a platform for engineering therapeutic gene circuits.

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W-2061

EVOLUTIONARY CONSERVED MECHANISMS OF HEMATOPOIESIS, LESSONS FROM A COLONIAL CHORDATE

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Hematopoiesis is an essential process that evolved in multicellular animals. At the heart of this process are hematopoietic stem cells (HSCs) which are multipotent, self-renewing and generate the entire repertoire of blood and immune cells throughout life. While there are comprehensive studies on HSC self-renewal, differentiation, regulation and niche occupation, relatively little is known about the evolutionary origin of HSCs, their progeny and their niches. We study the hematopoietic system of Botryllus schlosseri, a colonial chordate with vasculature containing circulating blood cells, and interesting characteristics of stem cell biology and immunity. Self-recognition between compatible Botryllus colonies leads to formation of natural parabionts with a common vasculature, whereas genetically incompatible colonies reject. This self–nonself recognition process is controlled by a highly polymorphic histocompatibility gene BHF: at least one shared BHF allele is required for fusion to take place. Using flow cytometry, whole-transcriptome sequencing of defined cell populations, and diverse functional assays, we identified HSCs and progenitor cells, immune effector cells and an HSC niche in this organism. This study revealed a significant evolutionary conservation between the gene repertoire of the Botryllus and mammalian hematopoietic stem cells (HSC) and blood progenitor populations. It also suggests that hematopoietic bone marrow and the Botryllus endostyle niche evolved from

the same origin. The immune functional assays we performed further revealed that cellular rejection between genetically incompatible colonies is mediated by cytotoxicity and that BHF is a self-recognition inhibitory factor for cytotoxic cells, similar to NK inhibition by MHC. Botryllus has cells that share morphological and molecular characteristics with the vertebrate HSC and myeloid lineages, including cells that take part in phagocytosis. Its cytotoxic cell population on the other hand, highly express more than 50 genes with no human or mouse homologues. Studying these gene sets and the BHF inhibition pathway are likely to reveal novel mechanisms to delimit self from non-self and target pathogens.

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W-2063

IDENTIFICATION OF A RETINOIC ACID-DEPENDENT DEFINITIVE HEMATOPOIETIC PROGENITOR FROM HUMAN PLURIPOTENT STEM CELLS

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The generation of the hematopoietic stem cells (HSCs) from human pluripotent stem cells (hPSCs) is a major goal for regenerative medicine. HSCs derive from a population known as hemogenic endothelium (HE) that is specified at the onset of definitive hematopoiesis, and HSCs are specified from HOXA+ HE in a retinoic acid (RA)-dependent manner. We have previously identified a WNT-dependent (WNTd) CDX4+ mesodermal population that gives rise to a clonally multipotent, HOXA+ definitive HE. However, this HE lacks HSC-like capacity in the absence of exogenous transgenes, and is RA-independent, with exogenous RA treatment failing to confer HSC potential. Thus, identification of RA-dependent HE has remained elusive. We therefore asked if the developmental stage of RA-dependence is developmentally earlier than currently appreciated. Through single cell RNAseq and pseudotime analyses, we identified that WNTd mesoderm is actually comprised of two distinct KDR+ populations, prior to HE specification. These subsets are distinguishable by mutually exclusive CDX4 and CXCR4 expression. Interestingly, CYP26A1, a RA degrading enzyme, was exclusively expressed in CDX4+ CXCR4- mesoderm, and it was this population that solely gives rise to HOXA+ CD34+ HE. Thus, all WNTd definitive hematopoiesis described to-date is derived from a KDR+CXCR4- mesodermal population. In sharp contrast, WNTd CDX4- CXCR4+ mesoderm instead displayed robust expression of ALDH1A2, a key enzyme in the synthesis of RA, suggesting this population may be responsive to RA signaling. Either retinol or all-trans retinoic acid application to this CXCR4+ mesoderm, during an early, narrow developmental window, resulted in the specification of CD34+ cells with remarkably higher expression of medial HOXA genes than RA-independent progenitors. Critically, following RA signaling this population now possessed HE, which robustly gave rise to definitive erythroid, myeloid and lymphoid progeny. Collectively, this represents the first ever characterization of stage-specific RA-dependent hPSC-derived definitive hematopoiesis, and its mesodermal progenitor. This novel insight into human hematopoietic development enhances our understanding of human definitive hematopoietic development, and provides the basis for the de novo specification of HSCs.

PANCREAS, LIVER, KIDNEY

W-2065

A GLOMERULUS ON A CHIP THAT RECAPITULATES THE PATHOPHYSIOLOGY OF THE HUMAN GLOMERULAR FILTRATION BARRIER

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With increasing rates of renal failure and limited options for its treatment, there is an urgent need of implementing our knowledge and resources to guide new strategies for understanding disease- and patient-specific glomerular pathophysiology and for developing more efficient drug screening tools. Recreating an ex-vivo functional glomerulus depends on our ability to generate an in vitro 3D multicellular system that allows fluid perfusion and proper interactions between podocytes and glomerular endothelial cells (hGEC) in a platform that mimics the complex architecture of the glomerular filtration barrier (GFB). In our laboratory, we have developed an innovative, barrier-free, glomerulus-on-a-chip (GOAC) system that closely mimic the GFB. Amniotic fluid derived podocytes (hAKPC-P), human immortalized podocytes (hiPod), primary human podocytes (hpPod) and human fibroblasts or human lung endothelial cells

(negative controls) were seeded on microfluidic chips with hGEC. Cell phenotype was confirmed by immunofluorescence and de-novo deposition of GBM components such as collagen IV and laminin alpha5 was verified by staining and western blotting. Albumin permselectivity was successfully confirmed and albumin impermeability was impaired following PAN. In the presence of Membranous nephropathy (MN) serum, the chip displayed IgG deposition on the podocytes and loss of permselectivity to albumin, to an extent proportional to urinary protein loss in vivo. ACTH successfully rescued MN-mediated damage, confirming feasibility for drug screening. Chip structure and function were impaired when we used podocytes from individuals with Alport Syndrome, a kidney disease due to genetically determined collagen abnormalities. In conclusion, we have successfully developed a GOAC system that closely mimics the GFB structure and provides a powerful tool for studying renal regenerative and disease mechanisms, toxicity effects and will help the discovery of new drugs.

W-2067

ALLEVIATION OF LIVER FIBROSIS USING MESENCHYMAL STEM CELLS DERIVED EXOSOMES: A CELL FREE THERAPEUTIC APPROACH

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Mesenchymal Stem Cells (MSCs) administration as a therapy for liver disease holds great promise. Currently there are more than 33 registered clinical trials using MSCs for treating liver diseases. However, there are few concerns associated with these cell based therapy which still remains unresolved like long term storage, mal-differentiation, etc. Therefore, use of MSCs derived exosomes has evolved as a safer cell free approach. Although, these exosomes mediated tissue repair and regeneration is still in its initial phase and needs in-depth understanding. One of the major concerns is their isolation with high yield and membrane integrity. In this regard, we have identified an improvised ultracentrifugation based approach for exosome isolation. For this, exosomes isolated from various tissue sources like Bone marrow (BMSCs) and adipose tissue (ADSCs) were used and characterized for their size; morphology and surface marker profiling. We have obtained exosomes with the characteristic cup shape and size ranging from 30 to120 nm. Also, using NTA, we observed that this method yielded significantly higher yield of approximately 4.6 × 109 particles from 5 million cells while maintaining their integrity because of the cushioning effect of sucrose. This method used is cost-effective and less time-consuming. These exosomes were further evaluated for their content via RNA sequencing and mass spectrometry. Subtle tissue specific variation was observed in their content. Also, these exosomes were evaluated for their therapeutic role in CCL4 induced liver fibrotic mice model. The exosomes were initially labeled to track their localization to the targeted organ using various route of administration. It was observed that exosomes were successfully localized to the fibrotic liver in mice and alleviated liver fibrosis as observed by decreased oxidative stress and apoptosis of hepatocytes in the liver. The ECM marker expression was reduced and EMT transition was reverted as confirmed by PCR. Therefore, these smaller and less complex vesicles which are easier to produce and store as off the shelf therapeutics, constitute a compelling alternative over the corresponding MSCs. This study comparing tissue specific MSC exosomes paves way for future preclinical and clinical studies using exosomes as cell free approach.

Funding Source: The work done in this study was generously supported by All India Institute of Medical Sciences and Department of Biotechnology, India.

W-2069

EFFECT OF HUMAN PROGENITOR CELLS ON AN ANIMAL MODEL OF TYPE 1 DIABETES

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This project focuses on a novel progenitor cell therapy for type 1 diabetes, a chronic illness that is categorized by pancreatic beta cell death. The current advancements in diabetes research has brought attention to innovative cellular applications that can help increase insulin production. Here, human progenitor cells were used to mimic and replace beta cells in a diabetic rat model induced by a one-time intraperitoneal injection of streptozotocin that selectively damages beta cells. The effects of inducing diabetes were examined by testing blood glucose levels daily using Accu-Chek glucometers. After establishing diabetes in the

rats (>400mg/dL glucose), preparation for transplantation began by immunosuppressing the diabetic rat using Alzet pumps that chronically administer cyclosporine over the course of 23 days. During this time, blood glucose and weight was monitored to ensure the rat's viability for a successful transplant surgery and post-surgery outcomes. Next, live human progenitor cells, live differentiated pancreatic progenitor cells and dead human progenitor cells (control) were injected into different regions of the diabetic rat: the circulatory system, peritoneal cavity, pancreas, and kidney capsule. The last two treatments required an additional surgery of exposing either the pancreas or kidney in order to transplant the human progenitor cells. Finally, immunohistochemistry of the rat's organs (pancreas, kidney, etc.) showed the presence of surviving human progenitor cells in the kidney capsule 37 days post-transplantation. One day after cell treatment, pancreatic progenitor cell treatment group via kidney capsule had significant decreased blood glucose levels ($p<0.005$) compared to blood glucose levels of rats who received dead progenitor cells injected into the kidney capsule. This experiment aimed to alleviate pancreatic beta cell death and possibly be one step closer to curing type 1 diabetes.

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W-2071

KIDNEY ORGANOIDS GENERATED THROUGH HETEROCHRONIC RECOMBINATION RESULTS IN NEPHRON SEGMENTATION AND VASCULAR CONNECTION WITH HOST IN VIVO

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The increasing prevalence of end-stage kidney disease warrants research into technologies to understand how new kidney tissue can be generated. Procedures have been developed to differentiate human induced pluripotent stem cells (hiPSC) into renal organoids but these organoids were not fully packed with tubular clusters. Here, we investigated the potential of heterochronic recombination (HT) of hiPSC derived kidney progenitors to increase tubular density in kidney organoids. Characterization of this technique included HT of hiPSC derived kidney progenitors to generate organoids and their engraftment and, evaluation of nephron segmentation, graft vascularization and perfusion in vivo. For HT, we differentiated hiPSC derived kidney progenitors for 2 days on air-liquid interface and then mixed with fresh progenitors. Approximately 40% Six2+ and WT+ cells were used up after two days of differentiation on air-liquid interface. Tubules stained with molecular markers for proximal and distal tubules and revealed segmentation. Podocytes (Podxl+), stromal cells (Meis1+) and endothelial cells (CD31+) were abundant. Engrafted organoids showed differentiation of complex graft-derived glomeruli with vascular networks (CD31+ Endomucin+), podocytes (Podxl+ WT1+), mesangial cells (Pdgfrβ+), and juxtaglomerular cells (Renin+). Proximal tubules (E cadherin- LTL+), thick ascending limb of the loop of Henle (Tamm-Horsfall protein+), Distal tubule (BRN1+), Connecting tubule (E cadherin+ GATA3- DBA+), collecting duct (Troma-1+ GATA3+ DBA+), and stromal cells (Meis1+ Pdgfrβ+) were also present in the grafts. Engrafted organoids also showed vascularization (CD31+ Endomucin+) and direct connections with host vasculature. All nephron structures and stromal cells in the graft were iPSC derived (HuNu+) whereas endothelial cells were derived from the host (HuNu-). HT of kidney progenitors results in robust differentiation of human iPSC-derived kidney tissue in vitro and in vivo.

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W-2073

LARGE-SCALE DERIVATION OF FETAL-LIKE IPSC-DERIVED PANCREATIC PROGENITOR CELLS TO IDENTIFY FUNCTIONAL ASSOCIATIONS BETWEEN GENETIC VARIANTS AND MOLECULAR PHENOTYPES

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Experimental systems that enable a cell-type specific characterization of molecular phenotypes are necessary for the identification and functional annotation of putative regulatory variants. Learning the proximate effects of regulatory variants in both the adult and developmental stages (including fetal) is critical to fully understand genetic contributions to health and disease. Here, we are using the iPSCORE collection (a bank of hundreds of human induced pluripotent cell (iPSC) lines generated from individuals of multiple ethnicities) to derive "fetal-like" pancreatic progenitor cells (iPSC-PPCs) using a highly reproducible protocol. Here we present a robust standardized protocol for derivation of iPSC-PPC lines based on the STEMdiff™ Pancreatic Progenitor kit (STEMCELL Technologies) that we have modified for scale and reproducibility. To date, we have used this protocol for 40 different differentiations from 40 individuals obtaining high-quality iPSC-PPCs and are currently differentiating an additional 150 iPSCORE iPSC lines. Using the optimized protocol, we obtain on average 9.76 x107 iPSC-PPCs

from one 6-well plate. The purity of the iPSC-PPCs measured by flow cytometry as PDX1+NKX6.1+ double positive cells is 75.7% (median; range: 49 - 91.7%). We are collecting cell pellets for future molecular assays (RNA-seq, ATAC-seq, ChIP-seq for H3K27ac, H3K27me3, H3K4me1 and H3K4me3) as well as cryopreserving live iPSC-PPCs. To elucidate the proximate effects of regulatory variants in fetal-like pancreatic progenitor cells, we will perform integrative analysis of the proposed molecular phenotypes in conjunction with the whole-genome sequences that we have for all individuals in the iPSCORE collection. In addition, this study will result in the generation of a large-scale systematic data set that will serve as a model for the development of new statistical approaches for predicting genome function and will result in a better understanding of the molecular mechanisms by which genetic variation affects disease.

W-2075

ARID1A LOSS POTENTIATES β-CELL REGENERATION THROUGH ACTIVATION OF EGF/NRG SIGNALING

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Mechanisms that regulate β-cell expansion during development and disease are mysterious. We reasoned that machinery that orchestrates epigenome structure within β-cells might influence cell number and regeneration. We show that ARID1A, a member of the SWI/SNF chromatin remodeling complex, is a key regulator of β-cell proliferation and survival. In β-cells, Arid1a levels are high during quiescence and suppressed after partial pancreatectomy and during pregnancy, conditions that demand β-cell expansion. Inducible whole-body deletion of Arid1a using Ubc-CreERT followed by exposure to the β-cell toxin streptozotocin (STZ) showed that Arid1a deficient mice produced more insulin and were almost completely protected from type 1 diabetes. β-cell restricted Arid1a deletion also suppressed diabetes after STZ, supporting β-cell intrinsic activities. After 50% pancreatectomy as well as STZ, β-cell survival and proliferation were coordinately increased. RNA-Seq on islets before and after 50% pancreatectomy revealed hyperactivation of the Neuregulin and EGF pathway in the Arid1a KO setting. Functionally, pan-ERBB family inhibition with the small molecule inhibitor Canertinib abolished the pro-proliferative and anti-diabetic effect of Arid1a loss in vivo. Interestingly, the rs2292239 polymorphism within ERBB3 is significantly associated with T1D, but functional roles for ERBB3 have remained speculative. We also showed that ERBB3 overexpression in mice protects against diabetes and is a likely mechanism downstream of Arid1a loss. In conclusion, the potent effects of Arid1a loss in β-cells are mediated through increased ERBB signaling, revealing mechanisms relevant to diabetes pathogenesis and therapeutic strategies.

W-2077

AMNIOTIC FLUID STEM CELLS AMELIORATE CISPLATIN-INDUCED ACUTE RENAL FAILURE THROUGH AUTOPHAGY INDUCTION AND INHIBITION OF APOPTOSIS

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Amniotic fluid stem cells (AFSCs) have been shown to ameliorate Acute Renal Failure (ARF), however, the mechanisms responsible for its renoprotective effects still remain unclear. Therefore, the aim of the present study was to evaluate the therapeutic efficacy of AFSCs and to investigate the underlying mechanisms responsible for its renoprotective effect. We culture expanded rat AFSCs, characterized them and evaluated their therapeutic potential in cisplatin induced rat model of ARF. The AFSCs grew in culture as adherent spindle shaped cells and expressed mesenchymal markers viz. CD73, CD90, CD105 as well as renal progenitor markers viz. WT1, PAX2 and SIX2. Administration of AFSCs in ARF rats resulted in improvement of renal function and attenuation of renal damage as reflected by decreased blood urea nitrogen and serum creatinine levels and alleviation in renal tubular cell apoptosis as assessed by lower Bax/Bcl2 ratio and decreased levels of pro-apoptotic proteins viz. PUMA, Bax, cleaved caspase-3 and cleaved caspase-9 as compared to saline-treated ARF group. The infused AFSCs in ARF rats also exhibited increased activation of autophagy as evident by increased LC3-II, ATG5, ATG7, Beclin1 and phospho-AMPK levels with a concomitant decrease in phospho-p70S6K and p62 levels. In order to confirm whether the protective effects of AFSCs on cisplatin-induced apoptosis are dependent on autophagy, we used an autophagic inhibitor, chloroquine. Chloroquine significantly blunted the protective effects of AFSCs therapy as evident by increased caspase-3 activation and aggravated deterioration in renal structure and function caused by cisplatin, thereby suggesting the renoprotective role of autophagy in this disease model. Collectively, our results put

forth autophagy induction as a major mechanism for AFSCs mediated renoprotective effect against cisplatin-induced ARF leading to suppression of renal apoptosis and recovery of structural and functional parameters.

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EPITHELIAL TISSUES

W-2079

CONSECUTIVE HYPOXIA AND HYPOXIA-REOXYGENATION REGULATE MUC5AC AND FOXJ1 EXPRESSION IN HUMAN BRONCHIAL EPITHELIAL CELLS VIA DISTINCT HIF1A/BMP4/NOTCH1 AND NKX2-1/NOTCH3/HEY1 GENE EXPRESSION MODULES

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Hypoxia-reoxygenation (H/R) in vitro has been shown to mimic ischemia-reperfusion in vivo and induce oxidative stress and injury in various types of cells. Nonetheless, it has not been reported whether intermittent H/R and consecutive hypoxia differentially regulate proliferation and differentiation of human bronchial epithelial (HBE) cells. In this study, we investigated effects of consecutive hypoxia and intermittent 24/24-hour cycles of H/R on HBE cells derived from the same-race and age-matched healthy subjects (i.e., NHBE) and subjects with chronic obstructive pulmonary disease (COPD) (i.e., DHBE). To analyze gene/protein expression levels during HBE differentiation into 3D tissues, both the NHBE and DHBE cells at the 2nd passage were cultured at the air-liquid interface in the differentiation medium under hypoxia (1% O2) for consecutively 9 days and then returning to normoxia for another 9 days, or culturing under 24/24-hr cycles of H/R (i.e., 24 hours of 1% O2 followed by 24 hours of 21% O2, repetitively) for totally 18 days, so that all differentiating HBE cells were exposed to hypoxia for a total of 9 days. We found that both consecutive hypoxia and H/R significantly increased BMP4, HIF1A, MKI67, MUC5AC and NOTCH1 expression in differentiated DHBE tissues, whereas only H/R rather than consecutive hypoxia significantly increased BMP4, HIF1A, MKI67, MUC5AC and NOTCH1 expression in differentiated NHBE tissues. On the other hand, both consecutive hypoxia and H/R significantly decreased FOXJ1, HEY1, NKX2-1 and NOTCH3 expression in DHBE tissues, while H/R significantly increased whereas consecutive hypoxia decreased FOXJ1, HEY1, NKX2-1 and NOTCH3 expression in NHBE tissues. Transfection of HIF1A siRNA was capable of decreasing BMP4, MKI67, MUC5AC and NOTCH1 expression in both the NHBE and DHBE tissues cultured under H/R to the levels comparable to those in the NHBE tissues cultured under normoxia. Transfection of NKX2-1 cDNA overexpressor was sufficient to increase FOXJ1, HEY1 and NOTCH3 expression in both the NHBE and DHBE tissues cultured under consecutive hypoxia. Taken together, we show for the first time that HIF1A/BMP4/NOTCH1 and NKX2-1/NOTCH3/HEY1 expression modules play different roles in regulating proliferation and differentiation toward distinct mucous and ciliary pathways in HBE cells.

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W-2079

IDENTIFICATION OF MARKERS TO UNIVOCALLY DISTINGUISH HUMAN ORAL MUCOSA EPITHELIUM FROM CORNEAL AND CONJUNCTIVAL TISSUES.

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Limbal stem cells deficiency (LSCD) leads to corneal conjunctivalization and opacification producing loss of vision and pain. A small biopsy from a healthy part of limbus can be expanded in vitro and the resulting tissue can be transplanted over injured eye to treat LSCD. In case of total bilateral LSCD both eyes are damaged and an alternative stem cells source is needed. Oral mucosa epithelium has been used for its accessibility and regenerative capacity. So far, the univocal characterization of transplanted epithelium has been challenging due to the absence of tissue specific markers. Previously proposed markers (e.g. K3, K13) were shown to be coexpressed by corneal epithelium or by conjunctiva. We obtained holoclones (stem cells) from human oral mucosae, limbal and conjunctival epithelial cultures, by single cell clonal analysis. The analysis of their transcriptomes by microarray assay revealed high expression of SOX2 and PITX2 factors in oral mucosa holoclones compared to both cornea and conjunctiva epithelia, while PAX6 was highly expressed only in cornea and conjunctiva in comparison to oral mucosa. The results were validated by real time PCR and by immunofluorescence confirming the expression/absence of proposed markers in the different cultured cells or in in vivo sections of the three epithelia. These new findings have at least two important implications: a) they will support the follow up analysis of patients transplanted with oral mucosa; b) they will help to shed lights on the mechanism of repair and regeneration of the cornea (e.g. oral mucosa engraftment or stimulation of the residual limbal stem cells).

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W-2081

EFFECT OF DIET ON LUNG STEM AND EPITHELIAL CELLS, AND ITS INTERACTION WITH AGING

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Lung function is known to decrease with aging, with obese people having lower lung function parameters. High-fat diet (HFD) and calorie restriction (CR) are associated with cardiovascular diseases, and health and longevity, respectively. In this study, we determined the effects of HFD and CR, alone or in combination with aging, on lung epithelial and stem cells. Young and aged mice were fed on HFD, CR or HFD followed by CR for 6-12 weeks. Control age-matched mice were fed standard diet. Histological assessment revealed that CR significantly reduced the HFD-induced increase in lung inflammation in young mice, and the aging/HFD-induced increase in lung inflammation in old mice. CR also reduced the HFD-induced increase in the number of AT2 cells in both young and old mice. CR also reversed many of the mitochondrial changes induced by HFD, but its effects were less prominent in old mice. Regarding the lung stem cell in vitro colony forming “organoid” assay, both CR and HFD induced lung stem cells colony formation. In conclusion, HFD induces lung inflammation, alveolar cell proliferation and impairs mitochondrial function. CR reduces the aging and/or HFD-induced inflammation, mitochondrial impairment and activates lung stem cells.

W-2083

TARGETING THE P53 PATHWAY FOR THERAPEUTIC EXPANSION OF AIRWAY EPITHELIAL STEM CELLS

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Cell-based therapies for treating airway defects would require replacement of lung epithelial stem/progenitor cells. We sought to determine mechanisms that regulate stem/progenitor cell quiescence with the goal of developing strategies for therapeutic stem cell expansion. The tumor suppressor p53 is a well-known regulator of cell fate and one of the most commonly mutated genes in lung cancer. We hypothesized that p53 controls airway epithelial progenitor self-renewal and differentiation both during steady state and following injury. In vivo genetic manipulation was used for generation of loss- and gain-of-function models, respectively. We found that p53 loss decreased ciliated cell differentiation and increased the self-renewal and proliferative capacity of club progenitors. Conversely, an additional copy of p53 increased quiescence and ciliated cell differentiation, suggesting that tight regulation of p53 was a critical determinant of epithelial stem/progenitor cell activity. We next sought to determine whether short-term pharmacologic regulation of the p53 pathway could be used for modulation of stem cell clonogenic and differentiation potentials in vitro. Total epithelial cell fractions prepared from freshly dissociated mouse lung tissue were cultured as 3D organoids. Colony-forming ability was evaluated in the presence or absence of SB431542 (Alk 4/5 inhibitor), Y-27632 (ROCK 1/2 inhibitor) or pifithrin-alpha (p53 inhibitor). Pharmacological inhibition of p53 promoted survival and self-renewal capacity of the lung stem/progenitor pool, as indicated by an increase the size and colony forming efficiency of organoids. Effects of pifithrin-alpha treatment were further augmented by inhibition of ROCK1/2 and Alk 4/5, suggesting the non-overlapping roles for each pathway in regulating stem/progenitor cell expansion. We are currently exploring the effect of transient p53 inhibition on the expansion and differentiation of region-specific epithelial progenitors of the lung, by utilizing

lineage labelling strategies. We conclude that loss of p53 function enhances "stemness" of progenitor cells and transient inhibition of the p53 pathway may provide a strategy for expansion of stem cells for cell based therapies.

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W-2085

CELLULAR SENESCENCE AS A DRIVER OF PROGENITOR CELL DYSFUNCTION IN PULMONARY FIBROSIS

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Idiopathic pulmonary fibrosis (IPF) is a fatal form of interstitial lung disease which results in the progressive scarring of lung tissue leading to declining pulmonary function. Progenitor cell exhaustion with associated cellular senescence, hallmarks of ageing, have been proposed as pathogenic drivers of progressive lung fibrosis. We have shown previously that Sin3a is a critical determinant of lung endoderm development and that its absence leads to cell cycle arrest and induction of cellular senescence. We hypothesized that conditional loss of Sin3a in postnatal alveolar type 2 cells (AT2) cells would initiate a program of senescence and fibrogenesis. Sin3a loss of function was induced in postnatal AT2 cells by tamoxifen exposure of SftpcCreER/Sin3af/f mice. Conditional loss of Sin3a was associated with activation of p53 signaling and mitochondria dysfunction, leading to senescence of AT2 cells and progressive lung fibrosis. To determine whether fibrosis was the result of AT2 senescence versus apoptosis, AT2 cells were ablated by tamoxifen exposure of SftpcCreER;RosaDTA mice. DTA-mediated ablation of AT2 cells resulted in a transient fibrotic response that resolved over time. Removing senescent AT2 cells by senolytic drug cocktail attenuates lung fibrosis. We conclude that senescence rather than apoptosis of AT2 cells drives lung fibrosis.

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W-2087

KLF4 MAINTAINS QUIESCENCE IN MOUSE ADULT HAIR FOLLICLE STEM CELLS

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Adult stem cells are required for epithelial tissue homeostasis, yet the mechanisms through which these cells are maintained, activated, and direct their progeny to specific fates are not fully understood. The hair follicle is an excellent model in which to address these questions as it contains accessible and well-characterized SC populations. Hair follicles undergo cyclical periods of growth (anagen), regression (catagen), and rest (telogen); these cycles are partially driven by hair follicle stem cells. Our data show that the pioneer transcription factor KLF4, which can act as a transcriptional activator or repressor, is excluded from proliferating hair follicle cells upon anagen onset, is highly expressed in telogen hair follicle stem cells, and shows decreased expression in anagen hair follicle stem cells. To delineate the functions of KLF4 in adult hair follicle stem cells, we used a mouse model that permits doxycycline inducible deletion of Klf4 in adult epidermis and hair follicles. Klf4 deletion in postnatal epidermis and late anagen hair follicles results in premature entry into the subsequent anagen as well as inflammation. To determine whether this phenotype is due to an intrinsic requirement for KLF4 in hair follicle stem cells or is a consequence of inflammation, we utilized K15-CrePR to induce deletion of Klf4 specifically in the hair follicle stem cell compartment. This experiment showed that hair follicle-specific Klf4 loss promotes anagen in the absence of inflammation, suggesting that KLF4 is required within hair follicle stem cells to maintain hair follicle stem cell quiescence. Hair follicle stem cell quiescence is controlled in part by secreted factors such as FGF18 and by the repressive actions of TCF3 and TCF4 transcription factors that block expression of Wnt/ß-catenin target genes. We find that KLF4 binds the Fgf18 promoter, and Fgf18 mRNA expression is decreased following Klf4 deletion. In parallel, KLF4 directly interacts with TCF3 and TCF4 in quiescent hair follicle stem cells, suggesting that it may function as part of the repressive complex that antagonizes Wnt/ß-catenin signaling. Together, our data suggest that KLF4 maintains hair follicle stem cell quiescence via several mechanisms including activation of Fgf18 expression and repression of Wnt/ß-catenin signaling.

W-2089

CHOOSING THE RIGHT MODEL SYSTEM FOR HEPATOTOXICITY: A COMPARISON OF 3-D LIVER ORGANOIDS, HEPG2 SPHEROIDS AND IPSC-DERIVED HEPATOCYTES USING MTT AND DNA COMET ASSAYS

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The liver is the primary organ system for drug metabolism and detoxification. In this role, it is also highly susceptible to damage from pharmaceuticals and other chemical toxicants. Animal models and traditional in vitro assays modeling liver metabolism often fail to recapitulate the in vivo toxicity of drugs in human patients. A classic example is the cancer drug Trovafloxacin mesylate, which cleared preclinical animal models for safety and achieved FDA clearance, but was later retracted due to acute liver toxicity resulting in numerous fatalities. Failures, such as this, can have devastating health outcomes as well as result in huge financial costs for pharmaceutical companies. Therefore, it is imperative to use appropriate preclinical models that can accurately predict human toxicity. Although regulatory guidelines still mandate the median lethal dose test (LD50) in rodents for most new drugs, there is momentum at the FDA to approve cell culture models that can reap substantial time- and cost-savings. Hepatocyte cell culture models are increasingly being used for high throughput liver toxicity screening early during the drug development pipeline. Moreover, human model systems may more accurately reflect human physiology than animal models. In this study, we compare three in vitro hepatotoxicity model systems: induced pluripotent stem cell (iPSC)-derived hepatocytes, 3-D liver organoids, and HepG2 spheroids. Using a panel of known hepatotoxic compounds we evaluated the performance of these models using high throughput toxicity assays, including the MTT Assay, a robust test for cellular toxicity, and the DNA Comet assay and CometChip technology to assess genotoxicity. We conclude that each of the different hepatocyte model systems tested offer advantages for evaluating toxicity, and that these advantages can be used in a complementary fashion to accurately probe dose responses for liver toxicity.

EYE AND RETINA

W-2091

REGULATION OF LIMBAL EPITHELIAL HOMEOSTASIS BY MIR146A

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MiR-146a plays a role in numerous biological processes including cell migration, proliferation, differentiation, and inflammation. Previously, we showed that miR-146a upregulation impaired corneal epithelial wound healing and expression of putative limbal epithelial stem cell (LESC) markers. We investigated the regulatory roles of miR-146a in normal corneal limbus evaluating the effects of miR-146a on Notch signaling and inflammatory pathways. Human autopsy corneas were procured by National Disease Research Interchange. Primary cultures of LESC-enriched limbal epithelial cells (LEC) were used. RNAseq was performed on total RNA from primary LEC treated with miR-146a mimic, inhibitor and their corresponding controls. Sequencing identified several genes as possible targets of miR-146a. Overexpression of miR-146a decreased mRNA levels of both Numb and Notch-2, with a subsequent increase in Notch-1 levels. Western blot confirmed sequencing data at the protein level. Numb and Notch-2 protein levels were decreased by miR-146a overexpression. In addition, miR-146a overexpression led to an increased expression of keratin 15 (K15), a putative LESC marker. RNAseq also identified a marked decrease in mRNA levels of Traf6 and Irak1, two NF-kB regulators, with miR146a mimic treatment. To further investigate this change, increased TRAF-6 and IRAK-1 expression was induced by LPS treatment in LEC in vitro and in ex vivo organ-cultured corneas. MiR-146a overexpression suppressed TRAF-6 and IRAK-1 levels, whereas its inhibition led to the increase of TRAF-6 and IRAK-1 in LPS treated LEC. Our results indicate that MiR-146a plays an important role in limbal epithelial cell maintenance via regulation

of the Notch signaling pathway, supported by upregulation of K15 by miR-146a overexpression. Additionally, its downregulation of Numb, an inflammation inducer, combined with its targeting of Traf6 and Irak1 to regulate the NF-kB pathway, contribute to its regulation of inflammatory pathways.

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W-2093

MODELING HUMAN SYNDROMIC CILIOPATHIES USING IPSC-DERIVED PHOTORECEPTOR SHEETS

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Modeling human developmental and degenerative diseases has been always logistically challenging in particular in the case of rare diseases where no exhaustive animal models are available. Generation of sustainable human disease models that allow in-depth analysis of the molecular mechanism is one of the big challenges nowadays. Stem cell technology holds great potential in disease modeling and represents a new powerful tool for generating scalable and animal-free models that can more accurately illustrate clinical phenotypes of complex human diseases. Ciliopathies are a group of heterogeneous genetic diseases affecting proteins involved in primary cilium structure and function. Syndromic ciliopathies have a broad spectrum of symptoms ranging from retinal degeneration to skeletal and neuro-development anomalies, including polydactylism and mental retardation. Herein we describe the generation and molecular characterization of iPSC-derived photoreceptor sheets from patients affected by ciliopathies. Photoreceptor sheets are characterized by a polarized, multi-layered tissue expressing outer segment, connecting cilium, and nuclear photoreceptor markers. Ciliopathic photoreceptors displayed significant common alterations in the expression of hundreds of developmental genes. Moreover, they showed several anomalies in the formation and maintenance of cilia, the positioning of the mother centriole and the activation of a stress response to misfolded proteins. Furthermore, we observed genomic instabilities and accumulation of DNA damage in the photoreceptors progenitors of one of the patients. This study reveals how combining cell reprogramming and organogenesis technologies with next-generation sequencing enable the elucidation of molecular and cellular mechanisms involved in human ciliopathies. The same approach, combining photoreceptor sheet differentiation and wide genome expression profile could be applied to model many genetic, developmental and degenerative diseases affecting photoreceptors. These patient-derived retinal sheets may be useful for elucidating the molecular mechanisms underlining these diseases, for drug screening of compounds with potential therapeutic effect and predicting drugs side effects.

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W-2095

HUMAN MESENCHYMAL STEM CELLS AS A THERAPEUTIC APPROACH FOR THE TREATMENT OF CORNEAL SCARS

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Severe corneal injuries and diseases are a major cause of blindness around the world. Although cornea transplantations are routine in some parts of the world, it is an invasive procedure associated with post operational complications. Thus, alternative treatments for corneal diseases are desired. Mesenchymal stem cells (MSCs) have been shown to have regenerative potential and may also play a role in modulating the immune system. The goal of this study is to provide insights about MSCs' plasticity towards corneal keratocyte lineage, anti-inflammatory properties, and their putative application for the treatment of corneal stromal scars. We investigated four different tissue sources of human mesenchymal stem cells (MSCs): adipose tissue (ASC), bone marrow (BM-MSC), umbilical cord (UC SC), and corneal stroma (CSSC). These MSC populations were evaluated for their expression of mesenchymal stem cell markers using immunohistochemistry and their potential for differentiation by gene expression analysis. The capacity to respond to inflammatory stimuli was evaluated by the expression level of TNFAIP6 gene after treatment with pro-inflammatory cytokines (IFN-γ and TNF-α) and by inhibition of inflammatory RAW 264.7 differentiation. MSCs were tested for their regenerative capacity in a mouse model of acute stromal scars. All four types of MSCs expressed and retained the surface antigens and MSC markers including CD90, CD73 and CD105. All MSCs expressed keratocyte-related genes, including lumican (LUM), carbohydrate sulfotransferase (CHTS6), and keratocan

(KERA). Under keratocyte differentiation conditions, ASC and CSSC cell lines demonstrated a significant increase ($p < 0.05$) of those genes. All MSCs reduced inflammatory RAW 264.7 differentiation; however, TNFAIP6 transcripts in CSSC increased >3 fold, significantly greater ($p<0.05$) than non-stimulated MSCs. In vivo, the scar opacity was reduced after treatment with the MSCs. Based on our findings, CSSC showed the highest differentiation potential towards keratocyte lineage and anti-inflammatory properties, but all MSCs investigated reduced corneal scars in vivo. Our results support the hypothesis that MSCs could serve as a model for generating stem cell-based therapies for corneal stromal diseases.

W-2097

DEVELOPMENT OF AN AUTOMATED PROTOCOL FOR LARGE SCALE DIFFERENTIATION OF RETINAL PIGMENT EPITHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Retinal pigment epithelium (RPE), the monolayer of pigmented cells localized between the neural retina and the choroid plays crucial roles in sight mainly by contributing to the blood/retina barrier, absorbing light energy but also by transporting nutrients from the blood to the photoreceptors. Therefore, dysfunction or death of RPE is followed by the death of photoreceptors and is responsible for a group of rare hereditary diseases called retinitis pigmentosa and to some extend for age related macular degeneration (AMD). Since there is currently no treatment for dry AMD and for most of the retinitis pigmentosa, the replacement of RPE cells is an attractive solution. The ability of human pluripotent Stem Cells (hPSCs) to spontaneously differentiate into RPE has allowed their use for clinical applications. We have recently developed a cell therapy product consisting of human embryonic stem cells (hESC) disposed on a biological substrate. RPE cells were differentiated using spontaneous differentiation of hESCs. However, even if this protocol allows obtention of a nearly pure population of RPE, it remains a long and largely inefficient method that requires a fastidious manual enrichment by dissecting pigmented area. This process is therefore, not compatible with industrial large scale production and marketing which would be the next step to treat larger cohort of patients. We have developed a simple automated protocol with limited exogenous factors and obtained a pure population of RPE. With this protocol the main steps of the retinal development are recapitulated with the generation of retinal progenitors that could be differentiated into all retinal cell types such as retinal precursors. Finally, such protocol should allow cost reduction, large scale and reproducible production of RPE cells. This new protocol could open avenues for AMD clinical trial but although for retinal disease modeling and large high through put drug screening.

W-2099

INVESTIGATING THE ROLE OF THE MIR-182/96/183 SENSORY CLUSTER IN PHOTORECEPTOR DEVELOPMENT IN A HUMAN RETINAL ORGANOID CULTURE SYSTEM

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MicroRNAs (miRNAs) are potent post-transcriptional regulators of gene expression and evidence from different model organisms indicates they are important for determining developmental timing of neurogenesis and neuronal maturation and survival. The miR-182/96/183 Sensory Cluster is transcribed as a single 4kb pre-miRNA which is processed into three mature miRNAs. It is strongly expressed throughout differentiating sensory epithelia and is the most highly expressed miRNA family in the retina, but the role it plays in retinal development remains unclear. Here, we describe an approach to elucidate Sensory Cluster function in human photoreceptor cell development using a loss-of-function approach in an embryonic stem cell (hESC)-derived retinal organoid culture system. Optic vesicle-like structures formed by 5-7 weeks from hESCs and were isolated and cultured as floating organoids, which express photoreceptor markers in polarised neuroepithelia. We used qPCR to profile expression of the Sensory Cluster and markers across photoreceptor cell development in human foetal retinal development and compared it to that seen in undifferentiated ESC and in human retinal organoids from culture week 7 to 25. A CRISPR/Cas9 gene editing strategy utilising two guide RNAs was used to delete a 5.8Kb region of chromosome 7 containing the miR-182/96/183 Sensory Cluster and several putative regulatory elements in three hESC lines (including fluorescent reporters to facilitate isolation of photoreceptor cells). PCR analysis confirmed deletion of the Sensory Cluster. Despite high expression in pluripotent ESCs, we show that the Sensory Cluster is not required for maintenance of pluripotency and we investigated whether loss of the Sensory Cluster precluded neural induction and optic vesicle formation in human PSC-derived retinal organoids. To

test the hypothesis that Sensory Cluster expression is important for photoreceptor commitment and maturation comparative analysis of photoreceptor developmental marker expression in Sensory Cluster knockout and isogenic control organoid cultures was performed. Sensory Cluster knockout retinal organoids represent a novel tool to investigate miRNA function in the context of human retinal development.

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W-2101

A HUMAN ORGANOID-BASED MODEL FOR RETINOBLASTOMA

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Retinoblastoma is the most common tumor of the eye in early childhood and is caused by biallelic inactivation of the retinoblastoma gene RB1. Efforts to model retinoblastoma in mouse were not satisfactory as the mutation of Rb1 alone is not sufficient for tumor formation, indicating that the development of retinoblastoma in these two species follows different routes. Therefore, we have created a human cell-based model for retinoblastoma using organoid technology. For the establishment we started with the human embryonic stem cell line (hESCs) H9 and were able to generate retinal organoids containing mature photoreceptors (rods and cones) in the outer layer and ganglion cells, Müller glia cells, amacrine cells and horizontal cells in the inner layer. Using the CRISPR/Cas9 system we have generated H9 hESCs carrying a mutation in exon 3 (close to the splice donor site) either on one or both RB1 alleles. By now, we have characterized 10 heterozygous, one compound heterozygous and one homozygous clone by DNA, RNA and protein analysis. For comparative differentiation we have chosen two clones, G12LS and C7, heterozygous and homozygous for the RB1 variant c.374_380del (LRG_517t1), respectively. The variant results in a premature stop codon on protein level (p.(Glu125Valfs*9). Presence of different cell layers in the retinal organoids was analysed by immunostainings. RB1 wildtype, RB1 heterozygous (G12LS RB1+/-) and homozygous (C7 RB1-/-) knock-out organoids stain positive for ganglion cells, immature photoreceptors and cone photoreceptors on day 35, 61 and 96 of differentiation, respectively. Immunostainings on d126 indicated enhanced proliferation, a decrease in rod photoreceptors and horizontal cells and absence of amacrine cells in C7 RB1-/- organoids. In addition, over time the neural retina layer of C7 RB1-/- organoids took on a loose and disordered appearance. Overall, the presence of cones in our organoid-based model demonstrates its applicability for studies in retinoblastoma research as these are the cell-of-origin of retinoblastoma. Moreover, we could detect first differences between the wildtype and RB1 knock-out organoids. Immunostainings and histological examination for retinoblastoma-specific features (e.g. Flexner-Wintersteiner rosettes) are ongoing.

W-2103

DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TOWARDS CORNEAL ENDOTHELIAL LINEAGE

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Located at the outermost surface of the eye, the cornea provides the majority of its refractive power. The cornea is composed of five main layers: epithelium, Bowman's, stroma, Descement's and endothelium. The endothelium acts as a nutrient transporter from the aqueous to the stromal compartments. However, in contrary to epithelial or muscle cells, that can regenerate upon damage, the corneal endothelium (CE) does not regenerate. Given their lack of regenerative potential, differentiation human induced pluripotent stem cell (hPSCs) towards the CE lineage and assessment of its translational potential emerges as an attractive alternative approach in relation to post mortem endothelial sheets transplantation. Here, we were able to differentiate hPSCs towards CE-like cells (iCEs) based on the transient formation of neural crest (NC)-like cells and subsequent CE maturation. At mRNA and protein levels, we observed progressive downregulation of pluripotency markers, such as OCT4 and NANOG. Initial upregulation and subsequent downregulation of neural crest markers was observed, e.g PAX3, SOX10, SIX3, SIX6 and RAX. Finally, corneal endothelial markers, such as COL8A1, COL8A2, ZO1, CDH2 and ATP1A1, were significantly upregulated, however at different expression levels compared to primary CE cells. We are currently performing RNA-seq and functional analyzes of iCEs. In summary, we were able to obtain iCEs exhibiting characteristics of primary CE, which can be further explored prior translational applications.

STEM CELL NICHES

W-2105

HEPATOCELLULAR CARCINOMA SERUM MICROENVIRONMENT ENHANCES THE EXPRESSION OF ANTI-CANCER PROPERTIES IN ADIPOSE MESENCHYMAL STEM CELLS

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Although mesenchymal stem cells (MSCs) were found to be recruited to the tumor site, the effect of the tumor microenvironment on MSC plasticity and fate remains poorly understood. We have previously shown that co-culture of cancer cells along with MSCs induced their reprogramming to acquire cancer stem cell (CSCs) characteristics, which may contribute to tumor progression. Herein, we hypothesize that the tumor microenvironment of hepatocellular carcinoma (HCC) modulates the cell fate and plasticity of adipose MSCs (A-MSCs), and its potential to acquire tumor properties. Serum samples were collected from 15 HCC patients and 15 healthy volunteers. A-MSCs were cultured in the presence of HCC patient serum, and control normal human serum for 6 days. Total RNA was extracted and genetic analysis using standard quantitative PCR were performed on the treated cells. Our results demonstrated that HCC serum-treated A-MSCs showed significant decrease in proliferation rate when assessed by MTT assay. Their colony forming ability was also decreased, and migration in cell scratch assay was compromised, when compared to cells cultured in normal serum. There was significant down regulation of the pluripotency gene expression, OCT4, NANOG, and SOX2, detected by q RT-PCR. The mesenchymal genes, vimentin and snail, were also downregulated, while the epithelial marker E-cadherin was upregulated. The expression levels of cancer genes KRAS and TP53 were downregulated. Similarly, CD44 expression was downregulated while CD24 was depleted. The HCC biomarker, α-fetoprotein (AFP) was also found to be significantly decreased in HCC serum-treated A-MSCs compared to the control group. These findings suggest that exposure of A-MSCs to circulating cancer soluble factors in the blood stimulates the generation of bio-modulatory response against the cancer. Our findings provide novel insights into the interactions between MSCs and cancer microenvironment, with the potential of identifying novel molecular targets for cancer therapy.

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W-2107

DECIPHERING STEMNESS IN THE NEURAL CREST TISSUE LINEAGE

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The neural crest cell lineage arises from the primitive ectoderm during neurulation to form a multipotent stem cell population within the vertebrate embryo. Transient in nature, neural crest cells undergo epithelial-to-mesenchymal transitions, migrate throughout the embryo, and differentiate into a multitude of tissues and specialized cell types including craniofacial bone and cartilage, the peripheral nervous system, endocrine organs, and cardiac outflow tract. Within the neural plate border, neural crest progenitors receive signals from neighboring neuroectoderm and nonneural ectoderm to become specified into premigratory neural crest. While extensive efforts are underway to understand the gene regulatory networks that direct their specification, very little is known about the gene network responsible for maintaining their high degree of multipotency. Our previous studies using Spatial Genomic Analysis of avian cranial neural crest cells in vivo revealed a subpopulation that express pluripotency factor orthologs Oct4, Nanog, and Klf4, suggesting the presence of a neural crest stem cell niche. It is not known, however, if expression of these stemness genes has been maintained from the pre-gastrulation epiblast, or if the network was switched on at the time of neural crest specification. Therefore, here we 1) decipher the molecular basis governing neural crest multipotency; and 2) determine whether the network was continued from the epiblast lineage or induced post-gastrulation. To achieve our goals, we are using genetic, immunohistological, and biochemical approaches in both in vivo (mouse, chicken) and in vitro (mouse and human embryonic stem cell) model systems. We have previously developed cell culture conditions that generate self-renewing multipotent crestospheres from chicken neural crest explants. We have also optimized culture conditions that differentiate human embryonic stem cells in neural crest spheroids expressing both Nanog and neural crest markers including Sox10. This multi-system approach will increase our understanding of the molecular mechanisms underlying neural crest stemness and cell fate decisions that direct vertebrate development or erroneously contribute to congenital defects and associated diseases.

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W-2109

EXTRA-CELLULAR VESICLES ISOLATED FROM MOUSE BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS TREATED WITH SPECIFIC SIGNALING MODIFIERS AND THEIR EFFECTS ON THE FATE OF HEMATOPOIETIC STEM CELLS

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Bone marrow-derived mesenchymal stromal cells (BMSCs) are known to play critical role in the regulation of hematopoietic stem cell (HSC) fate. Our prior work has shown that signaling mechanisms prevailing in the BMSCs affect the fate of HSCs via intercellular communication involving extra-cellular vesicles (EVs) like micro-vesicles (MVs) and exosomes. MVs and exosomes therefore form a crucial medium through which stromal cells communicate with stem cells. We have extended this work further to examine the effect of the MVs on the functionality of HSCs. Our main aim is to identify specific signaling modifiers for preparation of MVs having HSC-supportive activity. We isolated the MVs and exosomes from murine BMSCs and characterized them on the basis of size using FE-SEM and CD63 and CD9 expression using immunofluroscence studies and western blot. We treated the BMSCs with hypoxia-inducing reagent COCl2 as we already have preliminary indicative data with these compounds. Simultaneously, we also incubated the BMSCs inside a sealed humidified hypoxia chamber containing 1% oxygen. Our intention was to check whether exposure to hypoxia would affect the morphology and size of EVs. We saw that COCl2 treatment and exposure to low oxygen levels did not affect the morphology of the MVs; however it led to a significant increase in the size of MVs. On the other hand the size of the exosomes was differentially affected each by COCl2 treatment and exposure to low oxygen level respectively. Our immediate objective is to assess the effect of hypoxia on the functionality of HSCs for which we will be incubating the Lin- cells with EVs and exosomes isolated from hypoxic BMSCs and checking their colony forming unit (CFU) ability. A suitable strategy is needed for the cryopreservation of EVs, and hence we have begun with cryopreservation experiments to evaluate the potential advantages/disadvantages of cryopreservation of EVs at different temperatures. A similar approach (as used in hypoxia experiments) will be used to study the effect of inhibitors of p38 and MEK/ERK MAPK pathways on the protein and gene expression of BMSCs and BMSC-derived EVs. We conjecture to develop a “ready-to-use” biologic comprising of EVs isolated from MSCs treated with selected signaling modifiers for boosting the engraftment ability of HSCs in clinical transplantations.

Funding Source: Department of Biotechnology, Ministry of Science and Technology, Government of India

W-2111

THE EXISTENCE OF NEURAL CREST-LIKE STEM CELLS IN THE HUMAN ADULT PERIPHERAL NERVE AND THEIR ROLES IN TISSUE HOMEOSTASIS

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Neural crest stem cells (NCSCs) are an embryonic cell population that generates diverse progenies from peripheral neuroglia to mesenchymal cells. Compelling evidence indicates the persistence of NCSCs in various adult tissues, where they contribute to postnatal neurogenesis and gliogenesis. However, the existence of NCSC-like cells in the adult peripheral nerve (PN) remains unclear. Here, we isolated and characterized adult PN-resident stem cells (PN-SCs) with NCSCs-like hallmarks. We employed a hydrogel-supported 3-dimensional organ culture of sciatic nerve with mitogenic stimulation of bFGF and EGF, which resulted in the robust cell outgrowth and enrichment of PN-SCs from nerve explants into the hydrogel. The PN-SCs expressed uniformly NC-related mRNAs or markers but no neuroglia-related genes and markers, indicative of an uncommitted NC cell phenotype. They maintained their capacity for continuous cell turnover, clonogenicity, and spheroid formation in vitro. Subcultured and clone-derived PN-SCs showed multipotency that yielded neurons, glia, and mesenchymal lineage cells. Intriguingly, PN-SCs expressed neurotrophic mRNAs and factors that enhanced the cell proliferation and neuritogenesis of neuroblastoma cells. Transplanted PN-SCs contributed to the regeneration of various cell types and the structural and functional recovery of the injured PN. Overall, adult PN-SCs might be useful for developing cell-based strategies to apply neurologic disease.

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W-2113

STEM CELLS-DERIVED MEMBRANE BOUND FACTORS FOR REGENERATIVE MEDICINE

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It is widely accepted that in parallel with direct cell replacement, the incriminated regenerative mechanisms in cell transplants resides in its secretome effect, mostly attributed to various soluble growth factors and cytokines. Less discussed, an equally important mechanism resides in the paracrine and juxtacrine signaling, consisting in production of factors that require cell-to-cell contact or proximity for physiological effect. The juxtacrine signals are recognized as powerful modulators in development, and include integrins, Notch, Hedgehogs (HH) and Wnt families. Wnt and HH ligands are lipid modified, insoluble in water and membrane bound. The current methods to capture these ligands use detergents and solvents that typically dissociate and remove the lipid modifications causing significant decrease of the physiological activity. Here we present a method that we developed to harvest membrane components and lipid modified ligands using a proprietary system that preserves the integrity of the molecules. We demonstrated the efficient capture of HH and Wnt family members by quantitative ELISA and the bioavailability by in-vitro and in-vivo testing. The originating cell population was human embryonic stem cells derived ectoderm. The partial differentiated population aimed to maximize the developmental signals and was produced using basic neuralization protocols. The resulting cell population was then exposed to a harvesting solution containing cyclodextrin. Cyclodextrins are glucose-based hydrophilic 3D constructs having a hydrophobic cavity that can capture hydrophobic molecules. Depending on the cyclodextrin size the capture can include one or more lipid molecules including membrane cholesterol and proteins that are lipid modified. Here we present the effect of the neuralized stem cells-captured lipid modified ligands on neuronal survival and stem cell niche activation. The results from human neuronal cell cultures, in-vitro human hair follicles, and in-vivo mouse and human hair regeneration are presented. The technology is an important advancement in regenerative medicine allowing the juxtacrine effect in the absence of transplanted cells with applicability in all organs and tissues with stem cell niches.

Funding Source: AIVITA Biomedical Inc.

W-2115

STEMNESS AND CELL FATE OF TRANSPLANTED SKELETAL MUSCLE PROGENITOR CELLS ARE REGULATED BY THE IN VIVO MUSCLE MICROENVIRONMENT

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Effective stem cell replacement requires donor cells to differentiate to functionally relevant cell types as well as self-renewal. We have generated skeletal muscle progenitor cells (SMPCs) from human pluripotent stem cells (hPSCs) able to form hundreds of new myofibers when transplanted into mouse models in vivo. However, retention of the progenitor/stem cell state has been limited. Skeletal muscle contains a well-defined niche that supports the endogenous muscle stem cells (satellite cells, SCs), but whether the SC niche can support hPSC-SMPCs, or whether endogenous SCs outcompete donor cells for SC niche space similar to that shown in other tissues, remains unclear. To evaluate retention of PAX7 by SMPCs in vivo, we intramuscularly injected hPSC-SMPCs into the TA muscle of mdx-NSG mice. Six-weeks post-engraftment, PAX7+ donor cells (marking stemness) were found only near regions of human-generated myofibers. No PAX7+ cells were found near mouse myofibers or in the SC niche. To evaluate cell fate, we re-isolated and profiled hPSC-SMPCs pre- and post-engraftment using single cell RNA-sequencing. Re-isolated SMPCs increased extracellular matrix, growth factor, and maturation genes, but decreased expression of all myogenic genes. We validated decreased myogenic potential of re-isolated SMPCs in vitro using live cell imaging and found 10-fold less efficient myotube formation compared to non-engrafted hPSC-SMPCs. To determine whether the decrease in myogenic cell fate in vivo was caused by endogenous SC competition for SC niche space, we crossed inducible knockout Pax7Cre/ERT2 DTA mice to mdx-NSG. We found donor SMPCs increased retention of PAX7 upon ablation of endogenous SCs and were better able to take up position in the SC niche. Understanding the competition between endogenous SCs and donor SMPCs to increase homing to the SC niche compartment may improve donor cell self-renewal and set a foundation for a long-term cell replacement therapy for skeletal muscle.

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CANCERS

W-2117

USING INDUCED PLURIPOTENT STEM CELLS TO IDENTIFY NOVEL BIOMARKERS OF AGGRESSIVE CANCER

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Reliable approaches to identify and target stem cell mechanisms that mediate aggressive cancer growth would have great therapeutic value, based on reports that have shown evidence of embryonic stem cell programs in highly malignant and metastatic cells. However, there remains limited understanding as to how some cancer cells repurpose embryonic mechanisms, and which embryonic mechanisms mediate aggressive outcomes. We harnessed the power of iPSCs to identify novel embryonic biomarkers that regulate aggressive cancer phenotypes, by first performing a cell surface proteomics screen of iPSCs and their somatic sources (to determine which may be iPSC-specific). To next determine if the list of iPSC proteins had potential clinical significance, we bioinformatically examined the overall mean gene expression changes occurring from normal samples vs. late stage disease for various cancer TCGA datasets. We found that a majority of the iPSC proteins showed a statistically significant increase in their respective gene's expression in advanced disease across a variety of cancer types. To next explore if the iPSC list contained proteins that may be more highly expressed in previously established 'stem-like' cancer populations, we tested gene expression in isolated 'cancer stem cells' (CSCs) from breast cancer and lung cancer cell lines. Both lung and breast CSCs showed a statistically significant increase in the overall iPSC-specific list. These results support that our list of potential iPSC candidate biomarkers are more highly expressed in previously established cancer populations that have been associated with 'stemness', and suggest that using iPSCs to identify targets could be applied to a variety of cancer types. Further analysis of iPSC proteins in cancer populations is ongoing, but thus far examination of selected initial protein candidates has identified proteins that are important for both stem and cancer cell functions (e.g. reprogramming, proliferation, survival), and that have been used to identify cancer cell line subpopulations that show increased tumorigenicity in vitro and in vivo. These combined findings demonstrate that examining pluripotent stem cells in relation to cancer can provide a powerful approach to gain insight into critical mechanisms regulating aggressive cancer.

W-2119

NANOPARTICLE DELIVERY OF SIRNA AGAINST TWIST TO REDUCE DRUG RESISTANCE AND TUMOR GROWTH IN AML CELL LINES

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TWIST1 and TWIST2 are two basic helix-loop-helix transcription factors that play a critical role in embryogenesis. These proteins are often aberrantly activated in many cancers. Emerging evidence suggests that dysregulation of Twist1 or Twist2 are implicated in the pathogenesis of various hematological malignancies. Acute Myelogenous Leukemia (AML) is a clinically and biologically heterogeneous clonal hematopoietic progenitor/stem cell malignancy. In the hematopoietic system, the expression of Twist1 is largely observed in CD34 hematopoietic stem cells, whereas Twist2 is mostly expressed in early myeloid lineages. Twist2 is a major negative modulator in both the development of myeloid cells and their proinflammatory responses. Transcription factors are difficult to target with small molecule drugs due to their nuclear localization. However, by using siRNA technology, we have been able to target and silence cytoplasmic TWIST mRNA and re-sensitize cancer cells to conventional chemotherapeutic agents. We have previously designed and validated two therapeutic siRNAs against TWIST, and have created a mesoporous silica nanoparticle (MSN) tagged with HA to be used as a nanoparticle-based delivery system in solid tumors. Also, we demonstrated that MSNs can deliver their siRNA cargo efficiently to the cytoplasm in AML cell lines. Furthermore, MSN-HA delivered TWIST siRNAs were able to reduce the expression of TWIST and increase in apoptosis markers such as annexin, BAX and P53 in AML cells. We predict that reduced TWIST expression will sensitize AML cells to chemotherapeutics like daunorubicin compared to cells treated with non-targeting control siRNA. These studies should reveal the TWIST family proteins as promising targets to address the compound problems of metastasis and acquired drug resistance in AML and provide evidence for MSN-siTWIST as a possible therapeutic agent. We utilized AML cell lines and patient derived cells to test TWIST1-2 knockdown as a therapeutic approach in combination with standard chemotherapy. Further investigations of the possible role of TWIST1-2 and other stemness genes may

provide a useful strategy for the management and treatment of leukemic stem cells. We hope that this novel platform will target leukemic stem cells and prevent acquired resistance in AML and other leukemia.

Funding Source: CIRM-Discovery Award

W-2121

THE EXPRESSION OF THROMBOMODULIN ON TUMOR STROMAL HOMING OF MESENCHYMAL STEM CELLS FOR TUMOR GROWTH

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Mesenchymal stem cells (MSCs) can mobilize into tumor stroma for affecting tumor progressions; however, the molecule's guiding the tumor-specific homing of MSCs remains mostly unknown. Thrombomodulin (TM), an endothelial anticoagulant, is up-regulated in tumor-conditioned MSCs. This study investigated the expressing and therapeutic significance of TM on MSCs-mediated tumor growth in vitro and in vivo using wild-type and TM gene-targeting bone marrow-derived MSCs and recombinant TM domain proteins. The present results showed that TM expression was up-regulated in B16F10 melanoma cells-conditioned medium (B16F10-CM) stimulating MSCs in plate-derived growth factor-BB (PDGF-BB)-activated PDGF receptor signaling dependence. Using Cre-mediated excision of loxP-flanked TM gene in MSCs, TM deficiency did not significantly affect MSCs' viability and chemotactic migration in normal culture condition but significantly enhanced MSCs' chemotaxis toward B16F10-CM. Moreover, TM deficiency markedly diminished MSCs-promoted B16F10 tumor growth and significantly reduced MSCs' homing capacity into B16F10 tumor stroma in vivo. Mechanistically, TM deficiency significantly declined the adhesion between B16F10-conditioned MSCs and endothelial cells (ECs), and this TM mediated MSCs-ECs adhesion was blocked by recombinant TM lectin-like domain (TMD1) and Lewis Y (LeY) glycan, indicating the reciprocal of TMD1-LeY interaction on directing tumor-activated MSC's into tumor microenvironment through adhesion to tumor-activated ECs. Therapeutically, recombinant TMD1 administration significantly enhanced the efficacy of radiation on suppressing human A2058 melanoma's growth by decreasing the amount of tumor-associated fibroblasts for establishing active tumor stroma. In conclusion, TM induction by tumor-secreted PDGF can navigate the specific homing of MSCs via interacting with LeY-expressed ECs-lining on tumor microenvironment for promoting tumor growth, suggesting that the interplay of TMD1 and LeY might contribute to tissue's specific delivery of MSCs. These findings may provide a new therapeutic option via blocking MSCs-mediated functional tumor stromal development for aiming tumor mediation.

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W-2123

RADIATION-INDUCED PHENOTYPIC PLASTICITY IN HUMAN GLIOMA STEM CELLS

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Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in adults with a median survival of just 14 months. The current standard-of-care treatment is ineffective and fails to significantly prolong survival. Moreover, these invasive tumors display extensive intratumoural heterogeneity and resistance to radio- and chemotherapy, posing a major clinical challenge due to inevitable tumor recurrence. Following exposure to aggressive multimodal treatment, GBMs frequently shift their biological features upon recurrence, acquiring a more resistant phenotype. However, the temporal dynamics and molecular mechanisms that facilitate GBM recurrence are poorly understood. The objective of our study was to determine how molecularly distinct patient-derived glioma stem cells (GSCs) temporally adjust their expression profile and phenotype in response to ionizing radiation in vitro and in vivo. We find that human GSCs undergo dramatic molecular and phenotypic changes in response to a single dose of ionizing radiation. The observed treatment responses differ depending on the genetic background of the patient-derived GSCs. We find that ionizing radiation causes a transient decrease in the expression of key stemness genes followed by drastic morphological changes and a concomitant increase in the levels of cell fate markers. These treatment-induced cellular alterations commence immediately following treatment (within 24-48 hours) and become more pronounced over the course of one week. If irradiated GSCs are allowed to recover for two weeks, we observe the presence of heterogeneous cell populations that were absent prior to treatment. We also performed single-cell RNA sequencing and mass cytometry at multiple different timepoints post-treatment to discover and characterize novel cell subpopulations that emerge from different human GSCs after irradiation. Moreover, cell viability experiments reveal that human GSCs previously

exposed to radiation are more radioresistant upon re-treatment compared to their naïve, untreated counterparts – suggesting that the aforementioned phenotypic shifts promote treatment resistance. Our results suggest that GSC responses to radiation are dynamic, and that surviving cells are capable of adopting novel cellular states over a matter of hours.

W-2125

SMALL MOLECULE THERAPEUTICS TO DOWN-REGULATE PANCREATIC CANCER 'STEMNESS'

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The average survival for patients with Pancreatic Ductal Adenocarcinoma (PDA) is merely 6 months, underscoring the need for new therapeutic approaches. During PDA progression, pancreatic acinar cells lose activity of the ClassI/II bHLH factors that regulate quiescence. MYC is reported to be amplified in 20–30% of pancreatic cancer cases. This amplification is sufficient to initiate cancerous phenotypes in pancreatic cells. We previously found that promoting transcriptional activity of the Class I bHLH factor E47 in highly aggressive PDA cells induced stable growth arrest in vitro and in vivo. In part by restoring tumor suppressor retinoblastoma (RB) activity. To translate these findings for clinical utility, we developed a high throughput screening platform to identify small molecule inducers of Class I/II bHLH activity. A screen of 4,375 known drugs identified 70 bHLH activators. Statins or (HMG-CoA reductase inhibitors) as a class of drugs, were identified as promising candidates. Pitivastatin was the most salient bHLH activator amongst them. Studies with pitavastatin in primary patient-derived tumor cells and established PDA lines, revealed dose-dependent growth inhibition. At the molecular level, pitavastatin induced expression of the cyclin-dependent kinase (CDK) inhibitor p21and blocked repressive phosphorylation of RB protein. Here we show that a second hit from the screen, Drug 2 (a chromatin modifier), acts in synergy with Pitavastatin to induce growth arrest by further activating RB and by ablating expression of the MYC oncogene. Together, these drugs reduce expression of the S phase genes Aur A, CCNB1 and 2, as well as survivin. The data establish the effectiveness of a novel drug combination to control PDA cell growth. Future studies are aimed at in vivo testing.

Funding Source: California Institute for Regenerative Medicine

W-2127

CLONAL COMPETITION OF HUMAN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS DURING CHEMO TREATMENT IN XENOGRAFT MOUSE MODELS

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The importance of cancer cell heterogeneity is increasingly recognized. However, the interactions between individual cancer cells remain poorly understood, particularly in response to therapeutic treatment. For instance, the combined regimens of an intensive and maintenance chemotherapy have proven beneficial in acute lymphoblastic leukemia (ALL) patients. However, it remains unclear why a long term low dose maintenance therapy is more beneficial than a short term intensive therapy. We hypothesized that maintenance therapy applies constant selective pressure and alters the growth competitions between leukemia clones. Past studies have used naturally occurring genetic mutations to examine heterogeneity and evolutionary trajectories of cancer cells. As these mutations occur at different phases, clones marked by these mutations cannot be directly compared to assess cellular interactions. To test our hypothesis, we use engineered genetic barcodes to track patient derived ALL clones under an unperturbed environment as well as under the selective pressure of chemotherapy using xenograft mouse models. We established a chemotherapy treatment protocol in our xenograft model that closely mimics the intensive and maintenance phases of ALL treatment used in clinic. We found that the clonal composition remains constant during the unperturbed progression of human ALL cells in mice. Surprisingly, intensive therapy did not change the clonal composition. Instead, the same group of ALL clones appear during relapse after intensive treatment. However, under maintenance treatment, some previously low abundant ALL clones strikingly increase in abundance. These clones may have been suppressed during the undisturbed progression of the disease, and may play a role in altering disease progression during the long-term maintenance treatment. Our findings indicate that the benefit of maintenance therapy to ALL patients may arise from altered clonal competition between ALL cells. Further characterization of these emerging leukemia clones that arise during the maintenance phase may help improve ALL treatment.

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W-2129

ROLE OF G-PROTEIN ALPHA-12, AND TISSUE FACTOR, IN THE IN VITRO GROWTH OF HUMAN GLIOMA STEM CELLS

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Glioblastoma multiforme (GBM) is the most common and lethal form of brain cancer with one of the worst survival rates of all cancers. The lab has gained considerable insight into the signals triggered through the S1P and PAR1 receptors, G-protein coupled receptors (GPCRs) that activate RhoA. Specifically RhoA signaling activates YAP and MRTF-A, transcriptional co-activators involved in the expression of genes involved in the growth of glioblastoma cells. We hypothesize that GPCRs for S1P and thrombin (PAR1) become and remain activated in GBM because they are continuously exposed to their ligands. We further hypothesize that it is through this pathway that glioma stem cells up regulate expression of stem cell genes and thus maintain their ability for self-renewal. To test the central role of signaling through GPCR and Gα12 in glioma stem cell self-renewal in vitro we are using patient derived glioma stem cells (GSC)-23 and lentiviral transfection with shRNA to knockdown Gα12. We compared both glioma stem cell self-renewal (growth in limiting dilution assay) and stem cell gene expression (qPCR analysis) in shRNA control and Gα12 knockdown cells. Expression of mRNA for a number of stem cell genes (NANOG, CCND1, MYC, OCT4 and SOX2) was decreased approximately 50% when Gα12 was deleted. Similarly, data from the limiting dilution assay revealed significant loss of GSC self-renewal as assessed by sphere formation in the Gα12 knockdown cells. Tissue factor (TF), a gene downstream of YAP is regulated in response to thrombin and S1P through YAP induced gene transcription and has the potential for autocrine feedback regulation of the RhoA signaling cascade. We are currently using a similar knockdown strategy to test the role of TF in maintenance of GSC stemness. Future experiments will include in vivo studies establishing whether tumor growth is slowed and mortality from PDX cell xenografts is decreased when Gα12 or TF are downregulated. The goal is to target glioma stem cells to improve patient survival.

NEURAL DEVELOPMENT AND REGENERATION

W-3001

OLIGODENDROCYTE PRECURSOR CELLS DERIVED FROM FETAL AND ADULT NEURAL STEM CELLS-DERIVED DIFFER IN PHYSIOLOGICAL REGULATION AND RESPONSE TO NOXIOUS STIMULI

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Multipotent neural stem cells (NSCs) give rise oligodendrocyte precursor cells (OPCs) which maturate in myelinating oligodendrocytes (OLs) in a process driven by thyroid hormone (TH) and mediated by nuclear thyroid receptors (TRs), which form heterodimers with retinoic acid receptors (RXR). This process occurs during developmental myelination but is also activated in adult NSCs during remyelination. In this study we investigated the differentiation process and response to noxious challenges of OPC obtained from NSCs derived from fetal and adult brain. We show that several nuclear receptors display strong changes in expression levels between fetal and adult NSCs, with an overexpression of TRβ and lower expression of RXRγ in adult cells. Such changes in adult NSCs may determine their reduced capability to differentiate as showed by reduced yield of maturation and different mRNA expression of key genes involved in OPCs differentiation. RXRγ seems to be a main determinant of these differences, being involved as important regulator of cell cycle exit, as indicated by NSC derived from RXRγ KO-mice . In order to study if differences in physiological differentiation/maturation of OPCs from fetal and adult NSCs may lead to different responses to noxious stimuli, we investigated the response of these cells to the two main components of demyelinating diseases: inflammation and hypoxia/ischemia, focusing on survival and differentiation. The differentiation of both fetal and adult OPCs is completely abolished after exposure to inflammatory cytokines, while only fetal-derived OPCs degenerate when exposed to oxygen-glucose deprivation (OGD). Moreover, we found that vulnerability of fetal OPCs to OGD is based on the exclusive glycolytic metabolism of fetal OPC. No contributions of glutamate excitotoxicity was found. These results indicate that fetal and adult NSCs display substantial differences in NRs expression, leading to different capability of OPCs to maturate as myelinating OLs. These differences in early stages and throughout the whole differentiation process, may lead to differences in response to noxious stimuli. The understanding of the underlying molecular mechanism will strongly contribute to differentiate myelination enhancing and neuroprotective therapies for neonatal and adult CNS lesions.

W-3003

TARGETING CSPG SIGNALING IN HUMAN IPSC NEURAL GRAFTS FOR SPINAL CORD INJURY REPAIR

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Human induced pluripotent stem cells (hiPSCs) and their neural derivatives are promising cell sources for transplantation therapies for spinal cord injury (SCI). However, grafting hiPSC derived neural cells into SCI rodent models have not yielded the expected benefits, especially for locomotor functional recovery. Inhibitors, such as chondroitin sulfate proteoglycans (CSPGs), secreted by reactive astrocytes after SCI are major contributors to this low locomotor functional recovery. CSPGs, a group of molecules containing a glycoprotein core with an array of repeating disaccharide side chains, are a part of the glial scars which inhibit axon regeneration. Approaches to reduce CSPGs in an injury environment have the potential to improve functional recovery but have mainly focused on enzymatic administration that will be difficult to implement clinically. In the current work, we present a new strategy which removes CSPG receptors PTPσ and LAR using CRISPR gene editing in iPSCs reprogrammed from patients' urine cells (U-iPSCs). In addition, we have established a pipeline to differentiate and purify neural progenitor cells (NPCs) from single and/or double PTPσ and LAR knockout U-iPSCs. Our data indicates that these NPCs are able to integrate and migrate in the injured spinal cord. Currently, we are testing if PTPσ and LAR knockout NPCs will further improve locomotor function compared to unmodified iPSC-NPCs after being transplanted into acute cervical SCI. Our study emphasizes the role of combining iPSC-NPC stem cell therapy with CSPG signaling biology and aims to enhance neural regeneration and protection. The tools and reagents generated in this study can be conveniently extended to chronic SCI animal models as well as other CNS injury research, which could be more complex and challenging.

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W-3005

FRESHLY-THAWED CRYOBANKED HUMAN NEURAL STEM CELL TRANSPLANTATION IMPROVES COGNITION AND HOST NEURON SURVIVAL IN AN IMMUNODEFICIENT RODENT MODEL OF TRAUMATIC BRAIN INJURY

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Human Neural stem cells (hNSCs) have promising application for cell therapy following traumatic brain injury (TBI). However, while various studies have demonstrated the efficacy of NSCs from on-going culture, there is a significant gap in our understanding of freshly thawed cells from cryobanked stocks – a more clinically-relevant source. To address these shortfalls, we aimed to test the therapeutic potential of our previously validated Shef-6 human embryonic stem cell (hESC) derived hNSC line following long-term cryostorage and thawing immediately prior to transplant. Specifically, immunodeficient athymic nude (ATN) rats received unilateral cortical contusions using a pneumatic controlled cortical impact (CCI) device. At 30 days following injury, 6x105 freshly thawed passage-matched hESC-derived hNSCs were transplanted into 6 injection sites (2 ipsilateral and 4 contralateral to the injury). Rats also received peripheral injection of asialo-GM1 antibody 1 day prior, and 14, 35, 56, and 77 days post transplantation to deplete NK cells and increase engraftment. Cognitive function was assessed via a battery of standardized tasks. Briefly, we observed a decrease in anxious behavior between animals treated with cells versus those with vehicle ($p=0.038$), measured as a 34% reduction in the time spent within open arms using an elevated plus maze. Cell transplantation also resulted in modest effects of improved learning and spatial memory determined by Morris water maze navigation. Through unbiased stereological assessment, approximately 45% of hNSCs survived 3 months post-transplantation, differentiating into neurons (NeuN), astrocytes (GFAP) and oligodendrocytes (Olig2). Importantly, as found in our previous work, there was also a significant increase in host hippocampal neuron survival following hNSC transplantation that may account for cognitive benefits. Taken together, these findings not only validate the potential of hNSCs therapy to restore cognition after chronic TBI, but also demonstrate that long-term bio-banking of cells and thawing aliquots prior to use is a suitable approach for clinical deployment.

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W-3007

ASSESSING THE DEVELOPMENT OF STEM CELL-DERIVED SPINAL SENSORY INTERNEURONS USING CRISPR/CAS9 MEDIATED GENE EDITING

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The spinal cord is responsible for mediating both coordinated movement and our ability to experience the environment (somatosensation). These functions depend on distinct populations of neurons that arise along the dorsal-ventral axis of the spinal cord. We are interested in the six classes of dorsal interneurons (dI1-dI6) that decode somatosensatory information from the periphery including nociception (pain), mechanosensation (touch) and proprioception (body position). Damage to the spinal cord can be devastating, resulting in the loss of both movement and sensation. However, while cellular replacement therapies using embryonic stem cells (ESCs) have been developed for spinal motor neurons, there has been limited progress to restore sensation through in vitro methods. Recently, the Butler laboratory defined the conditions to direct mouse and human ESCs towards four classes of dIs. While these studies demonstrated that both the dI1 proprioceptors and dI3 mechanosensors are dependent on the activity of bone morphogenetic protein 4 (BMP4), the mechanism by which BMP4 directs ESCs towards the dI1/3 fates remains unclear. BMP signaling is canonically mediated by a Smad complex of intracellular messengers, including Smad1, Smad5 and Smad8. We have found that Smad1 expression is specifically upregulated in differentiating dIs, suggesting Smad1 as the candidate Smad that regulates BMP-mediated dI specification. To test this hypothesis, we are using CRISPR/Cas9 methods to delete Smad1 function in ESCs. We have designed guide RNAs (gRNAs) to target exon2 of the Smad1 gene, which codes for the majority of the Smad1 protein. The most effective Smad1 targeting gRNAs have been identified by transfecting the gRNAs into mouse C2C12 cells, due to their high transfection efficiency, and using PCR genotyping to assess whether Smad1 exon2 has been deleted from the genomic DNA. The most efficient gRNAs will now be transfected into ESCs to obtain Smad1 deficient ESC lines. Once these lines have been established, we will use the directed differentiation protocol developed by the Butler laboratory to investigate how deletion of Smad1 affects the differentiation of dI1 and dI3, i.e. the BMP-dependent populations of sensory interneurons.

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W-3009

IMPAIRED CELL CYCLE PROGRESSION AND SELF-RENEWAL OF FETAL NEURAL STEM CELLS IN MOUSE FETUS WITH INTRAUTERINE GROWTH RESTRICTION (IUGR)

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Individuals with IUGR are at significantly increased risk for neurodevelopmental abnormalities. Clinical imaging showed decreased total brain and cortical grey matter volume in IUGR infants, suggesting decreased neurogenesis. Fetal cortical neurogenesis is a time-sensitive process in which fetal NSCs follow a distinct pattern of layer-specific neuron generation to populate the cerebral cortex as the gestation progresses. It has been shown in an in vitro system that profound hypoxia induces cell cycle arrest in NSCs. However, the in vivo effect of antenatal maternal hypoxia in fetal NSCs remains unclear. We used a murine maternal hypoxia-induced IUGR model to study the impact of IUGR on fetal NSC development. In this model, timed-pregnant mice were exposed to hypoxia during the active stage of neurogenesis, followed by fetal brain collection. In the IUGR fetal brains, we found a significant reduction in cerebral cortical thickness accompanied by decreases in layer-specific neurons. Using EdU labeling, we demonstrated that cell cycle progression of fetal NSCs was delayed. Interestingly, we also observed a defect in self-renewal in a subset of NSCs, leading to premature neuronal differentiation. Following relief from maternal hypoxia exposure, the remaining fetal NSCs re-established their neurogenic ability and resumed production of layer-specific neurons. Surprisingly, the newly generated neurons matched their control counterparts in layer-specific marker expression, suggesting preservation of the fetal NSC temporal identity despite IUGR effects. As expected, the number of neurons generated in the IUGR group remained lower compared to that in the control group due to premature depletion of fetal NSCs. Transcriptome analysis identified hundreds of genes affected by maternal hypoxia-induced IUGR. Finally, the IUGR offspring mice exhibited poorer cognitive functions than the control offspring mice. Taken together, maternal hypoxia-induced IUGR is associated with a defect in cell cycle progression and self-renewal of fetal NSCs, and has a long-term impact on offspring cognitive development. Our findings also pointed to a possible scenario where the temporal identity of the fetal NSCs may not be affected by IUGR.

W-3011

MODELING THE IMPACT OF GENETIC BACKGROUND ON NEURODEVELOPMENTAL MUTATIONS USING MOUSE AND HUMAN PLURIPOTENT STEM CELLS

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Genetic background can have a profound impact on the variability and severity of phenotypes in individuals with deleterious mutations in neurodevelopmental genes. We are developing a combined platform that integrates analysis with human pluripotent stem cells (hPSC) and genetically diverse mouse embryonic stem cell (mESC) lines in vitro with whole organism studies in vivo. Our aims are to investigate how genes involved in neurodevelopmental disorders affect brain development and reparative processes in the adult central nervous system, and to discover how genetic background influences phenotype. We found that most mESC lines derived from the Collaboative Cross (CC) founder strains respond poorly to standard differentiation protocols developed using mESC from the 129 background. Therefore, we established a robust neural differentiation protocol that enables efficient production of a wide range of neuronal cell types, astrocytes and oligodendrocytes from mESC lines of all CC founder strains. mESC are differentiated as embryoid bodies for a brief period, then transferred to adherent cultures in the presence of small molecules to direct further lineage specification. Within four days, the cells display robust Pax6 expression, and by day eight neural precursors cells (NPC) comprise almost the entire population. These NPC can be cryporeserved or expanded for further differentiation. In proof of concept studies, we are examining the effects of heterozygous mutations in DYRK1A, a gene associated with a severe autism syndrome, on various stages of neural specification and neuronal maturation in hPSC and mESC. Using CRISPR-Cas9 knockout, we have confirmed our previous studies showing that chemical inhibition of DYRK1A inhibits neural specification of hPSC. High content screening for the effects of Dyrk1a inhibition on the expression of pluripotency factors and early lineage specific markers in our mESC panel revealed clear differential strain-dependent responses in the stabilization of stem cell phenotype. Medium throughput, high content screening in vitro provides for rapid and efficient determination of genetic background effects on gene action at multiple stages of central nervous system development, and identification of candidate modifier loci that can subsequently be further investigated in vivo.

W-3013

ESTABLISHING A NOVEL RAT-HUMAN CHIMERIC MODEL FOR STUDIES OF NEURAL CONVERSION OF HUMAN GLIA IN VIVO

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Direct in vivo reprogramming of resident glial cells in the brain represents a promising therapeutic strategy for brain repair. To date, the majority of pre-clinical studies to explore this strategy have been performed using rodent glia, and the question whether human endogenous glia have the same capacity to convert in vivo is unclear. In vitro studies have demonstrated the ability to convert different types of human glia into neurons, but it remains an outstanding question if human glia can be converted into neurons within the adult brain. In this project, we aim to establish a novel rat-human glial chimeric animal model whereby human glia are transplanted into the rat brain, yielding a unique model that allows us to study in vivo conversion of human glia into clinically relevant neurons in the rodent brain. To do this, we have generated human glial progenitor cells (hGPCs) from human embryonic stem cells (hESCs). hGPCs can be transplanted and survive in the rodent brain, both when delivered to adult animals maintained under daily immunosuppression and delivered to nude athymic animals. We found that when these cells are transplanted to intact or dopamine-depleted rats at specific target sites, they proliferate and migrate throughout the host brain. The grafts were first analysed with STEM121 and HuNu to detect human cells which confirmed graft survival and migration in both gray and white matter over time. Further analysis showed that the hESC-derived glia express markers of astrocytes and oligodendrocyte progenitors, but no neuronal markers. Thus, this model is suitable to study the core cellular and molecular events controlling subtype-specific identity during in vivo conversion of glia into neurons. The results will enhance our understanding of how cell differentiation and reprogramming operates during in vivo neural conversion and define the key factors that control this process. When comparing lesioned and intact animals we found that cell survival, migration and phenotype is not affected by lesioning the host dopaminergic system, allowing for future studies aimed at conversion of human glia to neurons in a model of Parkinson's disease.

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W-3015

DYNAMICS OF STRIATAL NEUROGENESIS IN RESPONSE TO ISCHEMIC STROKE

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Stroke is the second most common cause of death and the third leading cause of disability globally. After stroke, a limited spontaneous recovery occurs, however the mechanisms are not fully understood and despite large efforts to study various therapy approaches, there is no effective treatment to date. Since the discovery that the brain continuously adds new neurons in discrete areas of the adult brain, a process called adult neurogenesis, stimulation of endogenous neural stem cell to generate new neurons has appeared to be a promising strategy for brain regeneration. Adult neurogenesis in humans remains a controversial topic, but cumulative evidence suggest that humans have a unique distribution of adult neurogenesis. For example, there is substantial neurogenesis in the healthy human striatum, an area that is not a neurogenic niche in healthy conditions in other mammals. However, in rodent models, neurogenesis can be found in the striatum as a response to stroke. Whether humans also have this regenerative response is not known. Measuring the integration of 14C derived from nuclear bomb tests during the Cold War in neuronal DNA allows us to measure the average age of the neuronal population. We provide data concerning the dynamics of neuronal generation in the striatum following stroke by assessing the age of neurons in the striatum of individuals who had suffered from a unilateral striatal stroke, using a retrospective birth-dating method. We compare the average age of neurons in both striatal hemispheres and compare these results with striatal neurons isolated from donors that died from unrelated non-neurological causes. Characterizing the neuronal turnover dynamics in the human striatum before and after stroke will help us understand the capacity of self-repair of the human brain, as well as translate the results from animal studies into the clinical situation and thus help guide the future development of therapies for brain regeneration.

NEURAL DISEASE AND DEGENERATION

W-3017

A MODEL OF TRAUMATIC BRAIN INJURY USING HUMAN IPSC-DERIVED CORTICAL BRAIN ORGANOIDS

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Traumatic brain injury (TBI) represents a major and progressively increasing burden on global healthcare, resulting in temporary to permanent neurocognitive and psychosocial impairment. It is estimated that 1.7 million people in the US are diagnosed annually with some form of brain injury caused by insults such as sudden acceleration/deceleration, impacts, penetration and blast shockwaves. Following this initial mechanical disruption of brain tissue, long-term injury propagation via secondary mechanisms include the spreading of necrotic tissue, perturbations in calcium and glutamate homeostasis and inflammation. Modelling the pathogenesis of TBI has been largely limited to rodent models that recapitulate some, but not all, aspects of human brain injury. As a result, in vitro models are likely an essential complement to these experimental procedures providing a more scalable and genetically-relevant system. Here, we generated human cortical organoids from induced pluripotent stem cells (iPSC) by dual SMAD inhibition and subjected organoids (aged 100-200 days) to high intensity focused ultrasound (HIFU) in order to mimic the effects of shockwaves on the brain. Following injury at pressures of 0.4 MPa and 0.6 MPa, cortical organoids exhibited features similar to TBI in humans. The ratio of phosphorylated tau (T231) to total tau was increased in organoids that experienced HIFU two days prior. Histological analyses showed enhanced phosphorylated TDP-43 intensity accompanied by a significant increase in neuronal cytoplasmic aggregates compared to controls 4 days after injury. Additionally, increased neurogenesis has been reported in rodent models following traumatic brain injury. Here, we observed a significant increase in neuronal coverage, accompanied by increased staining for synapsin I. These findings suggest that acutely following HIFU, cortical organoids exhibit hallmarks of TBI observed in humans, including increases in phosphorylated tau and TDP43. The increased proportion of neurons and synapsin I suggest perturbations in synaptic homeostasis that may attribute to the deficits in brain function observed in cases of TBI. In aggregate, we present here a novel in vitro model of TBI that recapitulates aspects of the human pathology and can be utilized to discover new therapeutics.

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W-3019

EARLY STEM CELL AGING IN THE MATURE BRAIN

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Stem cells are responsible for tissue generation throughout life. In order to do so, they must balance the production of newborn cells with their own maintenance. However, this homeostasis is progressively lost during aging and drives age-related disease. Neural stem cells (NSCs) continually generate new born neurons and astrocytes (neurogenesis) to augment learning and memory function within the hippocampus. Despite this, neurogenesis and hippocampus function are markedly lower in older animals. We developed in vivo single cell linage tracing, computational modeling approaches, single cell RNA-sequencing and systems level data science to comprehensively investigate NSC aging in the adult mouse hippocampus. We identify NSC loss, slowing kinetics and cell fate choice switches as reasons why neurogenesis declines during aging. Strikingly, we elucidate that these changes occur in mature adulthood, between 3 and 6 months of age in mouse. Further, NSC display an aging stem cell transcriptomic signature. Comparative analysis of NSC with old epidermal, hematopoietic, and muscle stem cells reveals a common metabolic decline and alteration in Protein and RNA homeostasis with age. Our study elucidates cellular and molecular origins of neurogenesis decline and may serve as a new mammalian stem cell model to study early-onset cellular aging.

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W-3021

DEVELOPMENT OF A HUMAN NEURONAL CELL MODEL OF BETA-PROPELLER PROTEIN-ASSOCIATED NEURODEGENERATION (BPAN) AS A DRUG SCREENING PLATFORM

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BPAN is an X-linked subtype of neurodegeneration with brain iron accumulation (NBIA) due to mutations in WDR45, encoding a beta-propeller protein with a postulated role in autophagy. Affected patients present with infantile neurodevelopmental delay and epilepsy, developing parkinsonism-dystonia usually in the 2nd or 3rd decade of life. The mechanisms linking autophagy, iron overload and neurodegeneration are poorly understood. In order to address these issues, we have developed a patient-derived, induced pluripotent stem cell (iPSc) midbrain dopaminergic (mDA) neuronal model of BPAN; we have used this to further elucidate disease mechanisms and develop novel therapies for this pharmacoresistant disorder. Three patient-derived iPSc lines, two age-matched controls and two CRISPR-corrected isogenic controls have been differentiated into mDA using a dual SMAD inhibition protocol. When plated on multiwell plates at low density, patient-derived ventral midbrain progenitors at Day 11 of differentiation exhibit fewer LC3 puncta per cell when compared to controls, both in basal conditions and after inhibiting or inducing autophagy. Using this high content imaging assay, we have performed a drug screen using both the FDA- approved Prestwick library (1,280 compounds) and a series of novel autophagy activators. A number of compounds significantly enhance LC3 puncta production in all tested lines, and further validation is underway. In summary, our work in developing a mDA model of BPAN has provided an innovative platform for high content imaging-based drug screening with the aim of identifying new treatments for this medically resistant condition.

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W-3023

HUMAN INDUCED NEURONAL CELLS AS A PATHOLOGY MODEL FOR APP SWEDISH MUTATION IN ALZHEIMERS DISEASE

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Alzheimer's disease (AD) is the most common dementia affecting half of Americans over 85. To better understand the mechanisms underlining AD, here we generated human wild-type and APP Swedish mutant (K670N;M671L) neuronal cells from human iPSCs following the protocol of neuronal induction with Ngn2 overexpression. Both wild-type and mutant neuronal cells are derived from the same genome edited-iPSC lines with a conditional APP Swedish mutant allele to avoid variation resulting from different genetic backgrounds and iPSC generation processes. Because of the previous reports showing that AD pathophysiology is triggered by synapse loss and mitochondrial dysfunction. we have been focusing on synaptic and mitochondrial abnormalities. By comparing to wild-type cells, we have observed some interesting phenotypes in APP Swedish mutant induced neuronal cells, including increased synapse formation and reduced mitochondrial migration. These phenotypes could reveal the pathogenesis at early stages of AD and potentially inspire therapy development and drug discovery for AD.

W-3025

PROFILING SEIZUREGENIC LIABILITY COMPOUNDS USING HIGH THROUGHPUT 3-DIMENSIONAL HUMAN IPSC-DERIVED NEURONAL CULTURES

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Spheroid-based cellular platforms are considered to enable more complex, biologically relevant, and predictive assays for compound screening, safety evaluation and toxicity studies. Thus, here we deployed a high throughput spheroid co-culture of cortical glutamatergic and GABA-ergic neurons as well as astrocytes, more closely resembling the tissue constitution of native human brain tissue. High content imaging indicated that this platform shows robust well-to-well size homogeneity, as well as the neural networks established in this model express typical neuronal and astrocytic identity and functional markers, which altogether enable a highly functional neuronal circuitry. High throughput kinetic fluorescence imaging of Calcium-sensitive dyes indicated robust spontaneous, synchronized, readily detectable calcium oscillations, with reproducible baseline activity patterns across wells and and inter- plates. In order to validate the capabilities of the platform in toxicology and safety pharmacology, a set of 12 compounds including drugs known to cause seizures in animal models through independent mechanisms was evaluated. The compounds consisted of Pentylenetetrazole (PTZ), Picrotoxin, Strychnine, Pilocarpine, Cholopromazine, Amoxapine, Enoxacin, Phenytoin, Linopirdine, 4- aminopyridine, Amoxicillin and Acetominophen. In order of potency, 4-aminopyridine, Strychnine, Linopirdine, Cholopromazine, Phenytoin, and Pilocarpine induced drastic changes in calcium oscillation patterns in the natural network bursting observed in this model. 4-aminopyridine was the only compound that increase the frequency in calcium oscillation patterns whereas the remaining active compounds all reduced the frequence calcium oscillation. The negative controls (Amoxicillin and Acetominophen) had no significant effect when compared to DMSO/vehicle. In conclusion, high throughput functional assays using the human iPSC-derived 3D neuronal spheroids platform deployed in this study provided robust data for assessment of functional neurotoxicity and seizure liabilities.

W-3027

SCREENING FOR POTENTIAL THERAPEUTICS AGAINST NEURODEVELOPMENTAL DISORDERS USING A 3-DIMENSIONAL HUMAN CORTICAL NEURAL PLATFORM

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The human Central Nervous System (CNS) has a unique structural organization that is critical to its complex functions. Efforts to model this intricate network in vitro have encountered major bottlenecks. Recently, much work has been focused on obtaining 3D brain organoids in an attempt to better recapitulate

the brain development and function in vitro. Although self-organized 3D organoids can potentially more closely recapitulate key features of the human CNS, current protocols still need major improvements before being implemented in a drug discovery scenario. We have recently described a highly homogenous off-the-shelf human induced Pluripotent Stem Cells (hiPSCs)-derived cortical spheroid screening platform in 384 well format, composed of cortical neurons and astrocytes. Using high throughput calcium flux analysis, we showed the presence of quantifiable, robust and uniform spontaneous calcium oscillations, which is correlated with synchronous neuronal activity in the spheroid. Our platform is optimized to have a highly homogenous and consistent functional signal across wells, plates, and batches. Finally, we demonstrated the feasibility of using this platform to interrogate large libraries of compounds on their ability to modulate the human CNS activity. Here, we describe the use of this platform to investigate neurodevelopmental disorders. When introducing hiPSCs derived from Rett Syndrome (RTT) patients into our platform, a clear functional disease phenotype was observed. RTT 3D neural cultures displayed calcium signal that indicates a compromised neural network with slow, large, synchronized frequency of oscillations. We also performed a pilot screen using a library of 296 selected compounds for their ability to alleviate the observed RTT phenotypes in vitro, and identified some potential targets. In summary, we demonstrated the feasibility of incorporating a neurodevelopmental disorder in a high-throughput screening platform. The system presented here has the potential to dramatically change the current drug discovery paradigm for neurodevelopmental disorders and other neural diseases.

W-3029

A SELF-ASSEMBLING PEPTIDE BIOMATERIAL TO ENHANCE HUMAN STEM CELL-BASED REGENERATION OF THE INJURED SPINAL CORD

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Human induced pluripotent stem cell-derived neural stem cells (hiPS-NSCs) are a promising therapeutic approach to regenerate the traumatically injured spinal cord (SCI). Unfortunately, the harsh post-injury microenvironment is a significant barrier to regeneration. QL6 (K2(QL)6K2; Medtronic Inc.) is a novel, biodegradable, peptide biomaterial which self-assembles into an extracellular matrix-like lattice in vivo. After acute SCI it has been shown to reduce inflammation and astrogliotic scarring resulting in significant neurobehavioral improvement. QL6 also reduces tissue loss and supports the survival of allogeneic mouse NSCs when co-delivered in SCI. However, its ability to support translationally-relevant hiPS-NSCs in the more common chronic SCI niche has not yet been determined. hiPS-NSCs were cultured on QL6 versus a Geltrex control. The mechanism of adhesion was assessed by EDTA assay and qPCR. hiPS-NSC survival, proliferation (Ki-67 ICC), and neurosphere formation were extensively characterized in vitro. T-cell deficient RNU rats (N=70) capable of supporting a human graft were given a translationally-relevant C6-7 clip-contusion injury or sham surgery (laminectomy alone). In the chronic injury phase, animals were randomized to receive: (1) vehicle, (2) hiPS-NSCs transplant, (3) QL6 transplant, (4) QL6 + hiPS-NSCs co-transplant. All rats receive delayed daily (5d/wk) treadmill rehabilitation. hiPS-NSCs proliferated robustly on self-assembled QL6 versus a geltrex control as demonstrated by Ki67+/DAPI+ ICC (29% vs 6%, $p<0.01$). EDTA adhesion assay demonstrated that human NSC binding to QL6 is largely driven by calcium-independent mechanisms. qPCR of hiPS-NSCs on QL6 showed downregulation of apoptosis markers after 3 days, upregulation of early pro-neuronal differentiation markers, and upregulation of select calcium-independent cell adhesion molecules. Importantly for NSCs, QL6 enhanced the formation of adherent neurospheres, the native conformation of NSCs. Blinded sensorimotor and transcriptomic assessments of transplanted rats are ongoing with a 22-week post-injury endpoint. This work provides key proof-of-concept data that QL6 self-assembling peptide can support translationally-relevant human iPS-NSCs for use in traumatic SCI.

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W-3031

PROBING THE MOLECULAR ETIOLOGY TO LINK MUTATIONS TO NEURONAL DYSFUNCTION IN INTELLECTUAL DISABILITY SYNDROMES

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We used Rett patient derived human induced pluripotent stem cells (hiPSCs) to make cortical interneurons to model Rett syndrome in vitro. We discovered that mutant interneurons exhibit increased expression of p53 target genes, indicated the state of stress the cells are experiencing. These findings agreed with the analyses of human Rett patient brain samples, which also exhibit induction of p53 target genes. This implies that we are able to recapitulate Rett syndrome phenotype in vitro. Additionally, neurons lacking MeCP2 undergo premature senesce accompanied by elevated levels of H2AX and PML expression. Analyzing neuronal phenotype revealed significant decrease in complexity of dendritic arborization. This lead to our hypothesis that it is possible to alleviate the stress the cells experience by blocking p53 pathway and, thus, promote healthy

interneuron development and rescue dendritic branching. We used pifithrin, a potent p53 inhibitor, to test our hypothesis. Treating the cells with pifithrin did indeed restore dendritic complexity of mutant neurons compared to the wild type. Our future work will focus on modeling ICF, ATRX and Angelman syndromes in addition to Rett, and investigating whether there are common pathways involved in the development of each disorder.

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W-3033

NUCLEOCYTOPLASMIC PROTEOMIC ANALYSIS HIGHLIGHTS ETF1 AND NONSENSE MEDIATED DECAY AS THERAPEUTIC TARGETS IN C9ORF72-RELATED ALS/FTD

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The transport of mRNA and proteins between the nucleus and the cytoplasm has emerged as a critical cellular pathway in neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS) and frontotemporal dementia (FTD). Here, we developed an approach to assess how the repeat expansion in C9orf72, which is the largest genetic contributor to ALS/FTD, impacts the nucleo-cytoplasmic distribution of proteins. We specifically utilized biochemical subcellular fractionation coupled with tandem mass spectrometry and identified 126 proteins, enriched for protein translation and RNA metabolism pathways, which collectively drive a shift towards a more cytosolic proteome in mutant C9orf72-expressing cells. Importantly, while other repeat expansion mutations associated with neurodegeneration also exhibited a similar cytosolic shift, the proteins affected were disease-specific. To address the functional relevance of these alterations we focused on the nuclear accumulation of ETF1, a protein that regulates the termination of protein translation and the degradation of aberrant mRNA transcripts by the nonsense mediated decay (NMD) pathway. We found that ETF1 becomes enriched within an elaborate network of nuclear envelope invaginations in C9orf72 iPSC-patient neurons and postmortem patient tissue. The redistribution of ETF1 mediated a protective shift from protein translation to NMD-dependent mRNA degradation. While the full spectrum of mRNAs degraded by NMD in patient neurons remains unclear, we found that NMD targets the expanded C9orf72 transcript to prevent its cytosolic accumulation and translation into toxic dipeptide proteins. Lastly UPF1, which is a master regulator of NMD, was a significant modulator of C9orf72-induced toxicity in vivo, suppressing it when overexpressed, and enhancing it when its levels were reduced. Our findings provide a resource for proteome-wide nucleocytoplasmic alterations across neurodegeneration-associated repeat expansion mutations and highlight ETF1 and NMD as therapeutic targets in C9orf72-associated ALS/FTD.

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W-3035

FUNCTIONAL CHARACTERIZATION OF PI3K-AKT-MTOR RELATED MEGALENCEPHALY USING PATIENT-DERIVED IPSCS AND CEREBRAL ORGANOIDS REVEAL DISTINCT MOLECULAR PATHOMECHANISMS

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Megalencephaly (MEG) and focal cortical dysplasia (FCD) are neurodevelopmental disorders characterized by variable brain overgrowth and cortical abnormalities. This spectrum is associated with significant pediatric morbidity and mortality including epilepsy, autism and intellectual disability. Importantly, FCD is the most common cause of pediatric focal epilepsy. Mutations in the PI3K-AKT-MTOR pathway have been identified to cause MEG and FCD, however the molecular cascade underlying brain overgrowth, dysplasia, and epileptogenesis are not well defined. We aimed to model disease pathogenesis in vitro, selecting patient-derived fibroblast lines with mutations in key upstream (PIK3CA) and downstream (MTOR) nodes in the pathway. We generated induced pluripotent stem cells (iPSCs) carrying the common PIK3CA(H1047R) and MTOR(T1977I) mutations to characterize these nodes. We performed functional assays including population doubling time, senescence, analysis of proliferation and apoptosis; and differentiated them to Neuronal Progenitors (NPCs), cortical neurons, and cerebral organoids. Our preliminary results show both overlapping and exclusive cellular phenotypes in PIK3CA and MTOR mutant cell lines. Specifically, MTOR mutant organoids had an average area at least two times larger than controls, recapitulating MEG, with abnormal organoid morphology suggesting neuronal migration

defects. PIK3CA mutants showed cellular hypertrophy at both iPSC and NPCs levels, a feature not present in MTOR mutants. Both mutant lines also showed increased iPSC proliferation, but only PIK3CA mutants displayed increased NPCs proliferation, while MTOR mutant NPCs had signs of premature neuronal maturation. Together, our results suggest that distinct mechanisms underlie PIK3CA- and MTOR- related MEG and FCD occurring at different windows during human embryonic development. Future directions include performing these experiments on patient-derived cell lines with mutations in AKT3, a central node in the pathway. We will also study the effects of select MTOR pathway inhibitors on these cell lines including Rapamycin. This work will help characterize the mechanisms of MEG and FCD caused by mutations in MTOR pathway and guide the design of future clinical trials using pathway inhibitors.

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W-3037

A PHENOTYPIC SCREEN USING PATIENT-DERIVED MOTOR NEURONS IDENTIFIES A STEROID AS BROADLY EFFICACIOUS IN C9ORF72 FAMILIAL AND SPORADIC FORMS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease resulting in the degeneration of motor neurons. Despite intense research efforts, only two drugs have been approved by the U.S. Food and Drug Administration (FDA) for treating ALS. It is conceivable that the low success rate of translating pre-clinical discoveries into clinically effective treatments may be due to heterogeneous patient response to a drug treatment. To test this notion, we employed a precision medicine based approach utilizing induced pluripotent stem cells to generate motor neurons from ALS patients. The induced motor neurons (iMNs) recapitulate ALS disease processes. Using these disease-relevant iMNs, we performed an unbiased phenotypic screen of 2,000 FDA approved compounds to identify small molecules that rescue C9ORF72 ALS iMN survival. Of the 74 primary hits, 68% of the compounds were associated with anti-bacterial, anti-hypertensive, anti-inflammatory, anti-neoplastic, antioxidant, and endoparasitic functions. To examine the efficacy of these compounds on expanded patient populations, we generated iMNs from three C9ORF72 patients, eight sporadic patients, and three healthy controls. Most validated hits from the C9ORF72 screen could only rescue iMN survival in a minority of sporadic ALS lines. However, one class of steroids potently extended iMN survival in the C9ORF72 lines and the majority of sporadic lines. Moreover, the steroid's protective effect was abrogated by knocking down the expression of its targeted receptor with antisense oligonucleotides (ASOs). In addition, bioinformatic analysis of existing RNA profiling data on FDA approved drugs and genetic knockdowns identified a multitude of genes whose suppression induces similar gene expression changes as steroid treatment. Genetic knockdown of these candidates also rescued ALS iMN survival, providing new genetic targets that can be modulated by ASOs. These results indicate that motor neurons from different ALS patients have highly varied responses to pharmacologic intervention, suggesting this may be a major cause of clinical trial failure in ALS. Our screening approach using motor neurons derived from a diverse cohort of ALS patients identified rare targets whose perturbation is broadly efficacious across motor neurons from many ALS patients.

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ORGANOIDS

W-3041

GENERATION OF TONSIL EPITHELIAL ORGANOIDS FROM HUMAN PALATINE AND ADENOID TONSILS IN A CHEMICALLY DEFINED MEDIUM

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Tonsils, a collection of the mucosa associated lymphoid tissues, are the gateway of respiratory and digestive tract as the first line of defense against ingested or inhaled pathogens such as bacteria and viruses. Tonsils are vulnerable to infections, which can lead to tonsillitis, tonsil hypertrophy and oropharyngeal cancer. There is no suitable in vitro model to recapitulate human tonsils, and experimental animal model for tonsils is not available, making it difficult to study the tonsil and its pathophysiology. Tractable

methods to identify and interrogate pathways involved in tonsil related disorders are urgently needed. We developed defined culture protocols of palatine and nasopharyngeal tonsil-derived epithelial organoids that preserve essential features of the tonsil epithelium, such as its cellular composition and microscopic structures. Tonsil organoids can be rapidly generated from resected biopsies, expanded over several months, and exhibit histologic characteristics of stratified squamous epithelium. Moreover, a substantial proportion of EpCAM-positive cells and subsequent culturing efficiently generate tonsil organoids containing tonsil epithelium-like structures expressing markers of all-lineage cells in an organized, continuous arrangement and pathogenic responses for that resembles the tonsil in vivo. Furthermore, lentiviral transduction of human papillomavirus 16-encoded E6/E7 induced precancerous changes such as aberrant differentiation and hyperplastic proliferation. Also, supernatant post-LPS challenge increases neutrophil chemotactic activity and to elucidate the role of CXCL8-CXCR1/CXCR2 pathways in this process. We developed an organoid technology established from human tonsils, and so offer the valuable complements to analyze human specific tonsil disorders using tissues from HPV infected or cancer patients and to test potential therapeutic compounds.

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W-3043

EXPOSURE TO CIGARETTE SMOKE ENHANCES THE STEMNESS OF ALVEOLAR TYPE 2 CELLS

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Emphysema is characterized by the irreversible destruction of alveolar structures accompanied by an increase in apoptotic cells. However, the mechanism by which alveolar type 2 (AT2) cells fail to repair is not clear. A variety of site-specific stem cells are known to play a key role in repairing and maintaining lung tissues. We hypothesized that AT2 stem cells are functionally disturbed in emphysematous lungs. We exposed 8-12 week old mice expressing green fluorescent protein (GFP) in cells expressing surfactant protein (Sftpc)(CBA/Ca x C57BL6J) mice to mainstream cigarette smoke (CS) through the nose, 5 days/week for 3 months. We then examined them for changes in their lung histology and their ex-vivo stem cell function and compared them to age-matched air-exposed mice. Histological assessment showed that the CS-induced emphysematous lungs had an increased number of AT2 cells in comparison to the air-exposed mouse lungs. When the lung AT2 cells were examined in the stem cell 3D organoid/colony forming assay, we found that the number and size of GFP+ colonies formed by the CS-exposed AT2 cells was significantly higher than that of the air-exposed control AT2 cells. When examined in the 2D in vitro culture, we found that the disappearance of GFP fluorescence after seeding, was delayed in the CS-exposed group at all time points examined (0-48 h) in comparison to the air-exposed group suggesting a trend towards apoptosis resistance. These results indicate that chronic CS exposure causes an increase in the number of AT2 cells in the alveolar area and that the increase in AT2 number is a result of activation of their stem cell function, with CS-exposed AT2 cells surviving apoptosis longer than their air-exposed control cells. In this study, we demonstrate for the first time that the “stemness” of AT2 cells was enhanced by exposure to chronic cigarette smoke, in the course of emphysema development.

W-3045

IDENTIFICATION AND CHARACTERIZATION OF MATURE DOPAMINERGIC NEURON SUBTYPES AT SINGLE-CELL RESOLUTION IN HUMAN VENTRAL MIDBRAIN-PATTERNED ORGANOIDS

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Parkinson’s disease (PD), one of the most common neurodegenerative disorders, is characterized by progressive loss of dopamine (DA) neurons in midbrain. Although the relatively focal degeneration in PD makes it a good candidate for cell-based therapies, the inaccessibility of functional human brain tissue and the inability of two-dimensional in vitro cultures to recapitulate the complexity and function of dopaminergic circuitries have made the study of human midbrain functions

and dysfunctions challenging. Despite intensive research efforts in recent years, the molecular mechanisms controlling the developmental program and differentiation of DA neuron subtypes remain largely unknown. In this study, we designed a method for differentiating human pluripotent stem cells into three-dimensional (3D) dopaminergic organoids, which mimic features of human ventral midbrain (VM) development by recreating authentic and functional DA neurons. Immunolabelling-enabled 3D imaging of solvent-cleared organs (iDISCO) of whole organoids provides an anatomical perspective useful for reconstructing regional identities, spatial organization and connectivity maps. By combing CRISPR-Cas9 gene editing – used for generation of tyrosine hydroxylase (TH)-Cre knock-in reporter cell line – with unbiased transcriptional profiling at single-cell resolution, we showed that TH+ neurons exhibit molecular and electrophysiological properties of mature DA neurons expressing functional receptors of A9 and A10 neurons as well as their ability to release dopamine, commonly affected in PD. Importantly, we also conducted a direct comparison with fetal VM organoids, which may serve as a valuable reference for creating the optimal conditions to differentiate pluripotent stem cells into human midbrain organoids, underscoring developmental similarities and differences.

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W-3047

MULTIMODAL AND VOLUMETRIC IMAGING REVEALS SPATIAL ORGANIZATION OF CELLULAR PHENOTYPES AND NEPHRONS IN KIDNEY ORGANOIDS DERIVED FROM HUMAN IPSCS

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Organoids are gaining significant interest in the field of regenerative medicine. Their ability to self-organize from pluripotent stem cells into functional organ-like structures makes them candidates for organ replacement or repair. We aim to produce functional kidney organoids to build an implantable kidney graft to reduce or replace dialysis for patients with end-stage kidney disease. Engineering kidneys is especially challenging due to their complex anatomy comprising a variety of cell types, but kidney organoids have been shown to self-organize into the important structures found in the kidney. We differentiate human induced pluripotent stem cells (iPSCs) into two progenitor populations: the ureteric bud and the metanephric mesenchyme. After 7 days of differentiation and a further 18 days of maturation, these organoids resemble human kidneys in the first trimester of embryonic development. They are several millimeters in size and comprise small nephron-like structures, with tubular segments and an immature endothelium. The field would benefit from the ability to generate high quality spatial information on kidney organoids as this has been a significant challenge with organoids of these dimensions and complexity. To solve this, we developed a robust imaging pipeline for volumetric light and electron microscopy in order to assess cellular composition, organization, functional ultrastructure, and connectivity of tubular structures. Various techniques, such as clearing and correlation of light and electron microscopy, were applied for this purpose. Mapping organoids over time enables us to assess and manipulate their self-organization, maturation and functionality. Ultimately, we aim to develop fully functional and mature organoids that will be suitable for regenerative medicine.

W-3049

JUXTACRINE SIGNALS FOR IN-VITRO PRODUCTION OF RETINAL ORGANOIDS FROM HUMAN EMBRYONIC STEM CELLS

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Blindness affects millions throughout the world. Surgical techniques have been developed that can re-introduce new cells into the deteriorating host retina. The cells can be in the form of a single cell suspension or better as assembled tissue, demonstrated by numerous in-vitro and in-vivo experiments on animals and in-human clinical trials. Besides quantitative limitations, sourcing the transplantable tissue from aborted fetuses is an important ethical concern, thus producing retina from established human pluripotent stem cell lines represents a fesible alternative for transplantation. The manufacturing of retinal organoids (ROs) from human pluripotent stem can produce photoreceptors and other retinal cell types including ganglion cells, Müller glia, rod bipolar cells, amacrine, and horizontal cells. The main challenges for the in-vitro derived RO remain 1) the limited size that is typically much smaller than the natural organ and 2) the limited lifespan that is reflected in advanced maturation deficiency. One of the causes of the observed deficiencies, besides imperfect cell culture, could be the lack of paracrine and juxtacrine signal, provided in-vivo

by adjacent tissue. Based on the observations that one of the conditions for retina organoids to develop is the presence of a mesenchymal stroma surrounding the developing eye fields and furthermore that in the absence of this stroma complete neuralization is observed resulting a pure culture of neuronal cells, we developed and tested a proprietary method that uses a cyclodextrin-based molecular trap to capture non-soluble, membrane bound ligands from the families of Wnt and Hedgehog (HH) that are produced by surrounding cells. We characterized and tested juxtacrine signals captured from partial differentiated pluripotent stem cells, mesenchymal cells and retinal pigmented epithelia (RPE) cultures on retina organoids differentiated from embryonic stem cells. Here we present the effect of the non-soluble membrane bound ligands produced by different type of cells on the size, structure and survival of the retina organoids. The encouraging results suggest that the methods can be applied to other type of organoids produced in-vitro, such as brain, kidney, liver pancreas with promising potential for future tissue engineering for regenerative medicine.

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W-3051

NOVEL CANINE ENTEROID MODEL FOR GENOME EDITING OF MULTI-DRUG RESISTANCE PROTEINS AND DOSE-EXPOSURE-RESPONSE OF CHEMOTHERAPEUTIC DRUGS

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The multi-drug resistance protein (ABCB1, MDR1) gene codes for P-glycoprotein (P-gp), an important drug efflux transporter which is involved in resistance to chemotherapeutic drugs, including doxorubicin (DOX). Inhibition of P-gp can lead to dangerous adverse effects and harmful drug-drug interactions, making characterization of P-gp-mediated drug transport and dose-exposure-GI toxicity response to candidate drugs critical during the preclinical evaluation phase. Dogs are routinely used for studying naturally occurring diseases, including GI disorders and colorectal cancer (CRC) and for pharmaceutical drug development. Our laboratory has recently developed an ex vivo 3D canine GI organoid (enteroid/colonoid, ENT/COL) system, which accurately models the physiological and molecular features of intestinal tissue in vivo. P-gp function in canine ENT was analyzed by 30 minute incubation with 10 µM rhodamine123 (Rh123), a fluorescent dye substrate for P-gp, and/or 20 µM verapamil, a P-gp inhibitor, and quantitated by fluorescent microscopy and ImageJ. CRISPR/Cas9 knockout of MDR1 in canine enteroids was achieved by Lipofectamine transfection and efficiency monitored by green fluorescent protein (GFP) expression. DOX concentration in canine 3D ENT in matrigel after 2 day exposure was determined by liquid chromatography mass spectrometry (LC-MS) and compared to a 6-point standard curve (50 to ~14,000 pg range). DOX cytotoxicity on canine ENT and two CRC cell-lines, HCT-116 and HT-29, was examined after 48 hours by MTT assay. In the P-gp function assay, the lumen of ENT incubated with Rh123 showed green fluorescence, but co-incubation with verapamil reduced Rh123 fluorescence by 50% ($P = 0.05$), indicating inhibition of P-gp-mediated transport. In addition, canine 3D ENT were stably transfected with control GFP or CRISPR/Cas9 plasmid to knockout MDR1. Uptake of DOX by canine ENT was about 10% or 34 ng/12 wells ENT after 100 nM DOX incubation for 2 days. We observed a mild, dose-dependent cytotoxicity of 15-25% by 100-1000 nM DOX ($P < 0.05$) in healthy ENT, but cytotoxicity up to 60% in two CRC cell-lines by 1000 nM DOX ($P < 0.05$). In summary, the analysis of DOX actions and P-gp function/expression shows that canine ENT/COL are a valuable tool for preclinical testing of drug transport, efficacy and toxicity.

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W-3053

MICROENGINEERING A 3D HUMAN IPSC-DERIVED "NERVE-ON-A-CHIP"

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The development of human iPSC-derived neurons has vastly expanded the predictive potential of 2D preclinical assays, as iPSC-derived neurons are more obtainable than neural stem cells for tissue engineering and screening applications. Concurrently, engineered microphysiological systems (MPSs), Organs-on-Chips and 3D organoids have seen enormous growth as disease models and drug screening tools because

they are more biomimetic than 2D assays. When modeling a complex tissue such as the human nervous system, 3D engineered cultures provide clear advantages by recapitulating cell-cell interactions. However, limited focus has been given to MPSs that mimic peripheral nerves (PNs) even though peripheral neuropathy is implicated in many disease states and is a significant side effect of many therapies. In this study, we fabricated a novel in vitro human iPSC-based 3D nerve that supports axon growth analogous to PN anatomy. This in vitro nerve can provide clinically relevant read-outs such as nerve conduction velocity (NCV) and histological ultrastructure. These metrics serve as the gold standard in neuropathy evaluation and were previously measurable only through in vivo studies. Using low adhesion microplates, self-assembling spheroids consisting of either iPSC-derived human neurons or co-cultures of iPSC-derived human neurons and human primary Schwann cells were fabricated. Over 4 weeks, the nerves were grown in a 3D environment to remarkable lengths of 5 mm in a growth-directing dual-hydrogel scaffold. Self-assembly of neurons was optimized by the addition of Schwann cells; co-culture spheroids self-assembled 7 days faster than iPSC-derived neurons alone. Population-level electrophysiological activity was seen in both cultures, with neuron-only spheroids producing a faster peak nerve conduction velocity (NCV) of 0.18±0.04 m/s while co-culture spheroids had a peak NCV of 0.13±0.02 m/s. Schwann Cell migration, ensheathing, and myelination was seen in co-culture spheroids as shown by S-100 staining, and lamination on TEM micrographs. A clinically analogous G-ratio of 0.57 was found and myelinated and unmyelinated axon diameters were measured to be 0.55±0.33 µm and 0.40±0.15 µm, respectively. This novel in vitro nerve is desirable for neurotoxicity, neurodevelopment and disease modeling purposes.

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W-3055

SELF-ORGANIZED SYNCHRONOUS CALCIUM TRANSIENTS IN A CULTURED HUMAN NEURAL NETWORK DERIVED FROM CEREBRAL ORGANOIDS

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The cerebrum is a major center for brain function, and its activity is derived from the assembly of activated cells in neural networks. It is currently difficult to study complex human cerebral neuronal network activity. Here, using cerebral organoids, we report self-organized and complex human neural network activities that include synchronized and non-synchronized patterns. Self-organized neuronal network formation was observed following a dissociation culture of human embryonic stem cell-derived cerebral organoids. The spontaneous individual and synchronized activity of the network was measured via calcium imaging, and subsequent novel analysis enabled the examination of detailed cell activity patterns, providing simultaneous raster plots, cluster analyses, and cell distribution data. Finally, we demonstrated the feasibility of our system to assess drug-inducible dynamic changes of the network activity. The comprehensive functional analysis of human neuronal networks using this system may offer a novel way to access human brain function.

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W-3057

CHARACTERIZATION OF PANCREATIC COLONY-FORMING PROGENITOR-LIKE CELLS ISOLATED FROM HUMAN CADAVERIC DONORS

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Stem and progenitor cells from adult human pancreas could be a potential therapeutic target for treating type 1 diabetic patients, but their existence is highly controversial. To begin to address this controversy, we investigated whether pancreatic progenitor-like cells from cadaveric human donors can be identified and studied. Human pancreases were dissociated into

a single cell suspension and plated into a methylcellulose-based semisolid medium containing Matrigel and defined factors. In this semisolid medium, a single pancreatic cell capable of multiplying and forming a group of cells, or a colony, is defined as a pancreatic colony-forming unit (PCFU). Our results showed that adult human PCFUs gave rise to cystic colonies three weeks after plating. Differentiation of PCFUs into pancreatic lineages was determined by assaying gene expression by qRT-PCR and protein expression by immuno-fluorescent staining. Addition of a Notch signaling inhibitor (DAPT) to 10-day-old colonies increased expression of NGN3, a transcription factor for endocrine progenitors. Colonies treated with DAPT were placed under the kidney capsule of diabetic NOD-SCID mice. Three months post-transplantation, intra-peritoneal glucose tolerance test (IPGTT) and glucose-stimulated insulin secretion (GSIS) assays were performed and revealed that the DAPT-treated colonies developed into glucose-responsive insulin-secreting cells. Self-renewal of PCFUs was determined by dissociating colonies into single cells, re-plating and observing the formation of new colonies. Addition of a Notch ligand and a ROCK inhibitor enhanced human PCFU self-renewal in vitro by 300-fold over 9 weeks. Single-colony gene expression revealed that colonies after expansion were similar to the original culture, suggesting that differentiation potential of a PCFU was preserved after self-renewal. Finally, we were able to maintain and enrich progenitor cells in a suspension cell culture with ~30% PCFUs among total cells. Together, our results demonstrate that adult human PCFUs are tri-potent, capable of in-vitro self-renewal and differentiation, and after transplantation functionally regulate blood glucose in recipient mice. Our results have implications in therapy for patients afflicted with insulin-dependent diabetes.

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TISSUE ENGINEERING

W-3059

CARDIOVASCULAR TOXICITY DETECTION FOR CARDIAC REGENERATED MEDICINE

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A mini-microscope-based system for multisite detection of cardiovascular toxicity was developed. The mini-microscope consisted of an image sensor and lens module extracted from an inexpensive webcam. The flipped lens module enabled cells to be magnified and monitored during testing. The portability and compactness of this system enables shortterm and potential long-term experimentation inside a conventional incubator. The toxicity test results demonstrated that the normalized beating rates of cardiac muscle cells selected from multiple regions increased over time when treated with 100 nM isoprenaline. The presented system could be a promising cost-effective cell-based testing tool for discovering and screening drugs.

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W-3061

MESOANGIOBLASTS PROMOTE THE REGENERATION OF AN ORGANISED, STABLE AND PERFUSABLE VASCULATURE IN A DECELLULARISED INTESTINE

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The lack of a functional vasculature is the main hurdle that separates whole-organ tissue engineering from the clinical translation. Diffusion of oxygen and nutrients in a non-vascularised tissue is limited to a few hundred micrometres and is not sufficient to generate tissues and organs which have clinically relevant size.The use of endothelial cells alone cannot regenerate a functional and stable vasculature since, pericytes are required to improve vessels organisation and durability. This study takes advantage of a co-culture approach to develop an organised, stable and perfusable vasculature within an decellularised rat intestine in order to understand the mechanisms by which endothelial cells (HUVECs) and pericytes (human Mesoangioblasts, MABs) crosstalk in the regeneration process. Co-culture of HUVECs and MABs into the vasculature of the decellularised intestinal scaffold resulted in HUVECs lining the vessels and MABs located in a perivascular position, sustaining the endothelial durability. Moreover, HUVECs guide the maturation of Mesoangioblasts towards smooth muscle, which repopulates the smooth muscle layer of the vessels and surrounds the undifferentiated pericytes. Interestingly, the smooth muscle migrates out of the vessel and repopulates

the visceral smooth muscle, supporting the hypothesis that pericytes could be the progenitors of their specific tissue mesoderm. Finally, Mesoangioblasts allow a better and longer-lasting engraftment in-vivo of the regenerated vasculature which anastomose with the host vasculature. In conclusion, we engineered an organised, perfusable and long lasting vasculature which features both the vascular and perivascular compartments, making a step forward for the delivery of human sized engineered organs.

W-3063

HUMANIZED MINI HEARTS, A RELEVANT IN VITRO MODEL

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The development of humanized mini organs through decellularization of rodent organs and recellularization with human cells will provide higher quality translational evidence for in vitro models from animal to human within basic research and pharmacological applications. Here, we have focused on the preservation of the vasculature in rat hearts by cannulating the superior vena cava (SVC), ascending aorta (A), pulmonary vein (PV), and pulmonary artery (PA) and aimed to access the whole heart to recellularize the decellularized rat heart with human iPS derived cardiomyocytes to investigate their maturation by long-term culture. Controlled perfusion was conducted with inflow via the SVC and A, and outflow via PV and PA. The resin cast of a decellularized heart demonstrates that the retrograde flow in addition to antegrade flow through coronary vascular networks permit efficient whole heart perfusion of cells and culture medium. In our ongoing analysis so far, we have shown cellular elongation and alignment with the matrix, sarcomeric bands and high density of mitochondria in mini hearts after 6 weeks in culture, using multiphoton microscopy and histology. Also, valves' functionality has been observed using ultrasound recording. We believe that the humanized mini hearts surmount organ mimicry challenges with intact complexity in vasculature and mechanical compliance of the whole organ providing an ideal platform for improving pre-clinical drug validation in addition to understanding cardiovascular diseases.

Funding Source: This work has been supported by AstraZeneca and Swedish Knowledge Foundation.

W-3065

HUMAN HEPATOCYTE-LIKE CELL SPHEROIDS FOR LIPOPROTEIN METABOLISM STUDIES

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Due to the scarcity and variable quality of human primary hepatocytes (PHHs), researchers have differentiated hepatocyte-like cells (HLCs) from induced pluripotent stem cells (iPSCs) for disease modeling and drug screening applications. However, lipoprotein metabolism, an essential function of hepatocytes, has rarely been investigated in HLCs. In addition, similar to PHHs, HLCs maintained as a 2D monolayer lose their hepatic functions within several days of maturation, while many hepatic markers are still subpar compared to PHHs. Our study aims to investigate the lipoprotein metabolism functions of HLCs on 2D, followed by generating 3D hepatic spheroids with microfluidic technologies, to better mimic liver physiology and provide long-term functional support. In this work, iPSCs were generated from peripheral blood mononuclear cells (PBMCs) isolated from donors' blood. 4-7 clones per donor were then differentiated to hepatocytes via 4 stages: definitive endoderm (DE), hepatic endoderm, immature and mature hepatocyes. Flow cytometry of DE markers—CXCR4 and C-Kit—revealed that >90% were positive in the representative iPSC clones. qPCR and immunostaining confirmed the hepatic phenotypes, followed by functional assays such as LDL uptake, bile duct staining, albumin and ApoB secretions. Omeprazole and phenobarbital induced the expression of their respective cytochrome P450 enzymes, CYP1A2 and 2B6, by 2 folds. We further measured the secretion profile of ApoB-containing lipoproteins and demonstrated that the major species was indeed Very Low Density Lipoprotein (VLDL), as observed in PHHs but not cancer cell lines. To form hepatic spheroids, an aqueous phase containing HLCs was passed through two microfluidic devices to generate HLC spheroids in double-emulsion droplets. After systematic optimization, hepatic spheroids 65um in size could be generated within 24 hours. Bile duct transport and LDL uptake could be observed in the spheroids 10 days post maturation.

In conclusion, we have differentiated iPSCs to mature and functional HLCs that are suitable for lipoprotein metabolism studies. We also developed a microfluidic platform capable of generating hepatic spheroids efficiently and consistently for long-term 3D culture.

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W-3067

A BIOMANUFACTURING PLATFORM FOR THE LARGE-SCALE NEURONAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITOR CELLS

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Human pluripotent stem cell derived neural progenitor cells (hNPCs) have the unique properties of long-term in vitro expansion as well as differentiation into the various neurons and supporting cell types of the central nervous system (CNS). Because of these characteristics, hNPCs have tremendous potential in the modeling and treatment of various CNS diseases. However, large-scale neuronal differentiation of hNPCs and cryopreservation of their neuronal derivatives are major challenges in utilizing hNPCs for basic and translational applications. Here, we used a fully defined peptide substrate- a vitronectin derived peptide (VDP) as the basis for a microcarrier (MC)-based suspension culture system that enables the highly efficient neuronal differentiation of several hNPC lines. We further used this MC-based system in conjunction with a low shear rotating wall vessel (RWV) bioreactor for the large-scale cortical neuronal differentiation of hNPCs from patient-specific hiPSCs. Specifically, using a 55 mL bioreactor vessel, we were able to reproducibly generate over 125 million hNPC-derived neurons. Neurons generated in this bioreactor system could be dissociated, cryopreserved and replated onto VDP coated 2-D surfaces with high levels of cell viability, which will be important for downstream high-content phenotypic drug screening assays where the culture of neurons in 2-D will be required. Cryopreserved neurons also maintained the expression of cortical neuronal markers and exhibited spontaneous calcium spikes. In the future, this fully defined and scalable biomanufacturing system will provide a platform for the generation and cryopreservation of hNPC-derived neurons under GMP/GLP standards in numbers (>109) necessary for many downstream drug screening and regenerative medicine applications.

W-3069

3D SPHEROID CULTURING IN WNT-RELEASING MICROWELLS BOOSTS THE STEM CELL PROPERTIES OF SALIVARY GLAND STEM CELLS

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Three-dimensional (3D) spheroid culture in nanofibrous microwells promotes stem cell properties by enhancing cell-to-cell interaction, however, the lack of in vivo-like biochemical cue and hydrophobicity remain challenging for optimal culture conditions. In this study, we fabricated a WNT-releasing microwells by incorporation of WNT3a into cell-repellent polyethylene glycol hydrogel walls which were micropatterned onto cell-adhesive polycaprolactone nanofibrous scaffold. We determined whether this novel microwell culture boosts stem cell properties regarding marker expression, paracrine function, and differentiation potential and investigated its underlying mechanisms. WNT3a-incorporated microwells were designed to enable sustained release of WNT3a for 7 days. Human salivary gland stem cells (SGSCs) were cultured in 2D tissue culture plastic (TCP) dishes, microwells (Microwell), microwells with WNT3a-containing medium (Microwell-CM) or WNT3a-releasing microwells (Microwell-WNT). SGSCs were aggregated and formed into 3D spheroid structure in Microwell, Microwell-CM, and Microwell-WNT. The SGSC spheroids assembled in Microwell-WNT expressed the significantly higher stem cell-related gene and protein levels compared to Microwell and Microwell-CM. The SGSC spheroids in Microwell-WNT demonstrated higher paracrine activity and greater differentiation potential to give rise to salivary epithelial cells than Microwells and Microwell-CM. WNT activation boosted in Microwell-WNT was suppressed by inhibition of WNT canonical pathway, which led to reduced stem cell properties of SGSCs in microwells. The 3D spheroid culture of SGSCs in microwells promotes stem cell properties and WNT-bound microwell culture can be used to activate WNT signaling that enables boosting paracrine and differentiation functions of SGSCs.

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W-3071

HOST BONE-MARROW-DERIVED MESENCHYMAL STEM CELLS AND INFLAMMATORY CYTOKINES IN A LONG BONE INJURERD MODEL

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Tissue regeneration during wound healing is still one of the mysterious phenomena in our body. Although many types of cell including immune cells, mesenchymal stem cells (MSCs) are considered as essential contributor for tissue regeneration, the detailed mechanism are still uncovered yet. On the other hand, accumulation of host bone marrow MSCs into an injured site, which is under an inflammatory cytokine rich condition, is well known. However, it is not clear that how inflammatory cytokines affect the accumulated MSCs function. Here we investigated the influence of an inflammatory cytokine on MSCs properties to understand the mechanism of tissue regeneration. A wound healing model in C57BL/6J mice (6-week old, female, n=5) were created by making hole in the femurs by using a round bar. Mice were sacrificed at 0, 1, 3, 5, and 7 day after surgery and fixed with 4 % paraformaldehyde. To check the accumulation of MSCs and inflammatory cytokines in the injured site, CD146 positive cells and tumor necrosis factor alpha (TNF-a) were analyzed by the immunohistochemical staining. To investigate the effect of TNF-a on MSCs properties, MSCs were stimulated with TNF-a (10 μg/mL) for 48hours and then used further experiments. In the histological analysis, the increased number of CD146+ cells along with accumulation of TNF-a were clearly observed at day 1 after surgery. Moreover, in the flow cytometric analysis, the number of CD146+ cells was significantly increased ($p<0.01$, one way ANOVA/ Turkey) at day-1 post surgery. In an in vitro study, TNF-a stimulation promoted the expression levels of CD146, FAS-ligand, and cell migration ability of MSCs , while cell proliferation and differentiation properties were not affected in MSCs. Interestingly, in flow cytometric analysis, the numbers of both FAS-ligand positive cells and apoptotic T cells were increased in bone marrow at 1 day and 3 day after surgery respectively. In this study, as we hypothesized, the increased number of MSCs and TNF-a were observed at the same timing in bone marrow and TNF-a promoted MSC's cell migration and immunomodulatory properties in vitro. These findings suggested that TNF-a might recruit bone marrow MSCs to initiate the tissue regeneration by inducing immunotolerance. Clarifying this mechanism will connect to develop the new strategy for tissue regeneration.

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ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

W-3075

INDEPENDENT STEM CELL RESEARCH EXPERIENCE HAS A POSITIVE INFLUENCE ON CHOICE GOALS AND PERSISTENCE IN STEM-RELATED CAREERS FOR UNDERREPRESENTED MINORITY STUDENTS

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Challenges of underrepresented minorities' (URMs) persistence in STEM majors, degree attainment, and career choice have been well-documented in literature. Although URMs are declaring STEM as an undergraduate major, they are not graduating with a STEM degree at the same rate as their White and Asian counterpart. Large gaps in STEM degree completion and the subsequent lost talent disproportionally affect historically underrepresented groups. Even in STEM majors where underrepresented groups have shown comparable outcomes in completion, they often do not pursue STEM careers. Underrepresented minorities who pursue STEM careers diversify the workforce and increase their economic worth with higher median salaries and lower unemployment rates compared to those who pursue non-STEM careers. As the structural diversity of the country is changing, there is an increasing need in the workforce to fill and diversify STEM occupations to accommodate this change. Identifying and understanding factors influencing URMs and their decision to complete a postgraduate degree will shed light on challenges related to the lack of diversity in the STEM workforce. A qualitative methods approach was used in this study to provide insights on whether undergraduate research experience for URMs attending a two-year institution influences choice goals and persistence in scientific research or related careers. Underrepresented minorities in STEM, specifically in biological sciences, who participated in the CIRM-funded program: Bridges to Stem Cell Research at Pasadena City College, including a one-year internship at local renowned research institutes, were recruited on a voluntary basis to participate in this study. The participants' perception regarding the benefits and challenges of performing independent stem cell-related research and the potential influence on career choice were evaluated. Results demonstrate that participation in the stem cell training program and related research experience was perceived by the URMs to have a positive influence on their choice to pursue a STEM-related career which may ultimately contribute to the diversity of the STEM workforce. The study may inform funding sources to develop programs that effectively increase STEM persistence, access, and retention for underrepresented minorities.

W-3077

STEM CELL BIOLOGY AND BIOENGINEERING FOR PRE-COLLEGE TEACHERS AT RENSSELAER: AN ALTERNATIVE APPROACH TO SUMMER RESEARCH EXPERIENCE

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Stem cell research has thrived in the United States and around the world due in part to tremendous public support and intense interest from budding biomedical science trainees. However, less than forty percent of Americans go on to earn a college degree, and only two countries worldwide have a tertiary education rate higher than fifty percent. Therefore, responsibility for educating and inspiring future stem cell scientists and consumers rests primarily with middle and high school teachers. Yet due to rapid progress in the field, today's teachers often have had exposure to only the most basic stem cell concepts. Together with eagerness for stem cell "cures," this leaves the public vulnerable to sensational claims about unproven therapies and endangers public enthusiasm if expectations are not met. In order to sustain public investment, research scientists must partner with pre-college educators to enhance teachers' knowledge and promote more informed public discourse. Rensselaer's Pre-College Teachers Training Program in Stem Cell Biology and Bioengineering is an intensive six week summer course for teachers of grades 7 - 12 in New York State. The goals of the program are to 1) provide a foundation in key areas of stem cell science and bioengineering, 2) facilitate development of new teaching resources, curriculum and classroom activities, and 3) establish a dynamic teaching community among pre-college educators and Rensselaer faculty and students. In contrast to a typical summer research experience that pairs each participant with a researcher for most of the program, teachers spend the majority of the course working as a group. Approximately sixty percent of their time is spent in research and learning new material, while thirty percent is devoted to curriculum development and team-building, and ten percent is given to forming on-going professional relationships with mentor labs. To date, twenty two teachers serving twelve school districts with an aggregate enrollment of nearly 20,000 students have participated. Dissemination of teaching modules online and at professional conferences will increase impact of the program within and beyond New York State.

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W-3079

AUTOLOGOUS STEM CELL THERAPIES: DO I LEGALLY OWN MY OWN STEM CELLS

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The growing industry of unproven stem cell treatments around the world has focussed on using autologous cells as services that enable patients to access the medical treatment of their own cells. There have been efforts to regulate untested stem cell therapies in some nations including Australia (the new TGA regulation). However, some patients, their carers and patient support groups support reducing regulations to make these therapies more accessible, especially treatments that use cells collected from the patients. These patients believe that they have an inherent legal right to ownership and thus unlimited use of their own stem cells ie 'my cells are my cells'. Accordingly, they argue that government regulatory authorities (eg Food and Drug Administration/ FDA) should not intervene. This presentation will explore the arguments for and against patients legally owning their own bodies, body parts and tissues as well as the legal interpretations of the ownership rights of cellular materials with a focus on Australian law.

Funding Source: None

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

W-3081

MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR VESICLES IN REGENERATIVE THERAPY AND IMMUNE MODULATION: A PRECLINICAL SYSTEMATIC REVIEW

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Mesenchymal stromal cells (MSCs) have been used extensively in preclinical and clinical studies for a wide range of diseases based on their abilities to promote healing and reduce inflammation. It is widely recognized that their therapeutic effects are mediated by paracrine mechanisms, and recent interest has focused on the role of extracellular vesicles (EVs). This systematic review aims to provide an evidence map of all in vivo studies using MSC-derived EVs as a therapy. MEDLINE and Embase were systematically searched to May 2018 for in vivo interventional studies using MSC-EVs. Two reviewers screened articles by abstract and full-text. The following information was extracted from eligible studies: (1) interventional traits, (2) methods of EV isolation and characterization, (3) experimental design and (4) outcomes. In 2015, we published a similar scoping review for only 17 articles, whereas this review identified 205 studies. Based on the size criteria for EVs (e.g. exosomes/small EVs ~30-150nm, large EVs ~150-1000nm), only 60% of studies used appropriate terms to describe their EV therapy. Ultracentrifugation (143/205) and isolation kits (42/205) were the most common isolation methods. Inconsistency was seen for methods to characterize size, protein and morphology, and 90% of studies did not include negative protein markers for EV identification. Xenogeneic EVs were administered in 66% of studies. EVs were commonly dosed by protein (138/205) or particle (32/205) amount. Disease models varied across many organ systems including renal, cardiac, brain, respiratory and musculoskeletal. Of note, EVs were delivered after disease induction (treatment protocol) in 90% of reports. Benefits across all outcomes were reported in 80% of studies. However, half the studies did not incorporate randomization and only one-fourth included biodistribution experiments. Adverse effects from MSC-EVs were reported in only 3 studies; two of which showed increased tumour growth. In conclusion, this systematic review revealed extensive heterogeneity in methods and design for EV research. Despite this heterogeneity, most studies showed significant benefits. Our study highlights important opportunities to improve preclinical study design that could demonstrate the potential of MSC-EVs as a novel cell-free therapy.

W-3083

TISSUE-ENGINEERED BONE EQUIVALENT FOR TREATMENT OF COMBAT-RELATED BONE DEFECTS OF CRITICAL SIZE

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Combat-related injuries of limb bones (high-energy mechanism of trauma) are an unresolved clinical problem, especially regarding the bone defects of critical size. The gold standard management in this case is autologous bone transplantation. Temporary and economic costs for treating patients with alterations of reparative regeneration, the complexity of their social adaptation, justify the need to search for innovative alternative organ-saving technologies of regenerative medicine for bone integrity restoration. Our aim was to develop and assess the clinical effectiveness of transplantation of three-dimensional pre-vascularized tissue-engineered bone equivalent/graft (3D-OPG) for restoration of combat-related bone defects of critical size (ClinicalTrials.gov Identifier: NCT03103295). To fabricate 3D-OPG we used partially demineralized allo-/xenogeneic bone scaffold (ILAYAOSTEOGEN®) seeded with autologous cultured: BM-MSCs in a mix with periosteal progenitor cells (PPCs) and endothelial progenitor cells from peripheral blood (EPCs). Quality/identity of cultured cell types was assured by donor and cell culture infection screening, flow cytometry (cell phenotype), karyotyping (GTG banding), functional assays (CFU analysis, multilineage differentiation assay, FDA/PI combined staining). Bone defect treatment with use of 3D-OPG was applied to 50 combat-injured casualties with 52 bone defects. Restoration of the bone defects was observed after 6 months post-op and evaluated by X-ray examination. The results of the treatment are considered as the following: good – the formation of bone tissue with the restoration of the integrity of bone segments of a limb within 4-6 months post-op; satisfactory – patients who had partial graft lysis in the transplantation area, but bone was formed; patients who experienced a delay (more than 6 months) for bone tissue formation post-op; unsatisfactory – patients who had a complete lysis of the transplanted bone equivalent. Histological analysis of bone equivalent specimens 3 months post-op revealed immature bone tissue formation. The developed regenerative medicine and organ-saving approach allows restoring the bone integrity, forming new bone tissue in a site of bone defect, and significantly reducing the rehabilitation period of a patient.

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W-3085

COMBINED STRATEGIES FOR THE CREATION OF A CLINICAL GRADE IPS CELL BANK DERIVED FROM A NORTHERN EUROPEAN POPULATION

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We suggest a combination of strategies enabling the creation of a human iPS cell bank, providing clinical grade, HLA matched cells derived from and adapted for a northern European population. The establishment of a minimal mismatch iPS cell bank covering > 60% of the population is not feasible using a single approach. Based on the major five HLA haplotype alleles, retrieved from registered donors in the Swedish bone marrow register, the Tobias registry we found that an iPS cell bank composed of 191 selected but common HLA haplotypes could provide direct HLA match based on five alleles to 60% of the population. These 191 lines could be detected through a minimal screening effort of < 10 000 individuals, a fraction of the over 200 000 unique blood donors which annually donate blood in Sweden. To cover the entire registry of > 40 000 donors, 14734 lines would be needed. 40% of the population has rare HLA haplotype combination (1/2000), out of which, 8.5% of registered donors are unique. The remaining 40% can be covered by sharing the cell lines globally and by the creation of HLA super donor lines through genome-editing techniques. Two such super donor lines would cover 98% of the study population and 89% of the Japanese population.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

W-3087

METABOLIC CONTROL OVER MTOR DEPENDENT ENTRY AND EXIT FROM DIAPAUSE-LIKE STATE

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Embryonic diapause is a state of dormancy that interrupts the normally tight connection between developmental stage and time. To better understand the processes underlying diapause in mammals, we characterized the transcriptional and metabolite profiles of mouse pre-implantation, post-implantation and diapause embryos. We show that triacylglycerol (TAG) and diacylglycerol (DAG) levels are highly reduced due to mTOR inhibition induced lipolysis, while their products, free fatty acids and phosphatidylcholine (PC) are enriched to support cell survival in diapause. We furthermore identified a unique cellular regulation signature placing diapause at a distinct developmental state with highly activated glycolysis and metabolic pathways regulated by AMPK. Significant enrichment of AMP further indicated activation of the cellular starvation-sensor, AMPK. We show that starvation in pre-implantation ICM derived mouse embryonic stem cells induces a reversible dormant state, transcriptionally mimicking the in vivo hormonally controlled diapause stage. During starvation, a splice variant of an upstream kinase of AMPK, Liver kinase b1 (Lkb1), induces a reversible, mTOR controlled diapause-like, quiescence state in vitro through AMPK. We furthermore show that, paradoxically, forced expression of a non-diapause Lkb1 splice variant results in a constitutive diapause-like state due to a phospho-AMPK dependent increase in glucose transporters and decrease in mTOR activation. Leucine degradation intermediates are enriched in diapause and Slc38a1/a2, glutamine transporters, are essential for mTOR activation in diapause exit. These Glutamine transporters are downregulated due to forced expression of the non-diapause Lkb1 splice variant. Our data show that upregulation of the glutamine transporters SLC38A1/A2 and downregulation of the non-diapause Lkb1 splice variant primes the pluripotent cells for mTOR dependent exit from quiescence.

W-3089

ENERGY METABOLISM IN ES CELLS AND EARLY EMBRYONIC-LIKE CELLS

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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 have characteristic similar to that of early embryos, which can contribute to both embryonic and extra-embryonic lineages. In this study, we defined culture condition for ESCs that leads to increases the population of MuERV-L positive cells. We found that ascorbic acid and insulin play important roles in the transition from ESCs to early embryo-like cells. We compared expression of energy metabolic pathways associated genes between ESCs and early embryonic-like cells. As a result, Pdk1, Slc2a1, and Stk11 were significantly downregulated in early embryonic-like cells than that in ESCs. Pdk1 is considered to contribute to enhance glycolysis by suppressing entry of pyruvate into the mitochondrial tricarboxylic acid cycle. In addition, Slc2a1 and Stk11 are known to enhance glycolysis by facilitating glucose uptake. Thus, our results suggest that glycolysis pathway is suppressed in early embryonic-like cells compared to ESCs. In contrast, there are no significant differences in the expression levels of Cox7a1 and Cpt1a, which have central roles in oxidative metabolism, between ESCs and early embryonic-like cells. We will discuss whether changes in cellular metabolism influence cell stat transition or not.

W-3091

DERIVATION OF FUNCTIONAL OOCYTES FROM GRANULOSA CELLS

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Limited oocyte and ovarian reserve in vivo or chemo-therapy leads to reproductive aging or premature aging and associated diseases including infertility. Excitingly, oocytes have been successfully derived from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) by ectopic expression of transcription factors, showing great potential in fertility preservation or restoration. The accessible granulosa cells are a type of somatic cells that interact and evolve with oocyte development during folliculogenesis. Further, with stem cells-like property, granulosa cells are amenable to reprogramming to generate iPSCs and have been the first used for clone animals. These prompted us to explore the potential of granulosa cells in derivation of germ cells. Meanwhile, the strict genome fidelity required for germ cells inspired us to test reprograming by complete small chemical, which avoids genetic manipulation, cell transfection and destruction of embryos. Here we show that somatic granulosa cells of adult mouse ovaries can be converted to germ cells and functional oocytes that reproduce fertile pups. We are able to consistently induce granulosa cells to pluripotent state (gPSCs) like ESCs in both developmental competence and molecular signatures. Notably, crotonic sodium-facilitated crotonylation is critical not only for pure small chemicals-based reprogramming of granulosa cells to gPSCs, but also confers the gPSCs with high germline capacity. Consequently, the gPSCs and the derived primordial germ-cell like cells hold longer telomeres and maintain high genomic stability which is critical for germ cells. Taken together, we efficiently generate high quality gPSCs and functional oocytes from adult granulosa cells by significantly improving chemical reprograming approach.

W-3093

GLUCOSE METABOLISM DRUING PRE-IMPLANTATION MOUSE EMBRYO DEVELOPMENT

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The pre-implantation mouse embryo development requires synergistic interactions between signaling transduction pathways and metabolic pathways. During mouse pre-implantation embryos development, the totipotent blastomeres generate the first three cell lineages of the embryos: Trophectoderm (TE), the Inner Cell Mass (ICM) and Primitive Endoderm (PE). The whole developmental process to blastocyst can be recapitulated in vitro using a defined medium containing three metabolites (Pyruvate, Glucose, and Lactate), salts and buffer systems. Glucose deprivation in the culture blocked the embryonic development at 8-Cell stage. We characterize the role of glucose in the morula to blastocyst transition. Our studies show that in the absence of glucose the outer cells of the embryo fail to

differentiate into TE cells while the inner cells remain competent to differentiate into ICM cells. Our metabolomic studies further show that glucose does not contribute carbon to the TCA cycle, which is maintained exclusively by pyruvate and lactate and the catabolism of endogenous metabolites. Our studies show that at this stage critical pathways of glucose catabolism are the pentose pathway (PPP) and the hexosamine biosynthetic pathway (HBP) and blocking these pathways recapitulate distinct aspects of the glucose phenotype. Analysis of the roles of the PPP and the HBP further showed that these pathways have non-over lapping roles in the regulation of specific transcription factors that are essential for the establishment of the TE fate.

CHROMATIN AND EPIGENETICS

W-3095

GENOMIC ORGANIZATION OF UHRF1 IN MOUSE EMBRYONIC STEM CELLS (ESCS)

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UHRF1/ NP95 is a multi-domain, chromatin binding protein which has been implicated in multiple cellular processes and is found to be overexpressed in numerous human cancers. Uhrf1 plays an essential role in propagating DNA methylation patterns during DNA replication through binding 5mC and recruiting DNMT1. It can also bind to histone and other DNA modifications, interact with chromatin modulators and directly regulate protein ubiquitination. Although, Uhrf1 is not essential for stem cell self-renewal, it seems crucial for cell fate determination, however, the regulation of its different binding modules is not well characterized. The binding of UHRF1 to H3K9me3 is known to be necessary for DNA methylation. Surprisingly, our ChIP-seq in embryonic stem cells showed that UHRF1 does not co-localize with H3K9me3 but is primarily enriched in euchromatin, especially at gene enhancers and promoters including these of pluripotency genes. The functional relevance of UHRF1 binding at euchromatic regions is not known. Interestingly, we found UHRF1 enriched at sites that contain TET1 and 5hmC, and its binding at Nanog and Nodal is decreased upon TET1 depletion. Whether UHRF1 recruitment is mediated by 5hmC or through an interacting protein remains to be characterized. Taken together, our data suggests that UHRF1 has an alternative role in gene expression activation.

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W-3097

NETWORK ANALYSIS OF PROMOTER INTERACTIONS REVEALS THE HIERARCHICAL DIFFERENCES IN GENOME ORGANISATION BETWEEN HUMAN PLURIPOTENT STATES

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Naïve and primed human pluripotent stem cells (hPSCs) hold distinct developmental and epigenetic identities that encompass the properties of pre- and post-implantation epiblast cells, respectively. Investigating these hPSC states therefore has the potential to uncover the pathways that govern the early molecular events that occur during human embryonic development and cell differentiation. Comparative transcriptional and epigenetic profiling has revealed substantial differences that distinguish naïve and primed hPSCs. However, our understanding of how 3D chromatin organisation is affected by this changing epigenetic landscape, and how it contributes to gene regulation in these human cell types, is poorly understood. Towards this goal, we used promoter-capture Hi-C to generate a high-resolution atlas of DNA interactions in naïve and primed hPSCs. We developed network approaches to examine this chromatin interaction atlas at multiple scales, ranging from a global genome-wide overview to sub-megabase scale communities that recapitulate topologically associating domains, down to individual promoter-enhancer interactions. We uncovered numerous large and highly connected hubs that changed substantially in interaction frequency and in transcriptional co-regulation between naïve and primed states. Small interaction hubs frequently merged to form larger networks in primed cells, and these hubs were often linked by newly formed long-range Polycomb-associated interactions. We also identified state-specific differences in enhancer activity and interactivity that corresponded with widespread rearrangement in pluripotency factor binding and the transcription of target genes. Utilising network approaches to construct a global overview of chromatin conformation, annotated with transcriptional and epigenomic information, provides new insights into the hierarchical features of gene regulatory control in human development and pluripotency.

W-3099

UNCHARACTERIZED ROLE OF ATP-DEPENDENT CHROMATIN REMODELING FACTOR-CHD8 IN ACTIVITY DEPENDENT PLASTICITY OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS

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CHD8 is one of the highly mutated genes in autism spectrum disorder (ASD). It an archetypical ATP-dependent chromatin-remodeling factor, with essential roles in nucleosome positioning and regulating gene expression. Electrophysiological recordings of mouse neurons- carrying the heterozygous loss of function mutation in CHD8 gene, shows altered spontaneous excitatory activity. In general, evidence point that CHD8 regulates the excitability of neurons, but no molecular mechanism for this role supported in the literature. To demonstrate this role in "human" neurons and to investigate the molecular mechanism of activity regulation, we generated human pluripotent stem cells (hPSCs) carrying conditional CHD8 loss of function alleles, and we reprogrammed them to excitatory neurons with transcription factor (Ngn2). We then used Cre–recombinase to induce targeted heterozygous and homozygous loss of function mutation in neurons. We identified transcriptional and chromatin-remodeling targets of CHD8 by conducting the RNA-sequencing experiment, chromatin immunoprecipitation (ChIP), and the assay of transposase-accessible chromatin (ATAC-seq) experiment. To characterize specific role in the regulation of activity-depended gene expression, we stimulated the neurons with KCl and measured gene expression changes. Collectively, results show that "Inducible targets of CHD8" is a group of genes that either has no direct binding of CHD8 to transcriptional start (TSS) site or there is a signal on regions downstream of the TSS. The results suggest that the activity of CHD8 on inducible genes involves complex and different regulatory sequences: shortly after stimulating neurons, CHD8 inducibly binds to some of its target genes, and in another group of genes, the release of pausing from promoter-proximal region regulates the cognate gene. We are currently testing this hypothesis with ChIP-quantitative PCR (ChIP-qPCR) using an anti-CHD8 antibody, and with primers designed for upstream and downstream of TSS region of target genes. This work will bridge the gap in studies of mouse and human neurons, and it will provide mechanistic evidence for regulation of activity in neurons.

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W-3101

FEED-FORWARD PIONEER FACTOR ACTIVITY OF POU4F3 LICENSES ATOH1-DEPENDENT SENSORY HAIR CELL DIFFERENTIATION

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Synergistic expression of Atoh1 and Pou4f3 are essential for the generation of the induced mechanosensory hair cells during reprogramming, consistent with the requirement of both transcription factors for the elaboration of the sensory hair cell phenotype during development. We have investigated the epigenetic and gene regulatory mechanisms downstream of this apparent Atoh1-Pou4f3 synergy. We discovered that Pou4f3 is both a direct target of Atoh1 in the differentiating hair cells, and has "pioneer factor" activity that acts in a feed-forward manner to license an additional array of hundreds of Atoh1 targets necessary for hair cell differentiation, among which are several disease-causing genes involved in Usher syndrome, the most prevalent cause of deaf/blindness in the population.

W-3103

DISTINCT IMPRINTING SIGNATURES AND BIASED DIFFERENTIATION OF HUMAN ANDROGENETIC AND PARTHENOGENETIC EMBRYONIC STEM CELLS

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Genomic imprinting is an epigenetic mechanism that results in parent-of-origin monoallelic expression of specific genes. The functional non-equivalence of parental genomes imposed by imprinting precludes uniparental development, and aberrant imprinted gene expression is associated with developmental disorders and cancer. Although mouse models have provided

crucial insights into this unique phenomenon, studying imprinting in humans remains a challenge. To explore molecular and developmental aspects of imprinting in humans, we generated multiple human androgenetic embryonic stem cell (aESC) lines of exclusively-paternal origin. Sperm injection into human oocytes with removal of the maternal genome resulted in efficient preimplantation development and subsequent derivation of homozygous pluripotent aESCs. Thereafter, we established a human pluripotent-cell experimental system of distinct parental backgrounds, by comparing aESCs with exclusively-maternal parthenogenetic ESCs (pESCs) from unfertilized oocytes and bi-parental ESCs from in vitro fertilization. Analyzing the transcriptomes and methylomes of human aESCs, pESCs and bi-parental ESCs enabled us to characterize regulatory relations at known imprinted regions, and uncovered novel imprinted gene candidates residing within and outside known imprinted regions. Focusing on one putative imprinted gene revealed both its monoallelic expression and differential DNA methylation. We next utilized the pluripotency of our different ESCs to investigate the consequences of uniparental development, considering the known biases of the maternal and paternal genomes towards embryonic and extraembryonic development. Teratoma differentiation in vivo recapitulated both the tendency of androgenetic cells to placental contribution, and remarkably, revealed another significant paternal bias towards an embryonic tissue, the liver. We thus differentiated aESCs and pESCs in vitro into trophoblast cells and hepatocytes to further study the mechanistic roles of specific imprinted genes underlying these developmental biases. Our results emphasize the potential of pluripotent cells with different parental origins for studying the impact of imprinting on human development and disease.

PLURIPOTENCY

W-3105

INHIBITION OF NOTCH SIGNALING ON PRIMED HUMAN EMBRYONIC STEM CELLS (ESCS) PROMOTES FEATURES ASSOCIATED TO THE NAIVE PHENOTYPE

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During development mouse ESCs derived from the pre-implantation blastocyst, reside in a Naïve pluripotency state without lineage differentiation bias, in contrast to Primed human ESCs, derived from in vitro fertilized oocytes. Naïve ESCs are characterized by higher expression of pluripotent transcription factors, such as OCT4, KLF4 and REX1, and STELLA (which promotes global DNA demethylation). We recently showed that miR-363 promotes pluripotency in hESCs by post-transcriptionally inhibiting Notch components, including NOTCH1/2 receptors and the gama-secretase mediating their activation. Both receptors were recently identified as surface markers specifically associated with Primed hESCs. Therefore, we hypothesized that pharmacological inhibition of Notch signaling (by the gama-secretase inhibitor DAPT) would drive cultured hESCs into a Naive state. Primed H9 hESCs were cultured in a feeder-free condition (n=4) in the presence of DAPT (2μM) or in its absence (DMSO control) for 6 passages (22 days). Markers associated with Primed and Naive pluripotency states were evaluated by quantitative microscopy and PCR. Nuclear and cytoplasm cell compartments were stained (Hoechst and CellMask Blue), along with fluorescent antibodies against OCT4, CD90 and SSEA3 (Primed state markers), and CD7 and CD130 (Naive markers). Images were acquired with a High Content Analysis System, segmented and quantified with CellProfiler analysis software. Expression analysis by qPCR revealed that, compared to controls, Notch inhibition significantly decreased the levels of HEY1 (one of the main pathway targets) and also NOTCH1/2 transcript (although not statistically significant); while, significantly increasing (3x) levels of STELLA. No changes were observed for OCT, KLF4 and REX1; ZIC2 and OTX2 (primed markers) and DNMT3A/B. Quantitative microscopy revealed a striking increase in the number of OCT4 positive cells cultured with DAPT, as compared to control. This was accompanied by a significant increase in the mean intensity levels of the naïve marker CD7 and a significant decrease of the primed marker CD90. Our results suggest that Notch inhibition may promote Primed to Naïve conversion and may contribute to the development of protocols for the maintenance and differentiation of cell for regenerative medicine.

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W-3107

INSULIN STIMULATES PI3K/AKT AND CELL ADHESION TO PROMOTE THE SURVIVAL OF INDIVIDUALIZED HUMAN EMBRYONIC STEM CELLS

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Insulin is present in most maintenance media for human embryonic stem cells, but little is known about its essential role in the cell survival of individualized cells during passage. In this report, we show that insulin suppresses caspase cleavage and apoptosis after dissociation. Insulin activates IGF receptor and PI3K/AKT cascade to promote cell survival, and its function is independent of ROCK kinase regulation. During niche reformation after passaging, insulin activates integrin that is essential for cell survival. IGF receptor co-localizes with focal adhesion complex and stimulates protein phosphorylation involved in focal adhesion formation. Insulin promotes cell spreading on matrigel-coated surfaces and suppresses myosin light chain phosphorylation. Further study showed that insulin is also required for the cell survival on E-cadherin coated surface and in suspension, indicating its essential role in cell-cell adhesion. This work highlights insulin's complex roles in signal transduction and niche re-establishment in hESCs.

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W-3109

N6-METHYLADENOSINE READER YTHDC1 IS AN ESSENTIAL FACTOR FOR MOUSE PREIMPLANTATION DEVELOPMENT AND MAINTENANCE OF EMBRYONIC STEM CELLS

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N6-methyladenosine (m6A) has been shown to participate in various RNA metabolic processes, including splicing, translation efficiency, nuclear transport and stability, which is considered to be important for the differentiation and self-renewal capability of mouse embryonic stem cells. YTHDC1, a reader of m6A, has been shown to play important roles in alternative splicing, nuclear export and XIST-mediated gene repression. Here, we constructed mouse model and found that Ythdc1 deficient embryos show defect and lethality at E4.5. Many pluripotency related genes are down-regulated and trophectoderm specific genes are up-regulated in Ythdc1 deficient inner cell mass. We further found that loss of YTHDC1 in ESCs leads to flattened and less compacted colonies, dramatic decrease of the proliferation rate, down-regulation of the pluripotent markers as well as the impaired colony formation ability. Moreover, m6A-reading-mutated YTHDC1 could not rescue the defect of Ythdc1 deficient mESCs. Our data suggest YTHDC1 regulates the pluripotency in both early embryonic development and ESCs, through its m6A recognition function.

W-3111

HUMAN PLURIPOTENT STEM CELL QUALITY: A SCIENTIFIC WAKE-UP CALL

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As stem cell scientists, the quality of our research is directly related to the quality of the hPSC materials used. Poor quality cells can impact reproducibility, jeopardize results, waste time, and drain resources. In screening materials submitted to the WiCell Stem Cell Bank, we have identified a substantial and concerning variability in cell quality, highlighting the need for improved testing strategies and standards. As of this abstract, 1604 cell lines have been submitted to WiCell for banking and characterization by 31 providing laboratories. The vast majority of these cell lines were generated through grant-funded projects as a resource for the larger scientific community, and reportedly screened prior to submission. Various testing strategies were used, and available characterization information was provided to WiCell for reference. To date, 747 of these lines have been independently tested by WiCell for thaw viability, genetic stability (karyotype), identity via short tandem repeat (STR) analysis, sterility (bacteria and fungus), and mycoplasma. Of the 747 hPSC lines examined, 261 did not meet minimum quality standards (289 separate instances, due to some lines failing more than one test). Overall, more than one-third of WiCell screened cell lines failed routine quality testing. Unexpected abnormal karyotypes were noted in 175 lines tested (23%). STR anomalies, including cross-contaminated (mixed) cell lines, identity mismatch, and sex mismatch were noted in 26 cell lines (>3%). Seventy-five (75) cell lines (10%) were unrecoverable at thaw, exhibiting either no attachment or excessive differentiation, preventing establishment of the culture. Twelve (12; 2%) were not sterile, and 1 line was mycoplasma positive. These results show that current ad hoc screening strategies are variable and largely insufficient. Based on this data, we can assume that a substantial percentage of materials used in investigator laboratories have unidentified quality issues that will impact research. This underscores the need for routine testing prior

to initiating and following studies. Furthermore, it highlights the need for, and value of, centralized repositories with established quality standards that ensure distribution materials are routinely and appropriately screened.

W-3113

BALANCING PLURIPOTENCY IN MOUSE EMBRYONIC STEM CELLS THROUGH THE ACTION OF INTRACELLULAR TRAFFICKING PATHWAYS

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Cell fate determination in the early embryo and in embryonic stem cells are regulated by a number of mechanisms. Recently, vesicular trafficking has been shown to play an important role in cell fate choice, although the exact identity of pathways and molecules remains poorly understood. Using a combination of high-throughput screening approaches and data mining, we identify a novel regulation of embryonic stem cell (ESC) pluripotency by endocytic mechanisms, driven by both clathrin and caveolin. We show that clathrin-mediated endocytosis (CME) is required for maintaining the pluripotent nature of mouse ESCs (mESCs). We demonstrate that CME is required for the internalization and recycling of E-cadherin from the cell surface of pluripotent mESCs, along with the trafficking of the Transforming Growth Factor Receptor beta 1 (TGFBR1) to lysosomes for degradation. We further demonstrate that in the absence of CME, the epithelial nature of mESCs is compromised, resulting in an activation of the differentiation program. On the flip side, we also demonstrate that the expression of Caveolin1 (a critical component of caveolin-mediated endocytosis), and a number of other endocytosis-associated genes (EAGs), are repressed in mESCs, through a dual mechanism involving epigenetic repression by the action of the Polycomb Repressive Complex 2 (PRC2), and post-transcriptional regulation by the action of microRNAs. Together, our results suggest that cell fate choices in early development and pluripotency regulation may be controlled by specific intracellular trafficking pathways.

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W-3115

ADVANCED HUMAN PLURIPOTENT STEM CELL EXPANSION IN STIRRED TANK BIOREACTORS FACILITATING VERY HIGH DENSITY MANUFACTURING

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Human pluripotent stem cells (hPSCs) are a unique source for the production of functional human cell types, fueling the development of advanced in vitro disease models and future regenerative therapies. Most applications will require the constant supply of billions of cells generated by robust and economically viable bioprocesses. The expansion of hPSCs as matrix-free, cell-only aggregates in suspension culture is a superior strategy for producing required cell numbers by industry-typical stirred tank bioreactor (STBR) technology. We have recently established robust expansion of pluripotent hPSC in suspension in STBR. Subsequently, direct transition towards the efficient differentiation into highly enriched mesodermal lineages including cardiomyocytes, endothelial cells or macrophages was enabled, demonstrating the universal utility of the approach for the mass production of hPSC progenies. Here we describe an advanced process for hPSC expansion as cell-only aggregates in STBR enabling the production of up to 2.7 billion hPSCs in 150 ml process scale. The culture strategy includes perfusion-based feeding with control of pH and dissolved oxygen facilitating improved process control and superior cell yields. Moreover, standardized pre-culture handling and optimized inoculation supported kick-starting of the process and superior growth kinetics. Stirring-controlled

cell aggregation prevented growth limitations and improved aggregate homogeneity. Ultimately, by cell growth-adapted perfusion rates, unmatched cell densities for hPSC suspension culture of 18 x 10^6 cells/mL can be reached. Together, the study highlights the enormous potential for process development in hPS cell manufacturing, in particular by using well-monitored and controlled bioreactor systems, which also facilitates straightforward process upscaling. This advancement facilitates the clinical translation of hPSC-progenies paving the way for cost-efficient cell therapies.

PLURIPOTENT STEM CELL DIFFERENTIATION

W-3119

EFFECTIVELY DIFFERENTIATING HUMAN PLURIPOTENT STEM CELLS FOLLOWING A TRANSIENT DMSO TREATMENT

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The propensity for differentiation varies substantially across human pluripotent stem cell (hPSC) lines, greatly restricting the use of hPSCs for cell replacement therapy or disease modeling. In prior work, we showed that pretreatment of hPSCs with dimethylsulfoxide (DMSO) enhances differentiation across all germ layers. Here we investigate the underlying mechanisms and show that the DMSO treatment improves differentiation through the retinoblastoma (Rb) pathway and by regulating the cell cycle of pluripotent stem cells. While transient inactivation of the Rb family members (including Rb, p107, and p130) suppresses DMSO's capacity to enhance differentiation across all germ layers, transient expression of a constitutively active (non-phosphorylatable) form of Rb increases the differentiation efficiency similar to DMSO. Transient inhibition of downstream targets of Rb, such as E2F signaling, also promotes differentiation of hPSCs. Furthermore, using Fluorescence Ubiquitin Cell Cycle Indicator (FUCCI) technology in hPSCs, we show that gene expression patterns of signaling factors and developmental regulators change in a cell cycle-specific manner in DMSO-treated cells following RNA-sequencing of hPSCs isolated from early G1, late G1, and SG2M. Genes associated with cytoskeletal, cilium assembly, and cell adhesion factors were especially subject to regulation by the DMSO treatment in the SG2M phases, characteristic of a time when cells may need to duplicate centrioles in the S phase, change shape during mitosis, or exit the mitotic cycle to differentiate. Together, these results show that the DMSO treatment targets early modes of regulation to put hPSCs on a better trajectory for differentiation and ultimately improve their utility for regenerative medicine. Using these mechanistic insights, we highlight how these tools can be applied to improve differentiation of human pluripotent stem cells into any lineage.

W-3121

EXOSOME COMMUNICATION BETWEEN HUMAN IPSC-DERIVED VASCULAR CELLS AND LUNG PROGENITORS MEDIATES SITE-SPECIFIC EPITHELIAL CELL MATURATION

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Bioengineered lung grafts created with patient-specific induced pluripotent stem cell (iPSCs) derivatives could provide immune-compatible implants to treat end-stage lung diseases. Differentiation of iPSC-derived cells in vitro to mature epithelial cell phenotypes, which is paramount for restoring airway and alveolar structure and function, remains a major hurdle. We posited that a dynamic interplay between supportive cells (i.e. vascular smooth muscle and endothelial cells) and lung progenitors (LPs) may direct airway patterning. Stage-specific developmental cues were employed to differentiate iPSCs into CD34+PECAM-1+VEGFR2+endothelial cells (iECs), α-SMA+CALP+ smooth muscle cells (iSMCs), and NKX2.1+Sox2+Sox9+ LPs, confirmed by flow cytometry and immunocytochemistry. Matrigel assays revealed that iECs rapidly self-organized into interconnecting and branching networks that persisted ≤24 hrs. Furthermore, iSMCs demonstrated close apposition and assisted in structure stabilization (>72hrs), reflecting vasculogenic mimicry. LPs generated 3-D organoids harboring proximal/bronchial (FOXJ1, P63, SCGB1A1) and distal/alveolar (AQP5, PDPN, SFTPC) elements, evaluated by gene analyses. Intriguingly, LPs exposed to 50% iSMC-conditioned medium (iSMC-CM) favoured development of bronchiolar spheroids; whereas, 50% iEC-conditioned medium (iEC-CM) yielded more alveolar organoids. We postulated that this paracrine effect was mediated by small extracellular vesicles (sEVs). LPs treated in 2-D cultures with sEVs purified from iSMC-CM over a 3-week period, displayed ≥2-fold elevation in bronchial epithelial markers. Conversely, LPs cultured with sEVs from iEC-CM led to ≥3-fold increase in alveolar epithelial expression. Pre-treatment of iSMCs and iECs with GW4869, a pharmacological inhibitor of exosome biogenesis/secretion, prior to sEV collection markedly reduced their respective divergent effects on lung airway patterning by ≥70%. Collectively, our results implicate vascular cells as potent

sources of signals instrumental in guiding proximal or distal lung epithelial maturation by an exosome-mediated mechanism. This suggests that iSMC and iEC-derived exosomes could be used to enhance regenerative cell therapies for site-specific lung airway repair and regeneration.

W-3123

INVESTIGATING THE ROLE OF MITOCHONDRIAL DISULFIDE RELAY SYSTEM IN REGULATION OF HPSC FATE COMMITMENT

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Human pluripotent stem cells (hPSCs) are characterized by their capacity for self-renewal and their potential to differentiate into any body cell type. hPSCs hold great promise as tools in personalized regenerative medicine, however, a major obstacle in translating this promise is variable differentiation efficiencies resulting in potentially tumorigenic undifferentiated cells and phenotypically immature cells. MitoBloCK-6 (MB-6), a small molecule inhibitor of the mitochondrial ALR/Erv1 disulfide relay system, selectively induces apoptosis of hPSCs, with no measurable effects on differentiated cells, and can impair zebrafish development. Recent studies show that changes in mitochondrial morphology and function are germ-lineage specific and act as a developmental regulator of cell fate determination, but a role for ALR in hPSC pluripotency maintenance and differentiation remains unknown. To probe the dependence of early differentiated germ-lineage specific programs on the disulfide relay system, we treated hPSC cultures with MB-6 during different stages of directed differentiation. Here we show that although cells can exit pluripotency, lineage differences in sustained sensitivity towards MB-6 suggest lineage-specific dependency of the ALR-dependent disulfide relay system. Towards understanding the role ALR plays in lineage fate, mitochondria morphology, and metabolic activity, we are developing shRNA knockdown of ALR in hPSCs. Further understanding of dynamic mitochondrial regulation in pluripotency and cell fate is integral to progressing hPSC differentiation protocols for regenerative medicine.

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W-3125

CHEMICALLY-DEFINED, XENOGENIC-FREE SCALABLE PRODUCTION OF HUMAN PLURIPOTENT STEM CELL-DERIVED DEFINITIVE ENDODERM AGGREGATES WITH MULTI-LINEAGE POTENTIAL

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Human pluripotent stem cells (hPSCs) have been shown to be a useful tool for disease modelling and drug screening assays, and have great potential to be used in cell therapy applications. Culture and differentiation of hPSCs in suspension in chemically-defined, xenogenic-free conditions is a promising method to attain large quantities of cells for such applications. In particular, differentiating hPSCs towards definitive endoderm (DE) using suspension cultures is of great interest, as this could provide a large source of DE cells which can be used to generate multiple DE-derived cell types including liver, lung, pancreas, and intestinal cells. Here, we generate hPSC-derived DE cells in suspension and investigate their differentiation potential towards the multiple DE lineages. By day 4 of differentiation, greater than 90% of DE aggregates expressed the DE surface markers CXCR4, c-Kit, and EpCam. RT-qPCR analysis and fluorescent staining showed that these aggregates were also positive for the DE markers SOX17 and FOXA2. After applying lineage-specific differentiation protocols, DE aggregates were able to further differentiate towards different DE-derived cell types including HNF4αpos liver, PDX1pos pancreas, CDX2pos intestinal, and NKX2.1pos lung cells. In addition, DE aggregates were frozen, and upon thawing could reinitiate differentiation towards the liver, pancreas, intestinal, and lung lineages. Thus, hPSCs can

be differentiated in suspension towards definitive endoderm in a scalable manner. In addition, these DE aggregates can give rise to the different lineages of the definitive endoderm, and maintain their differentiation potential after freezing and thawing.

W-3127

DEEP LEARNING NEURAL NETWORKS HIGHLY PREDICT EARLY ONSET OF MOUSE AND HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION FROM LIGHT MICROSCOPY IMAGES

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Pluripotent stem cell (PSC) differentiation is a highly dynamic process in which both epigenetic, transcriptional and metabolic changes eventually lead to new cell identities. These modifications occur within hours to days and are generally identified by measuring gene expression changes and protein markers. PSC differentiation is also followed by important morphological transformations, but these are inherently subjective and thus are not used as a standard and quantitative measurement of cell differentiation. Our goal in this work was to use artificial intelligence techniques to automatically classify PSCs from early differentiating cells based on their morphology. For that, we made use of convolutional neural networks (CNNs), powerful algorithms that are particularly useful in computer vision. We induced differentiation of mouse embryonic stem cells (mESCs) to epiblast-like cells (EpiLCs) and took transmitted light microscopy images at several time-points from the initial stimulus. We found that several network architectures can be trained to recognize differentiating from undifferentiated cells and correctly classify images with an accuracy higher than 99%. Successful prediction started only 20 minutes after the onset of EpiLC differentiation. We also show that CNNs can be successfully trained to predict whether mESCs were cultured in standard serum + LIF medium or in the recently developed defined culture conditions using MEK and GSK3 inhibitors plus LIF, that sustain the ground state of pluripotency. Moreover, these algorithms also displayed great performance in the classification of undifferentiated human induced PSCs (hiPSCs) compared to hiPSCS-derived early mesodermal cells. Although high training accuracy required strong computational power and the use of hundreds of images for each condition, once the CNN was trained it allowed to rapidly and accurately classify the query images. We believe that efficient cellular morphology recognition in a simple microscopic set up may have a significant impact on how cell assays are performed in the near future, ranging from experimental biology to quality control of cell cultures for the eventual application of PSCs to the clinic.

W-3129

AN AUTOMATED PLATFORM FOR POPULATION-BASED DISEASE MODELING OF IPSC-DERIVED CELL TYPES

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Directed differentiation of pluripotent stem cells has become a cornerstone of cell-based disease modeling. Together with somatic cell reprogramming, which allows for the generation of patient-specific stem cells, it is a powerful tool for studying genotype-phenotype relationships in vitro. In severe monogenetic diseases, this relationship is often pronounced and can be demonstrated experimentally using cell lines from a small number of affected individuals. However, genetically complex diseases such as type 1 and type 2 diabetes require larger cohorts for effective disease modeling with patient-derived cells. The generation of functional insulin-producing beta cells is an ongoing pursuit as current protocols result in a mixture of cell types with significant variability in yield and function. Protocols for directed differentiation are frequently optimized using a single cell line and show inconsistent results when applied to cohorts of genetically diverse stem cell lines. This lack of robustness incorporates experimental variability that can mask subtle phenotypes, representing a major limitation for large-

scale studies where multiple cell lines have to be differentiated and analyzed in parallel. We have developed an automated pipeline for the optimization of directed differentiation protocols that has the capacity to simultaneously test a large set of culture conditions on multiple cell lines. Automated flow cytometry staining and analysis facilitates the precise quantification of developmental factor expression on a single-cell level. Guided by design of experiment (DoE), this approach offers a standardized path towards optimizing differentiation protocols for population-based disease modeling and personalized stem cell therapies.

W-3131

THYROID HORMONE ENHANCES CELL CULTURE CAPACITY AND PROMOTES LINEAGE SPECIFIC DIFFERENTIATION IN HUMAN EMBRYONIC STEM CELLS

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Thyroid hormone triiodothyronine (T3) improves early embryo development in vitro. Human embryonic stem cells (hESCs) are derived from the inner cell mass of blastocyst, but little it known how the does hESCs modulated by T3. In this report, we use hESCs to show that T3 improves different aspects of stem cell culture. T3 stimulates oxidative phosphorylation and activates mitochondrial activities. T3 promotes cell survival and passaging efficiency at high density, and boosts culture consistency. Further research shows that T3 promotes FGF/ERK pathway to maintain pluripotency, and also boost cell adhesion and survival in suspension culture. For in vitro differentiation, T3 inhibit neural crest differentiation but promote pituitary gland differentiation, T3 also promote mesoderm and extraembryonic differentiation. This study demonstrates T3 can enhance hESC culture and is a useful supplement to advance stem cell applications.

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W-3133

GMP-COMPLIANT MICROCHIP BASED CELL SORTING OF IPSC-DERIVED DOPAMINERGIC PROGENITORS

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In 2018 a first-in-human trial was initiated aiming at investigating the safety and adequacy of iPSC-derived dopaminergic progenitors for treatment of Parkinson's disease. Manufacturing of the cell product comprises a multistep procedure that involves a flow cytometry-based cell sorting (FACS) step at day 12 to 13 of differentiation. Here, Corin+ progenitor cells are isolated and subsequently aggregated in spheres until day 30 when transplanted into the striatum of PD-patients. Until recently, GMP compliant cell sorting could only be achieved by use of a classical high-pressure droplet-based flow sorter with disposable fluidics system. Though purity of Corin+ cells is sufficient to match the cell product specification (>90% purity), cell viability is impacted by the procedure as manifested by reduced replating efficiency of target cells leading to smaller sphere sizes after sort, and eventually low overall cell yields. For reaching desired cell numbers to conduct QC and surgery two days of cell sorting (d12 and 13) with constant operator interaction are currently needed. For assessing the suitability of the fully closed microchip-based cell sorter MACSQuant® Tyto® DA-progenitors were sorted from 2 iPSC lines and analyzed based on the standard release criteria used for the investigational cellular drug product. A head to head comparison to the FACSAriaTM-III system was carried out. Technical sorting results were comparable between both systems (Aria: Purity 96% / Yield 24.5%; Tyto: Purity 92.7% / Yield 25.2%), however Tyto-sorted cells exhibited a significantly increased post sort replating potential leading to a 20% larger sphere diameter and most importantly an 1.7 fold higher overall cell yield after final harvest of spheres. In summary, the data suggests that due to the gentle sort mechanism the fully closed cartridge based low pressure microfluidic sorting system MACSQuant® Tyto® could provide a means to flow sort the needed cell number necessary to generate a clinical patient sample within ~8 hours instead of ~16 hours using the BD InfluxTM system. The modified procedure would ease the sample preparation process at day 12 (1 day of cell processing instead of two consecutive days), significantly reduce operator hands on time and overall user interaction leading to increased usability and product safety.

W-3135

MANUFACTURING HUMAN EMBRYONIC STEM CELL-DERIVED CLUSTERS CAPABLE OF INSULIN SECRETION IN THE PRESENCE OF INCRETIN STIMULUS

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Type 1 diabetes (T1D) is characterized by chronically elevated blood glucose levels as a direct result of beta cell dysfunction and insulin deficiency. The most common treatment is multiple daily insulin injections, which prevents hyperglycemia but puts patients at risk of life-threatening hypoglycemia and a myriad of long term complications because injections are unable to mimic the precise glycemic control of pancreatic islets. Islet transplantation offers a superior alternative, yet its widespread implementation as a form of beta cell replacement therapy is limited by the paucity of cadaveric donor islets and the necessity of chronic immune suppression. Stem cells are potentially an unlimited source of beta cells for the treatment of T1D. Here, we attempt to recapitulate beta cell development starting with human embryonic stem cells (hESCs) using a stepwise differentiation protocol resulting in 3D aggregates comprising of >80% pancreatic progenitors co-expressing key transcription factors NKX6.1 and PDX1. The aggregates are further differentiated into hormone producing endocrine cells in bioreactors. We are also exploring the use of Raman spectroscopy, a label free optical technique, to monitor and detect heterogeneities within distinct stage-specific populations throughout the differentiation process. Perifusion analyses at later stages show the cells become glucose competent in the presence of the gut incretin mimetic exendin-4. However, like immature beta cells, the cells are unresponsive to high glucose alone. Immunodeficient mice implanted with macroencapsulated endocrine clusters had human C-peptide at median levels of 0.44 ng/mL (IQR: 0.3 – 0.5 ng/mL) one hour after oral glucose delivery at 2 weeks post implant. We are developing approaches for large scale production of mature hESC-derived endocrine cells in bioreactors. With further optimization, we hope to generate fully mature endocrine cells that have insulin secretion kinetics similar to human islets that can be tested in animal models of diabetes.

W-3137

INDUCTION OF FUNCTIONAL NOTOCHORDAL CELL DIFFERENTIATION FROM IPS CELLS USING SMALL MOLECULE AND BRACHYURY OVEREXPRESSION

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Back pain is reported to affect about 80% of the adult population. Previous studies have indicated a link between back pain and intervertebral disc (IVD) degeneration. The notochordal cells (NCs) appear to be the ideal cell type to regenerate the IVD: these cells disappear in humans as they mature, are replaced by nucleus pulposus (NP) cells, and their disappearance correlates with the initiation of IVD degeneration. Human NCs are in short supply, thus here we aimed for generation of notochordal-like cells from induced pluripotent cells (iPSCs). Human iPSCs were generated from dermal fibroblasts using non-viral overexpression of six factors: OCT4, SOX2, KLF4, L-MYC, LIN28, and p53 shRNA. Then the iPSCs were treated with GSK3i to induce differentiation to Primitive Streak Mesoderm (PSM), PSM markers were upregulated comparing to the control group and markers of pluripotency downregulated. PSM cells were transfected with Brachyury (Br) encoding plasmid and the cells were encapsulated in hydrogel that mimics the NP environment. The gene expression analysis of the hydrogels showed iPSC-derived NCs (iNCs) retained the NC phenotype consistently for up to 8 weeks. The iNC-condition media (iNC-CM) was found to induce significantly higher expression of NC and NP marker genes in human BM-MSCs than porcine NC-CM. The cells were also tested in vivo in a large animal model. IVD degeneration

was induced using annular puncture in pigs, which was evident as early as 2 weeks after the procedure. The cells were injected after 4 weeks and analyzed at 12 weeks after the injury using MRI, gene expression and histology. The iNC-treated discs were found protected from degeneration. This was evident in histological analysis and changes in the pH levels, indicative of degeneration state of the discs, observed using qCEST MRI. Immunofluorescence stains show that their phenotype was consistent with the in vitro study, namely they still expressed the notochordal markers. In the present study, we report a stepwise differentiation method to generate notochordal cells from human iPSCs. These cells not only demonstrate a sustainable notochordal cell phenotype in vitro and in vivo, but also show the functionality of notochordal cells and have protective effect in case of induced disc degeneration and prevent the change in the pH level of the injected IVDs.

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W-3139

HUMAN EMBRYONIC STEM CELLS DERIVED SERTOLI-LIKE CELLS CAN BE SUCCESSFULLY GENERATED BY CULTURE-INDUCED DIFFERENTIATION

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Sertoli cells (SCs) support germ cells in spermatogenesis. In addition, SCs have immunomodulatory properties against immune stress response. From these properties, SCs are considered useful cell sources for therapeutic application. However, mature SCs are quiescent somatic cells in the testis and have a lower activity for proliferation and primary immature SCs lost their unique properties during prolonged culture. To overcome these disadvantages, novel cell source of SCs independent of donor testis cells is required. Although the direct reprogramming of mouse fibroblasts into embryonic Sertoli-like cells (SLCs) using several factors was reported, this method cannot be applied clinically. Also, SLCs can be derived spontaneously from human embryonic stem cells (hESCs), the efficiency and purity of the SLCs are still unclear. Recently, we successfully established in vitro differentiated SLCs derived from mouse ESCs, which have high purity and function. Based on previous our study, we thus developed SLCs from hESCs using in vitro culture-induced differentiation. To end this, hESCs induced mesendoderm (ME), sequential induced intermediate mesoderm (IM) and SLCs differentiation were included for present study. For induction of ME, glycogen synthase kinase-3 inhibitor (CHIR), were treated in vitro culture. Marker genes for ME were the highest at day 1, suggesting commitment into ME by CHIR. Since then, for IM stage, the cells on the ME were treated with bFGF and RA for 4 days. The adhered cells on ME stage were changed their morphology into a stretched shape with proliferation. During specification into the IM, the markers for IM were simultaneously expressed at day 4. To differentiate into SLCs, bFGF, FGF9, prostaglandin D2, FSH and GDNF were supplemented in culture medium for 14 days. SC markers were expressed at SLCs induction day 7 and 14. And proteins for GATA4 and SOX9 were clearly co-expressed, suggesting putative SLCs (day 7, 31.4 ± 4.1%, day 14, 43.1 ± 2.5%). These results suggest that SLCs derived from hESCs could be differentiated under defined culture condition. Advancing methods such as a MACS sorting as a further study is necessary to acquire high purified SLCs. Based on the present study, generated SLCs derived from hESCs could be a useful source in infertility treatment and cell therapy in clinical application.

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W-3141

THE CHROMATIN REGULATOR - ZMYM2 RESTRICTS HUMAN PLURIPOTENT STEM CELL GROWTH BUT IS ESSENTIAL FOR TERATOMA FORMATION

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Chromatin modifications and conformations play a major role in gene regulation. Here, we aimed to identify the role of chromatin-related genes in the regulation of the growth and transformation of human pluripotent stem cells. We have thus examined the effect of mutations in 360 genes from more than 60 different chromatin-modifying complexes. Mutation in most chromatin-related genes negatively affected the survival of human embryonic stem cells (hESCs). However, 7 members of the LSD1-coREST-HDAC (LCH) complex showed growth advantage in the mutant cells and are thus involved in growth restriction of hESCs. Unintuitively, LCH complex members are highly expressed in undifferentiated hESCs in comparison to all examined somatic tissues and cell lines. The most potent growth restricting chromatic-related gene within LCH is Zinc

finger MYM-type containing 2 (ZMYM2), whose expression is down-regulated upon differentiation either in vitro or in vivo. In order to analyze the function of this gene we have genetically eliminated it using CRISPR-Cas9 technology. The cells with null mutation in ZMYM2 over-expressed pluripotent specific genes, and accordingly showed increased active promoter features by histone-3 acetylation ChIP-Seq analysis. These cells also showed refractory differentiation in vitro. Most amazingly, hESCs lacking ZMYM2 fail to produce teratoma upon their injection into immunodeficient mice. Our results suggest a central role for ZMYM2 in the exit from pluripotency of hESCs, and imply that this process is essential for the transformation of human pluripotent stem cells into differentiated teratomas.

W-3143

MICRORNA LET-7 EXPRESSION AND INFLUENCE ON GERM LAYER FORMATION IN EARLY MOUSE DEVELOPMENT

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MicroRNAs (miRNA) are small non-coding RNAs that negatively regulate gene expression by binding to the 3' UTR of RNAs. Abnormal regulation and expression of miRNAs have been implemented in various diseases including cancer. The let-7 family of miRNAs was discovered in C. elegans to control developmental timing, and is important for differentiation in mammals. In humans, loss of let-7 contributes to carcinogenesis due to an increase in its target oncogenes and stemness factors. Let-7 regulation is complex and poorly understood. Understanding let-7 regulation and function during normal development can assist in understanding its abnormal regulation in cancer. Let-7 has been reported to be absent from human embryonic stem cells and to increase upon differentiation. During early development, let-7 influences differentiation and germ layer specification, favoring ectoderm and mesoderm. We hypothesize that during early mouse development, let-7 levels are regulated on several levels, and rather than constantly increasing, they are tuned to allow the timely expression of targets as required during specific phases of development. We modeled early mouse development via embryoid body (EB) formation from mESCs. We demonstrated that expression of transcription factor Snail can be used to monitor primitive streak and formation of mesoderm and endoderm. Unexpectedly, our results show a drop in let-7 levels upon exit from pluripotency before its increase, correlating with previously published dynamic changes of let-7 targets HMGA2 and Lin-28. We use inducible let-7 overexpression to probe the effect of let-7 on germ layer formation. In conclusion, micro-RNA let-7 family members demonstrate dynamic expression during early mouse embryonic development in order to influence germ layer specification.

Funding Source: Loma Linda University School of Medicine Loma Linda University, School of Medicine, Anatomy Department

W-3145

INTEGRIN ALPHA-5 IS CRITICAL FOR THE EARLY STAGES OF HUMAN PLURIPOTENT STEM CELL CARDIAC DIFFERENTIATION

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Adult heart has limited regenerative capacity that is insufficient to compensate for cell death after myocardial injury. Recently, there have been significant advances in the differentiation of cardiomyocytes from human pluripotent stem cells (hPSC) for cell replacement therapies. It is well-known that stem cell niche plays a key role in growth and maintenance of pluripotency and it deeply influences stem cell differentiation potential. Therefore, our goal was to analyze how interactions between integrins and the extracellular matrix are involved in cardiac differentiation. First, we performed an expression profile of integrins, whose ligands are fibronectin (FN) and laminin, in hPSC and in two stages of cardiac differentiation: mesodermal progenitor cells (MPC) and cardiomyocytes (CM). We found a significant down-regulation of integrins α3, α4 and α6 and an up-regulation of α5 in MPC but a subsequent reduction at CM stage. These results were consistent in two different protocols: from a monolayer and through the derivation of 3D-embryoid bodies. We then focused on the α5 integrin regulation, thus we engineered a DOX-inducible CRISPRi-KRAB cell line to repress α5 expression in the first three days of differentiation. Our results showed a

significant down-regulation of T-brachyury and TBX6 by approximately 50% in dox-treated protocols. Furthermore, the mRNA expression of epithelial to mesenchymal transition (EMT) markers ZEB1 and ZEB2 was also decreased. Taken together, these results suggest that α5 is important for the EMT process and for giving rise to MPC. Finally, at day 15, CM yield was reduced from 60% ± 6% to 22% ± 5% and cardiac markers NKX2-5 and cTnT were significantly down-regulated. We also found that not only there was a reduction on CM differentiation but also on CM contractility. The latter was measured by using a specific contractibility software and found that the contraction and peak amplitude of CM were strongly impaired. In summary, we showed that integrin α5 has a significant impact in human cardiac differentiation from hPSC through the alteration of early stages of mesoderm commitment.

W-3147

CHARACTERIZATION AND FUNCTION OF IPSC-DERIVED ALVEOLAR EPITHELIAL CELLS

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The turnover time of alveolar epithelial type II cells (AECIIs) is very slow in a normal adult lung but is accelerated after experimental pneumonectomy in otherwise a healthy lung. However, this repair and regeneration capacity of AECIIs is harmed after lung injury. Induced pluripotent stem cells (iPSCs) are a promising cell source for lung repair since they can be differentiated into lung lineage cells. To examine their long-term phenotypes and behaviors of the iPSC-derived AECIIs as for a reliable cell source in lung repair and regeneration, we aimed at optimizing published differentiation protocols to achieve iPSC-derived AECIIs and maintain their phenotypes and function for future in vivo application. The two iPSC lines named HDF-SV and HDF-mRNA were differentiated into definitive endoderm, then into the anterior foregut endoderm fate, and subsequently into lung progenitor cells expressing NK2 homeobox 1 (NKX2.1), forkhead box protein A2 (FOXA2) and Transmembrane 4 L Six Family Member 1 (TM4SF1). The lung progenitors were then matured transforming into AECIIs expressing pro-surfactant protein C (ProSPC) and epithelial adhesion molecule (EpCAM) by exploiting small molecules and WNT activator, fibroblast growth factors, phosphodiesterase inhibitor etc. The AECIIs were eventually isolated by undergoing immunomagnetic enrichment using anti-EpCAM micro beads and passaged at 80% confluency every 5 – 7 days for up to 7 passages. We observed a doubling time of the AECIIs of about 4 days, and approximately 94% of the cells were positive for EpCAM and 98% positive for ProSPC at the 7th passage. Phenotype and functional assessments were performed up to 10 passages after the isolation. Our results suggest the feasibility of generating stable AECIIs from iPSCs, which may serve as a promising approach for regenerative research in lung injury.

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W-3149

APPLICATION OF SELECTIVE CYTOTOXIC VIRAL VECTORS FOR SENSITIVE DETECTION OF PLURIPOTENT STEM CELLS IN NEURAL PROGENITOR CELLS

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Cell-processed therapeutic products (CTPs) derived from human induced pluripotent stem cells (hiPSCs) have innovative applications in the regenerative therapy. Since undifferentiated hiPSCs possess tumorigenic potential, there is a potential risk of tumor formation if the products contain residual undifferentiated hiPSCs. The detection limit of the methods currently available for the residual hiPSCs is 1/100,000 (0.001%, undifferentiated hiPSCs/differentiated cells) or more, which could be insufficient for the detection of residual hiPSCs when CTPs contain more than 100,000 cells. To overcome this limit, we have previously constructed adenovirus (Ad) and adeno-associated virus (AAV) vectors expressing a suicide gene, iCaspase9, regulated by the CMV promoter, which is dormant in hiPSCs, for the selective expression of iCaspase9 in differentiated cells. The vectors possessed strong cytotoxicity to hiPSC-derived cardiomyocytes but not to hiPSCs, and concentrated hiPSCs to detectable levels

by some methods. These vectors have a potential to be applied to other cell types because Ad and AAV vectors can transduce various types of cells. In addition to cardiomyocytes, neural cells have been expected to treat diseases such as Parkinson's disease, cerebral infarction, and spinal cord injury, and studies toward the clinical use of neural cells have been conducted by various groups. In this study, we examined the capacity of these vectors to concentrate hiPSC in human neural progenitor cells (hNPCs). Among the viral vectors, we found that the Ad vector transduced into hNPCs with the highest efficiency, eliminating approximately 90% of the hNPCs. Next, we investigated whether the Ad vector concentrated a trace amount of hiPSCs in hNPCs. After 10,000,000 hNPCs supplemented with hiPSCs at a ratio of 0.0001% were infected with the Ad vector, hiPSCs became detectable, while they were undetectable without the infection. The number of hiPSCs detected in the infected cells was almost the same as in uninfected hNPCs supplemented with hiPSCs at a ratio of 0.001%, indicating the vector decreased the detection limit by a factor of 10. These results suggested that the selective cytotoxic viral vectors are useful tools for concentrating hiPSCs in hNPCs and decreasing the detection limit as well as cardiomyocytes.

W-3151

CONTROLLED, EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO DEFINITIVE TROPHECTODERM

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The in vivo embryonic counterpart of cultured human pluripotent stem cells (hPSC) are presumably the cells of the post-implantation epiblast, with a capacity restricted to giving rise to the embryo proper and no longer able to develop into trophectoderm (TE). Previous studies that reported TE differentiation from hPSC remain controversial, in part, due to incomplete differentiation or the use of undefined culture conditions. Here we describe highly efficient TE differentiation, including the generation of cytotrophoblast and syncytiotrophoblast cells, by manipulating specific cell signaling pathways in chemically defined conditions. We monitored the entire differentiation process by live-cell imaging and noted the emergence of cytotrophoblast cells, which was followed by frequent cell fusion events generating multinucleated syncytiotrophoblast cells. Gene expression profiling by using RNA-Seq and immunocytochemical analysis confirmed robust induction of genes associated with trophectoderm and placental development. Among the early markers, GATA3 and KRT7 were strongly induced, whereas CDX2 was expressed at lower levels. Fused multinucleated cells expressed typical markers of syncytiotrophoblast (e.g. DLX3, CGA, DAB2, TEAD3), further corroborating their appropriate molecular identity. Differentiated TE cells were then subjected to Zika virus experiments demonstrating their susceptibility to infection. In summary, although hPSC-derived TE cells remain to be fully characterized by ongoing experiments (e.g. epigenetic mapping), the present findings suggest that cultured hPSC may have a broader developmental potential than previously anticipated. Finally, these in vitro-generated TE cells may prove ideal for modeling diseases of the placenta, drug screening, and cell-based therapies.

W-3153

THERAPY OF HINDLIMB ISCHEMIA BY HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS AND SMOOTH MUSCLE CELLS

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Peripheral artery disease is a condition in which tissue necrosis occurs as blood flow decreases due to arterial occlusion, resulting in limb amputation in severe cases. Both endothelial cells (ECs) and vascular smooth muscle cells (SMCs) are needed for regeneration of peripheral artery in ischemic tissues. However, it is difficult to isolate and cultivate primary endothelial cells and smooth muscle cells from patients for therapeutic angiogenesis. Induced pluripotent stem cells (iPSC) are regarded as useful stem cells due to their pluripotent differentiation potential. In this study, we explored the therapeutic efficacy of human iPSC-derived ECs and SMCs on peripheral artery disease in a murine ischemic hindlimb model. After induction of mesodermal differentiation of iPSC, CD34-positive vascular progenitor cells were isolated by magnetic-activated cell sorting. Cultivation of the CD34-positive cells in endothelial culture medium induced expression of endothelial markers, including CD31, VE-cadherin,

and vWF, and endothelial characteristics, such as endothelial tube forming ability, eNOS expression, and Ac-LDL uptake. Moreover, the CD34-positive cells could be differentiated to not only ECs but also SMCs. Cultivation of the CD34-positive cells in SMC medium induced expression of SMC marker. In a murine hindlimb ischemia model, co-transplantation of iPSC-ECs with iPSC-SMCs accelerated blood perfusion and increased limb salvage rate in the ischemic limbs, compared to the ischemic limbs injected with either iPSC-ECs or iPSC-SMCs alone. Moreover, co-transplantation of iPSC-ECs with iPSC-SMCs further stimulated angiogenesis and that transplanted iPSC-ECs and iPSC-SMCs contributed formation of ILB4-positive capillaries and α-SMA-positive arteries/arterioles. These results suggest that combined treatment of iPSC-ECs and iPSC-SMCs differentiated from iPSC is useful for therapy of peripheral artery diseases.

W-3155

GENERATION OF MGE-LIKE INTERNEURONS FROM HUMAN EMBRYONIC STEM CELLS

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Amongst the different regions of the primitive brain, the medial ganglionic eminence (MGE) produces most of the cortical interneurons (CI) which mainly express parvalbumin (PV) and somatostatin (SST). These CI play an important role in the wiring of the developing nervous system and their dysfunction has been implicated in various psychiatric disorders. Therefore, the generation of enriched populations of MGE-like interneurons from human embryonic stem cells (hESCs) would greatly aid in understanding MGE CI dysfunctions in disease. Unfortunately, current protocols fail to obtain substantial amounts of the different subtypes of CI. To obtain MGE-like CI from hESCs, we optimised a differentiation protocol by varying the concentration of two key morphogens important for patterning; sonic hedgehog and WNT. hESCs (H7 and H9) were used for CI differentiation with various combinations of different concentrations of patterning molecules, resulting in a total of twelve conditions. Bulk quantitative real time polymerase chain reaction (qRT-PCR) was performed to measure the relative gene expression of markers for MGE progenitors and mature CI; subsequently four conditions were selected for further analysis. Single cell qRT-PCR and fluorescent in situ hybridisation with RNAscope probes were performed to measure the percentage of cells expressing the MGE CI marker LIM Homeobox 6 (LHX6) and the two MGE CI subtype markers (PV and SST). One condition clearly produced an enriched population of MGE-like CI with a majority of cells expressing LHX6 and SST and a significant increase of PV expressing cells in comparison to other conditions. To assess the functionality of the generated CI with this chosen condition, single cell patch clamping was employed. Active and passive membrane properties were investigated: neurons rested at around -45mV; fired action potential spontaneously and upon current injection; and showed spontaneous synaptic activity. Therefore, we have developed and validated a robust CI differentiation protocol which could be used to investigate mechanisms underlying MGE CI dysfunction and unravel biological pathways and markers implicated in the aetiology of disorders such as autism, epilepsy and schizophrenia.

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PLURIPOTENT STEM CELL: DISEASE MODELING

W-3159

BRAINSTORM: A NOVEL METHOD TO REVEAL NEURON SPECIFIC DEVELOPMENTALLY REGULATED EQTLS

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The World Health Organization estimates that the global burden of neuropsychiatric disorders exceeds that of cancer and cardiovascular disease combined. Despite vast unmet medical need, the development of novel therapeutics for neuropsychiatric disease like Schizophrenia (SCZ) has substantially lagged because the molecular underpinnings of disease remain unclear. Sequencing approaches have identified specific genes, a large number of haplotypes, ultra-rare coding variation, and chromosomal abnormalities that all affect the risk for neuropsychiatric disease, nominating networks related to neural synapse function, signaling, and development. Rapid methods are currently available to direct the differentiation of human induced pluripotent stem (iPS) cells into cells that resemble glutamatergic excitatory neurons. However, while these glutamatergic neurons fire electrical impulses and integrate into circuits, they lack morphological features of mature neurons such as dendritic spines and fail to express genes present in adult human neurons, vastly limiting their utility for investigating the relationship between natural genotypic variation that exists within human populations and expression of genes implicated as risk factors for SCZ. To overcome this limitation, we developed a novel technique termed BrainStORM (Stimulated Ontogenesis by Rodent-induced Maturation), in which early post mitotic iPS cell derived glutamatergic neurons are

implanted intracerebroventricularly into immunocompromised neonatal mice to allow integration into the developing rodent neural network. Single cell sequencing is used to determine whether implantation into a highly vascularized developing nervous system is sufficient to propel human iPS cell derived neurons beyond the maturation hurdle experienced in traditional 2D and 3D culture. By applying the BrainStORM technique to neurons derived from villages of human iPS cells, we capitalize on the power of large-scale population genomics to identify expression quantitative trait loci (eQTLs) that control neuronal gene expression in a developmentally regulated manner and reveal insights into novel genes whose therapeutic manipulation could benefit patients with psychiatric disease.

W-3161

USING HUMAN EMBRYONIC AND INDUCED PLURIPOTENT CELL MODELS TO DETERMINE CORTICAL NEURONAL CELL FUNCTION IN A GENETICALLY DETERMINED FORM OF DYSTONIA.

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Dystonia is a hyperkinetic movement disorder caused by co-contraction of antagonistic muscles, resulting in abnormal positions and postures. It is one of the most common and functionally disabling movement disorders, with significant associated lifetime disability. Due to a limited understanding of the underlying pathophysiology of the disorder, there are few therapeutic options available, with many patients resistant to currently available treatments. Myoclonus Dystonia (MD), caused by mutations to the autosomal dominant epsilon-sarcoglycan gene (SGCE), is fully penetrant when paternally inherited, making a neuronal cell model an ideal platform to further characterise dystonia pathophysiology. Using the CRISPR/Cas9 gene editing technique, we have generated two distinct human embryonic stem cell (hESC) homozygous SGCE knockout lines (SGCEko). Edited hESC lines show no off-target effects when analysed by SNP array. The SGCEko lines differentiate into cortical glutamatergic neurons in a similar manner to their wildtype parental cell line, with comparable levels of neural progenitor cell (NPC) markers PAX6 and FOXG1 ($p>0.05$), and cortical layer markers CTIP2 and TBR1 ($p>0.05$). Additionally, studies show a significant increase in calcium activity in the SGCEko hESC-derived cortical neurons compared to the wild-type parental line ($p<0.05$). Furthermore, we have derived induced pluripotent cell lines (iPSC) from the blood of two patients with distinct SGCE mutations (nonsense (c.289C>T, p.Arg97X) and missense (c.622G>A, p.Gly441Asp)). iPSCs maintain a normal karyotype and stain positive for the pluripotency markers NANOG and OCT4. These cells are able to undergo cortical neuronal induction, with no significant differences in NPC markers compared to controls ($p>0.05$). Investigations are ongoing to analyse deep layer cortical markers. Overall, these results suggest that although PSC models of MD demonstrate no significant differences in recognised markers of cellular differentiation, calcium imaging suggests potential functional differences which may contribute to the subsequent observed clinical phenotype. Further work is planned to better characterise these differences, and to relate these to potential variations in gene expression and gene ontological pathways.

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W-3163

IPSCS FROM AML CELL LINE HL-60 RETAIN LEUKEMIC ABERRANCIES WHILE IPSCS FROM RELAPSED/REFRACTORY PATIENT SHOW REPROGRAMMING CAN CIRCUMVENT LEUKEMIC GENOME IN ADVANCED AML

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Acute myeloid leukemia (AML) is the most common form of acute leukemia affecting adults, characterized by defects in hematopoietic differentiation and accumulation of immature myeloid cells. Although AML is a complex disease that presents with a variety of genomic aberrations affecting prognosis, a course of cytotoxic chemotherapy is used as the treatment standard. While this treatment can lead to a remission period in a majority of cases, many patients still suffer a fatal relapse. Patients in remission are candidates for hematopoietic stem cell therapy (HSCT), but autologous transplantations cannot correct for disease-causing genetic aberrations, and allogeneic transplantations are hampered by lack of matched donors and risk of severe complications. Further study of AML is required to gain a better understanding of how diverse disease genetics affect disease development and progression, and to develop more personalized and effective treatments. Induced pluripotent stem cells (iPSCs) provide a novel approach for studying AML. Here we report reprogramming hematopoietic stem cell-enriched (CD34+) cells isolated from the bone marrow of a relapsed/refractory AML patient containing a rare der(7)t(7;13) karyotype, and the AML cancer cell line HL-60. While iPSCs generated from both the primary AML sample and the HL-60 cell line display expression of pluripotent genes and cell surface markers at similar levels to iPSCs generated from disease-free cells, primary AML-derived iPSCs show no evidence of the original leukemic genetic aberrations and display restored myeloid differentiation potential. The generation of karyotypically normal iPSCs without gene correction from an AML patient with advanced disease provides support for reprogramming as a potential therapeutic option in complex AML. In contrast,

iPSCs generated from the HL-60 leukemic cell line retain many of the genetic and chromosomal abnormalities in the parent line, and appear to be deficient in differentiation potential. To our knowledge, this is the first successful generation of iPSCs from a human immortalized AML cell line. Together, our findings provide valuable tools and insight to study and treat complex AML disease.

W-3165

A CRISPR-I SCREEN IN HUMAN IPSC-DERIVED INEURONS TO DISCOVER NOVEL FOCAL CORTICAL DYSPLASIA GENES

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Focal cortical dysplasia (FCD) is a common cause of focal epilepsy and is often caused by somatic, mosaic mutations. Unfortunately, the sparse nature of these mutated cells presents many experimental barriers to FCD gene discovery; identified FCD mutations only occur in 1-6% of cells in resective tissue. Therefore, identifying novel genes is extremely challenging and may not be possible in many cases using patient tissues. Currently, ~30% of FCD samples are estimated to have mutations in identified FCD genes. These genes were identified by hypothesis-driven targeted sequencing rather than unbiased sequencing. Therefore, we have developed an unbiased screening platform for identifying novel FCD genes in vitro using a genome-wide inhibitory CRISPR library. We first reprogrammed human iPSC lines stably expressing both KRAB-dCAS9 and Neurogenin (NGN) 1,2 genes under control of a doxycycline (DOX)-inducible promoter. The inducible expression of NGN1,2 allows for the efficient, uniform differentiation of human excitatory cortical neurons. The KRAB-dCAS9 inhibits the expression of genes targeted by a guide RNA (gRNA) sequence introduced via lentivirus, with 80-95% knockdown of mRNA and protein after DOX treatment in prior studies. We are using phosphorylated S6 ribosomal protein (pS6), a biomarker for FCD type II, as the selection assay in our screen. Using a pS6 antibody and FACS sorting, we were able to obtain genomic DNA from pS6-high and -low cell populations. To validate our screen, we constructed a test library of 27 gRNAs that were expected to be positive, negative, or neutral regulators of pS6. The pS6-sorted neuronal genomic DNA samples were amplified using primers flanking the gRNA portion of the lentiviral insertion. The abundance of each gRNA sequence was then assessed using NGS. As expected, the gRNAs for FCD genes were significantly enriched in the pS6-high sample while gRNAs for genes necessary for S6 phosphorylation were significantly enriched in the pS6-low sample. A genome-wide gRNA library containing 5 unique sequences for each human gene (>100,000 total gRNAs) is now being applied to identify novel genes. Validated FCD gene candidates will offer additional targets for sequencing from patient tissues and the potential for new therapeutic strategies for FCD-associated epilepsies.

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W-3167

MEA-SEQ FOR COMBINED GENE EXPRESSION AND NEURONAL NETWORK MEASUREMENTS IN HUMAN IPSC-DERIVED NEURONS FROM KOOLEN-DE VRIES PATIENTS

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Koolen-de Vries syndrome (KdVs) is an intellectual disability syndrome caused by a mutation in KANSL1 or by a 17q21.31 microdeletion, a region which contains multiple genes including KANSL1. To study Koolen-de Vries syndrome in vitro, we generated induced pluripotent stem cells (iPSCs) from fibroblasts of KdVs patients and healthy controls. Additionally, we introduced a loss-of-function mutation in KANSL1 in healthy control iPSCs using the CRISPR/Cas9 system to study its effect in an otherwise congenic background. iPSCs were differentiated into neurons (iNeurons) by forced expression of neurogenin-2 (Ngn2) resulting in a homogeneous population of mature excitatory neurons within 30 days. Micro-electrode arrays (MEAs) were used to measure neuronal activity of iNeurons at different time points during differentiation. Interestingly, mature iNeurons from KdVs patients showed decreased network activity and generated less network bursts. Little is known about the mechanisms underlying this phenotype. Studying gene expression changes in iNeurons of KdVs patients gives insight in biological processes that are affected. Therefore, we optimized an RNA-seq method that can be used in combination with MEA experiments. The RNA-seq library preparation consist of generating cDNA molecules from RNA molecules with a poly(A) tail and labeling them with a sample-specific barcode, followed by pooling of samples. Subsequently, transposon 5 transposase is used for fragmentation of cDNA which at the same time inserts an adapter at the cut site, needed for further amplification and sequencing of molecules of interest. The

ability to pool samples together allows us to screen a large set of samples at low cost. We have now combined this RNA-seq method with MEA experiments performed on 24-well plates to allow for semi high-throughput combined experiments (MEA-seq). This enables us to correlate changes in gene expression to specific network phenotypes. MEA-seq can also be used to study neuronal phenotypes of any other neurodevelopmental disorder.

W-3169

SCHIZOPHRENIA RISK GENE DLG2 CONTRIBUTES TO CORTICAL NEURON DEVELOPMENT FROM HUMAN EMBRYONIC STEM CELLS

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Discs large homologue 2 (DLG2) is a membrane associated guanylate kinase protein with an established role as a scaffold in the postsynaptic density (PSD) to regulate receptor clustering and intracellular trafficking. As with other PSD proteins DLG2 is known to play an important role in higher cognition, while recurrent de novo deletions of DLG2 have been identified in individuals with schizophrenia. Unlike previous studies focusing on DLG2 in mature synapses this research investigated a potential neurodevelopmental role, using human embryonic stem cells as a model system. The phenotype of both DLG2 deficient and wild-type cell lines were characterised during cortical differentiation using RNA sequencing, western blotting and immunocytochemistry analysed by high content imaging. Results show that DLG2 is required for normal cortical neuron development, with deficient cells being able to form postmitotic neurons in similar levels to wild-type controls but with disruption in the expression of established cortical layer markers including TBR1 and CTIP2. In addition, DLG2 protein was found to be expressed in neural precursor cells (NPCs) and appears to have a key effect during this early stage of neural development, which is prior to PSD formation. This is supported by RNA sequencing data showing the greatest differential gene expression (DGE) occurred during the NPC stage of neuronal differentiation (almost 37% of protein coding genes), compared to the later postmitotic neuron stage (21.5%). Additionally, this DGE in NPCs showed a functional enrichment for gene ontology terms relating to key development processes including cellular differentiation, proliferation, adhesion and migration. This is in agreement with experimental data showing significantly increased proliferation and altered adhesion to various extracellular matrix components in DLG2-deficient NPCs. Therefore, this research strongly indicates that in addition to its established function of synaptic signal transduction as a component of the PSD, there is a currently unreported developmental role for DLG2. This has implications for understanding the nature of schizophrenia aetiology and suggests DLG2 may contribute to developmental insults leading to the disease as well as aberrant neuronal function in later life.

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W-3171

USING INDUCED PLURIPOTENT STEM CELLS FOR MODELING THE NEURODEGENERATION IN MUCOPOLYSACCHAIDOSIS TYPE II

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Mucopolysaccharidoses (MPS) are lysosomal storage disorders that are caused by deficiency of lysosomal enzymes for degrading glycosaminoglycan (GAG). MPS type II, which is a lack of the lysosomal enzyme iduronate-2-sulfatase (IDS), is the most common type of MPSs in Taiwan. Patients of certain subtypes of MPS II patients suffer from progressive neurodegeneration and currently no effective medicine is available. Recently, we have successfully reprogrammed the peripheral blood cells of four MPS type II patients with neurodegeneration into multiple iPSC clones. We have validated these MPS II-iPSC clones, including positive expression for pluripotency makers, normal karyotyping, and successful differentiation into three embryonic germ layers in vitro and in vivo. More importantly, we have successfully recapitulated the MPS II phenotypes in the neurons derived from these MPSII-iPSC clones, such as lower IDS activity, increased GAG accumulation, and enhanced express of LC3 and LAMP-1. In addition, RNA sequencing analysis revealed a distinct gene expression profile of MPS II-iPSC-derived neurons compared to control iPSC-derived neurons; electrophysiology study also showed unique action potential abnormalities of MPS-II-derived neurons. We also demonstrated that this platform can be used to test drugs for MPSII-related neurodegeneration. The ultimate goal of this study is to provide the scientific community a valuable in vitro MPS II neurodegeneration model that can be translated for multiple diagnostic and therapeutic purposes in the near future.

W-3173

MODELING CARDIOMYOPATHY IN FRIEDREICH'S ATAXIA WITH PATIENT-DERIVED CARDIOMYOCYTES

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Friedreich's Ataxia (FA) is the most commonly inherited ataxia and is caused by a GAA repeat expansion in the intron of the frataxin gene that results in reduced expression. Heart conditions are prevalent in patients with FA, severely affecting the quality of life and shortening life span. There is currently no treatment for FA, therefore developing small molecule therapeutics that increase frataxin expression is significantly needed. In this study, we aimed to establish an in vitro model of FA cardiomyopathy and to develop high-throughput assays for frataxin detection. To this end, we differentiated iPSCs obtained from patients with FA into cardiomyocytes and demonstrated that they express cardiac genes, contract spontaneously, and respond to chronotropic drugs (e.g., isoproterenol and hERG channel blockers). High throughput assays to measure frataxin mRNA and protein levels were established and demonstrated reduced expression of frataxin in FA cardiomyocytes (~20% of healthy control levels). Functional interrogation of FA cardiomyocytes was performed by microelectrode array recordings, plate-based calcium imaging (FLIPR-Tetra), and the Seahorse XF platform. Our data show significant reductions in field potential amplitude, spontaneous calcium transient amplitude, and oxygen consumption rates in FA cardiomyocytes when compared to healthy controls. Such functional deficits in FA cardiomyocytes were phenocopied by antisense oligonucleotide-mediated knockdown of frataxin in healthy controls. In summary, we have established an in vitro system to study FA cardiomyopathy with patient-derived cardiomyocytes that could be leveraged to perform chemical probe and genetic knockout screenings for the purpose of identifying novel targets and initiating drug discovery efforts to treat FA.

W-3175

PROBING THE AMYLOID CASCADE HYPOTHESIS IN A HUMAN PLURIPOTENT STEM CELL BASED 3D MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most prevalent age-related neurodegenerative disease. So far, no curative treatment for AD patients exists. Prominent hallmarks of the disease are aggregation of amyloid β (Aβ) with subsequent formation of Aβ plaques in the extracellular space, and the formation of intraneuronal neurofibrillary tangles composed of hyperphosphorylated aggregated tau. Aβ aggregation and tau pathology have been considered to conspire at an early stage of the disease process to elicit downstream alterations such as synaptic loss, metabolic dysfunction, neuroinflammation and eventually neuronal death. However, it has remained a challenge to experimentally validate the classic amyloid cascade hypothesis (ACH), and novel human-specific model systems are required to disentangle the pathogenic role of these diverse disease-driving events. Here we set out to probe ACH using pluripotent stem cell-derived neural stem cells conditionally expressing mutant APP (APPSwe/Lon – PS-1ΔE9) in a 3D matrix suitable for capturing extracellular Aβ. Eight weeks after transgene induction, differentiated 3D neuronal cultures contained Aβ deposits which strongly resemble human amyloid plaques and can be visualized by specific autofluorescence and amyloid staining dyes such as Thioflavin T. Transgene-expressing cultures not only displayed increased neuronal tau phosphorylation, but also evidence of intracellular tau aggregation. Interestingly, these alterations were associated with signs of mitochondrial dysfunction, dystrophic neurites and DNA damage. Over time, we observed an increased fraction of apoptotic cells. Our findings underpin the pivotal role of Aβ

in eliciting a cascade of AD-associated cellular alterations and depict 3D matrix cultures as a versatile in vitro model to explore the hierarchy and interrelationship of pathogenic pathways driving AD-related neuronal damage.

W-3177

X CHROMOSOME MONOSOMY IN HUMAN PLURIPOTENCY AND TROPHOBLAST DIFFERENTIATION

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X chromosome monosomy (45,X) is estimated to be the only viable human monosomy, and results in Turner's Syndrome (TS) in females. However, only 1% of XO conceptuses are estimated to survive to term. The cause of this developmental failure is currently unknown, but previous work, combined with the high rates of mosaicism seen in TS patients, suggests an impact on extra-embryonic cell lineages. We hypothesize that genes on the X chromosome that escape X inactivation and have Y-linked homologues are haploinsufficient during development. To investigate the effects of X monosomy, we have generated sets of isogenic aneuploid and euploid male and female iPSC lines. The use of isogenic lines excludes the impact of genetic variation when comparing cells from unrelated individuals. We performed RNA-seq to understand the impact of X monosomy in pluripotency and identified several significantly dysregulated genes in common among the male and female X-monosomic samples. To investigate the impact of X monosomy in an extra-embryonic cell lineage, we differentiated the iPSCs along the trophoblast lineage. Molecular characterizations of these cells, including assays for cell fusion capability, RT-qPCR and immunocytochemistry for trophoblast maker genes, and secretion of human chorionic gonadotropin, revealed no obvious impairment in the ability of X-monosomic cells to commit to the syncytiotrophoblast lineage. We present phenotypic analyses and gene expression studies of syncytial and mononuclear trophoblast cells. While X-monosomic cells appear capable of differentiating along the trophoblast lineage, further quantification of the cell fates will be necessary to fully assess cell-type specific impacts of X monosomy.

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W-3179

USING AN IPSC DERIVED MIDBRAIN DOPAMINERGIC MODEL TO ELUCIDATE THE ROLE OF MANGANESE IN PHYSIOLOGICAL AND PATHOLOGICAL DISEASE STATES

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Manganese (Mn) is an essential trace metal that is important for many physiological processes and crucial for neuronal cell function, as it acts as cofactor for multiple enzymes. Disorders leading to Mn imbalance in humans can lead to a broad spectrum of clinical phenotypes, including parkinsonism-dystonia, severe neurodevelopmental delay, chronic liver disease and polycythaemia. Bi-allelic mutations in SLC39A14, SLC39A8, and SLC30A10 have been identified in over 60 individuals, associated with manganese dyshomeostasis. All three genes code for membrane transporters postulated to shuttle manganese between different cellular/extracellular compartments. Mutations in SLC39A14 and SLC30A10 lead to Mn accumulation, whereas mutations in SLC39A8 lead to hypomanganesaemia and developmental delay. The disease mechanisms leading to Mn dyshomeostasis and associated neurological phenotypes are poorly understood. Therefore, we have developed a midbrain dopaminergic neuronal cell model, differentiated from patient-derived induced pluripotent stem cells (iPSC), to elucidate key pathophysiological processes governing these disorders. iPSC from patient fibroblasts carrying homozygous SLC39A14, SLC39A8, or SLC30A10 mutations, as well as age-matched controls, have been generated using a CytoTune Sendai reprogramming strategy. Patients and control lines have been directed into dopaminergic precursors with high efficiency, and further differentiated into midbrain dopaminergic neurons (mDA). Preliminary Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis reveals disease-specific neuronal phenotypes with abnormal Mn, iron and zinc uptake, suggestive of heavy metal dyshomeostasis in mDA. Overall, our neuronal disease model of Mn-related diseases will be an ideal platform to better understand putative disease mechanisms and will be of great interest to test new future novel therapies.

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W-3181

A HUNTINGTON DISEASE-RELATED PLATFORM FOR SCREENING HUMAN CARDIAC FUNCTION IN PATIENT AND CONTROL HIPSC-CARDIOMYOCYTES

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Huntington disease (HD) is an inherited neurological disorder usually diagnosed in midlife. The underlying genetic cause is an increase in the number of sequential cytosine, adenine, and guanine (CAG) trinucleotide repeats in the first exon of the Huntingtin gene (HTT), leading to a longer poly-glutamine (polyQ) chain within the HTT peptide; 36Q or more is usually regarded as pathogenic. HD clinical manifestation of motor, cognitive and psychiatric disturbances are due to neurodegeneration in the basal ganglia and cerebral cortex. However HTT is expressed in many tissues of the body, and recent studies have highlighted that HD patients show peripheral organ dysfunction such as severe metabolic phenotype, weight loss, HD-related cardiomyopathy and skeletal muscle wasting. In some cases these may even contribute or comprise HD patient cause of death, although mechanisms underlying these aspects of the disease are poorly understood. To begin modelling some aspects of these dysfunctions, we have generated human induced pluripotent stem cells (hiPSCs) from multiple HD-patients with a range of disease severity and polyQ, and begun correcting them using CRISPR-Cas9 to generate isogenic control lines. Following hiPSC-cardiomyocyte (hPSC-CM) differentiation, preliminary experiments indicate a difference in the metabolic activity of HD and control hiPSC-CMs. Establishing such a platform is enabling us to screen for pathogenesis of the mutant CAG repeats, and even identify problems with HD drug treatment regimes, on the cardiac function of specific HD patients.

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W-3183

FACILITATED GENOME EDITING IN HUMAN INDUCED PLURIPOTENT STEM CELLS TO ADVANCE DISEASE MODELING AND SCREENING

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Human induced pluripotent stem cells (hiPSCs) have been globally recognized as a multipurpose research tool for modeling human disease and biology, screening and developing potential therapeutic drugs, and implementing cell and gene therapies. The ability to differentiate human iPSCs into any cell type supports the study of biology and disease in these specified cells in vitro. The emergence of genome editing tools, inlcuding the CRISPR/Cas9 system or TALENs, enable genetic modification of these cells; such as introduction of single base changes or inserting reporters or bio-sensors, which can be used to study the effects of genetic differences or biological functions in the desired cell type. Given the challenges with genome editing efficiency, cell survival and clonal isolation, we have developed a number of reagents and processes to dramatically improve the success rate and timelines for a genome editing experiment in hiPSCs. Key areas of the genome editing workflow that have been addressed include the genome editing tools themselves, the delivery methods and the maintenance of healthy hiPSC cultures during these stressful manipulations. Using the generation of disease models relevant to cardiac and neuronal disease to explore and identify the best workflow for the genome editing process in hiPSC, we built a reliable approach that reproducibly supports the generation of hiPSC lines carrying small mutations such as SNPs or small deletions. Subsequent studies of the disease relevant cell types then identified cellular phenotypes that corroborated with those previously identified in patient-derived hiPSC-based models. Furthermore, we explored the use of these tools and workflows to insert larger DNA pieces into specific genomic loci, to generate fluorescent reporter cell lines for screening for example. Using a small number of loci, we found that introduction of large DNA donors into specific loci was dramatically lower compared to the introduction of SNPs, yet the efficiency obtained was significant enough to allow for the clonal isolation of the edited cells. In summary, we detail advances with tools, reagents and protocols that facilitate the genome editing workflow in hiPSC and demonstrate that the use of such tools can be readily implemented to generate hiPSC-derived disease models and reporter lines.

W-3185

IDENTIFYING SMALL MOLECULE REGULATORS OF COMPLEMENT COMPONENT C4 IN HUMAN STEM CELL DERIVED ASTROCYTES

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The complement system plays a crucial role in the elimination of synapses during development. While this process is necessary to create functional neuronal circuits, dysregulation of synaptic pruning has been associated with a variety of CNS disorders. Recently, genetic variation in the copy number and expression of complement component 4 (C4) has been shown to be associated with an increased risk of schizophrenia. This has led to the hypothesis that loss of synapses in schizophrenic patients might be due to excessive complement-mediated synaptic pruning. Targeted inhibition of the complement system, especially using blocking antibodies, is a realistic therapeutic avenue that is being explored broadly, especially in neurodegenerative diseases. To identify regulators of C4 levels that might be useful in treating a uniquely human disease such as schizophrenia, we performed an ELISA-based small molecule screen using stem cell-derived astrocytes. First, to produce astrocytes at sufficiently large scale, we developed a 3D protocol for the rapid differentiation of a substantially pure population of functional astrocytes. These cells were used to perform a small molecule screen of around 500 compounds, each tested in triplicate at 2 concentrations. Secondary validation of 25 hit compounds performed in unaffected and schizoaffective patient derived astrocytes, highlighted different pathways involved in the modulation of secreted C4. Among the identified hits, we explored the mechanism of action of an epigenetic modifier, (+)-JQ1. Treatment of astrocytes with (+)-JQ1 decreased several critical complement components (C1q, C3 and C4) via depletion of BRD4 on the chromatin fraction. Moreover, (+)-JQ1 was also able to suppress pro-inflammatory cytokine secretion. We then demonstrated that the decrease in secreted C4 from astrocytes affects the amount of synaptic C4 in neurons. Lastly, in vivo treatment of mice with (+)-JQ1 significantly reduced C4 mRNA in the pre-frontal cortex, a key area for higher cognitive function. In summary, using a novel screening approach, our work has start to shade light on mechanism of regulation of C4 in in vitro and in vivo, opening new avenue for the modulation of synaptic pruning in neuropsychiatric and neurodegenerative diseases.

W-3187

DEVELOPING IPSC DERIVED HUMAN NEURONAL CELL MODEL FOR MUCOPOLYSACCHARIDOSIS IIID

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Mucopolysaccharidosis type IIID (MPS IIID; Sanfilippo D) is a devastating pediatric neurodegenerative disorder with no cure or effective treatment available. The fundamental cause of MPS IIID is an inherited mutation in glucosamine (N-acetyl)-6-Sulfatase (GNS) required to catabolize heparan sulfate (HS). Without functional GNS, glycosaminoglycan accumulates in lysosomes. We have generated human induced pluripotent stem cells (iPSCs) from two MPS IIID patients. To model this brain disease, we are making iPSC derived neuronal cells and cerebral organoids by comparing with healthy control cells. This method can give rise to the developing cerebral cortex, ventral telencephalon, choroid plexus, among others, within one to two months. Furthermore, since organoids can be maintained for more than a year in long-term culture, they also have the potential to model later events such as neuronal maturation and survival. We will perform unbiased proteomic analysis using liquid chromatography tandem mass spectrometry to identify protein biomarkers. We will demonstrate disease-related phenotypes and model pathogenesis and test the effectiveness of different therapeutics.

REPROGRAMMING

W-3191

FUNCTIONAL UNCOUPLING OF INDUCING AND MAINTAINING PLURIPOTENCY BY OCT4

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The transcription factors (TF) OCT4 is essential for maintaining pluripotency in embryonic stem (ES) cells and inducing pluripotency from differentiated cells. Unlike maintaining pluripotency however, reprogramming is highly inefficient, and little is known about how OCT4 functionally-contribute to pluripotency from such diverse cellular contexts. Here, we have

dissected the reprogramming and pluripotency maintenance activities of OCT4 apart by systematically mutating the protein. We found that inducing pluripotency and maintaining pluripotency require functionally distinct regions of OCT4. We present extensive protein-protein and protein-DNA networks of both the essential and non-essential domains of OCT4, elucidating the divergence between reprogramming and pluripotency maintenance. We discovered a subset of OCT4 chromatin-associated partners that are essential for inducing pluripotency but not required for maintaining pluripotency. Altogether, our findings provide new insights into the functional-plasticity displayed by transcription factors to control specific cellular identity from diverse cellular environments.

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W-3193

IN-DEPTH META-ANALYSIS OF CELLS UNDERGOING REPROGRAMMING INTO PLURIPOTENCY AND TROPHECTODERM STATES

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During early embryogenesis, totipotent cells undergo asymmetric cell divisions, resulting in two compartments in the early embryo: the inner cell mass (ICM) that gives rise to the embryo proper and Trophectoderm (TE) that forms extraembryonic tissues such as the placenta. It is only at the 32-64- cell stage when a clear segregation between the two cell types can be observed. This proposes a 'T'-like model where cells of the early embryo undergo a relatively similar changes in their transcriptome and epigenome before specification (2-32-cell stage). Here, we sought to understand whether cells acquiring pluripotency and trophectoderm, by reprogramming factors, share similar processes between themselves such as those of the early embryo. To that end, we conducted a parallel meta-analysis on cells undergoing reprogramming into induced pluripotent stem cells (iPSCs) and induced trophoblast stem cells (iTSCs) and compared their transcriptome, epigenome and chromatin architecture along the process. Our analysis revealed that, in contrast to cells of the pre-segregation embryo that resemble each other in each stage, cells undergoing reprogramming to pluripotency and trophectoderm, exhibit a unique and specific trajectory from the beginning of the process till the end. Although similar processes such as somatic identity loss, proliferation, MET and metabolic shift occur in the two models, each of the processes uses a completely different set of genes and regulatory elements to induce its own state. This parallel meta-analysis may serve as a powerful tool to understand nuclear reprogramming and cell fate.

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W-3195

E6 AND E7 HUMAN PAPILLOMA VIRUS ONCOGENES FACILITATE REPROGRAMMING BY OCT4-SOX2-KLF4-MYC OF FIBROBLASTS AND ASTROCYTES INTO IPSCS WITH HIGH PLURIPOTENCY CAPACITY

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Cell proliferation is a requirement for the derivation of induced pluripotent stem cells (iPSC) from mouse embryonic fibroblasts (MEFs) using the reprogramming factors Oct4, Sox2, Klf4, and Myc (OSKM). In the present study we evaluated the influence of E6 and E7 Human Papilloma Virus (HPV) oncogenes in the dynamics and efficiency of iPSC derivation from MEFs and from primary postnatal astrocytes using a OSKM dox-inducible system. Actively proliferating MEFs (doubling time of 0.7-0.8 days) containing OSKM+E6E7 reprogrammed with 10-20-fold higher efficiency (2.45±0.75%) than in the presence of OSKM alone (0.16±0.06%); either OSKM+E6 or OSKM+E7 were also able to promote an increase in reprogramming efficiency (1.08±0.99%

and 1.49±0.78%, respectively). In addition, emergence of colonies with iPSC appearance occurred earlier in the presence of OSKM+E6E7 than with OSKM alone (7 days post-dox vs. 11 days post-dox) reaching the pluripotency commitment (i.e., with the ability to grow in 2i medium without dox) at about 14 days post-dox. Reprogramming efficiency of MEFs dividing very slowly (doubling time above 2.2 days) significantly decreased with OSKM alone (<0.05%) but, interestingly, in the presence of E6E7, E6 or E7 reprogramming efficiency remained high (2.74±0.84%, 1.89±0.53% and 2.63±1.21%, respectively). The iPSC identity of the colonies generated from these experiments were confirmed by their ability to grow in the 2i medium without dox. Similar experiments were performed with postnatal astrocytes at the doubling time of 2.2 days, which reprogrammed into iPSCs only in the presence of E6E7 at an approximate 2% efficiency. Cells from an iPSC clone derived from these experiments grew in the 2i medium without dox, showed a normal karyotype and were able to generate embryo chimeras with a high iPSC contribution to most tissues. Therefore, HPV oncogenes promote high reprogramming efficiency into iPSCs retaining high pluripotency capacity.

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W-3197

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM HUMAN RENAL EPITHELIAL CELLS USING A RNA-BASED REPROGRAMMING METHOD

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Induced pluripotent stem cells (iPSCs) demonstrate a tremendous potential in the study of disease and regenerative medicine. However, the use of iPSCs is limited by the availability of primary cells suitable for reprogramming and low reprogramming efficiency. iPSCs can be generated from primary cell sources including hair follicles, skin punch biopsies, blood and urine samples. Unlike other sources, exfoliated renal epithelial cells (RECs) found in urine samples can be quickly and non-invasively obtained, making them an attractive cell type for reprogramming. Current methods for the reprogramming of RECs include electroporation of non-integrating plasmids or the use of Sendai viral vector. These methods require time-consuming regimens and result in a relatively low yield of iPSCs. Here, we have utilized a non-integrative RNA based reprogramming method to reprogram urine-derived RECs with significantly faster reprogramming efficiency and kinetics. The approach depends on highly – tuned transfections of RECs with reprogramming modified mRNAs and mature miRNA mimics in combination with optimized culturing conditions. Using this methodology, we have successfully generated iPSCs from individuals with genetic diseases. All iPSCs exhibited normal karyotype and expressed markers of pluripotency. Our approach is ideal for generating high quality patient-derived iPSCs from a non-invasive source of somatic cells to be used for disease modeling and potential clinical applications.

W-3199

CREATION AND CHARACTERIZATION OF T CELL-DERIVED IPS CELL BANKS DERIVED USING REAGENTS AND WORKFLOWS OPTIMIZED FOR CELL THERAPY MANUFACTURING

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Advances in induced pluripotent stem cell (iPSC) research is moving the field towards clinical and translational applications. iPSC derived from T cells may be particularly useful in immune oncology applications; with proper differentiation T-iPSC could provide an indefinite source of therapeutic T cells. Cells intended for therapeutic applications require robust and consistent iPSC generation workflows that utilize high quality reagents, preferably xeno-free. In addition, there is an imperative need for accurate and high throughput characterization methods that qualify the identity, pluripotency, and genomic integrity of cells. Methods that enhance consistency and thorough characterization will minimize extra effort and costs associated with clones that fail to expand, or do not meet quality standards for downstream use. To streamline iPSC generation from T cells, and ensure the consistent creation of high quality iPSCs, xeno-free workflows were optimized to minimize the variability in reprogramming efficiency observed between donors. Prior to reprogramming, T cells were phenotyped, and a combination of conditions were tested including hypoxia, matrix, and seeding density. Optimization yielded consistent iPSC generation from potentially difficult to reprogram donor cells, with varying efficiencies. Following reprogramming, T-cell derived iPSC clones were analyzed for immune repertoire, leveraging a

next-generation sequencing based assay,. The resulting iPSC lines were also confirmed to be foot-print free, HLA profiled, and were further subjected to comprehensive characterization methods to assess the quality and safety profile. Pluripotency and differentiation potential of iPSC clones was confirmed using ScoreCard, a focused qPCR panel, and PluriTest, an array-based global gene expression platform. In addition, qualified clones were thoroughly investigated for genomic stability. Each of the clones tested showed a normal karyotype using both traditional G-banding as well as array-based methods KaryoStat and KaryoStat HD. The adoption of defined xeno-free workflows with qualified reagents , in combination with comprehensive and predictive characterization assays aids in easy transition of early investigational work towards translational and clinical research.

W-3201

MAPPING DE-DIFFERENTIATION 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 de-differentiation 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. Interestingly, these cells were characterized by KRT14 expression, a basal marker that it is not normally expressed in pancreas. We also observed that these atypical cells were able to contribute to organoid formation, a hallmark of cells with stem-cell properties. Moreover, these atypical KRT14 expressing cells were present in many different tissues upon transient expression of 4F. We believe that reprogramming in vivo may generate a type of plastic cells with progenitor-like capacities distinct from those already described during normal development and potentially important for regeneration.

W-3203

EFFECT OF HUMAN STIMULATED-OOCYTE EXTRACT ON INDUCING PLURIPOTENCY 0F BONE MARROW MESENCHYMAL STROMAL CELLS

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Reprogramming autologous adult cells to pluripotent cells allows for relatively safe cell replacement therapy. This can be achieved by nuclear transfer, cell fusion, or induced pluripotent stem cell technology. However, the epigenetic memory of the cell represents a formidable challenge for complete reprograming using these methods. Mimicking physiological reprogramming during fertilization by introducing oocyte-specific factors into differentiated cells may thus represent a promising reprogramming approach. However, earlier experiments showed that lower species oocyte extract did not lead to stable reprogramming. In this work, we present a novel technique to reprogram somatic cells using human oocyte-extract. Human MII-phase stimulated-oocyte extract was applied at different concentrations to reprogram human bone marrow mesenchymal stem cells (BM-MSCs). Our data show that the tested concentrations and treatment durations followed a bell curve with peak level of pluripotency genes expression at 10ng/ul and 4 days. After 4 days in culture, immature small and round mitochondria with low numbers of swollen cristae were localized proximal to the nuclei of the stimulated BM-MSCs. These cells also showed higher expression of pluripotency genes, with concomitant down-regulation of some mesenchymal-specific genes. Morphological analysis and epithelial marker expression failed to show evidence for mesenchymal-to-epithelial transition. BM-MSCs treatment showed reprogramming of the mitochondria into immature ones and entered the cells into the initiation stage of the reprograming phases (initiation, maturation and stabilization). These changes were optimally observed at day 4, while no significant differences observed at day 1 and day 7. The optimum concentration to induce pluripotency gene expression was achieved at 10ng/ul, while significantly higher proliferation and morphological changes were observed at the higher concentration of 30ng/ul. This is further prooved by the higher ability of the treated hBM-MSCs to form Embryoid bodies. We conclude the success of human oocyte-extract to initiate reprogramming of somatic BM-MSCs to pluriotency.

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W-3205

EFFICIENT DERIVATION, MAINTENANCE AND CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS) AT THE CEDARS-SINAI IPSC CORE FACILITY

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The Induced Pluripotent Stem Cell (iPSC) Core uses non-integrating episomal reprogramming to expand and characterize human iPSCs from blood of healthy and diseased subjects. These iPSCs are differentiated into specific cells of the human body for disease modeling and drug screening/toxicology studies by researchers. Notably, we are also developing clinically compatible methods for iPSC generation for development of regenerative cell therapies. The Core has derived approximately 700 non-integrated iPSC lines from control subjects as well as patients with diseases such as Spinal Muscular Atrophy, Huntington's Disease, and Amyotrophic Lateral Sclerosis. Briefly, human peripheral blood mononuclear cells (PBMCs) were isolated from individuals and cryopreserved in CryoStor CS10. The PBMCs were then nucleofected using the Lonza Amaxa 2D system and plated in 6-well plates. Individual colonies with iPSC-like morphology appeared between day 25-32 and were mechanically isolated and transferred onto 12-well plates. The resulting clonal iPSC lines were further expanded for characterization and distribution. iPSC pellets and embryoid bodies were collected for self-renewal and germ layer differentiation analysis, respectively, using hPSC Scorecard™ and cells were submitted to G-band karyotyping at early (p5-8) and late (p18-22) passages. Based on our expansive karyotype data, our cells lines report a 5% chromosomal abnormality rate. This is significantly lower than previously published rates, which would indicate that our cell lines are more karyotypically stable than those derived from alternative methods. Once each line is optimized and characterized, a distribution bank of ~25 vials is made at late passage. The iPSC lines generated at the iPSC Core are readily available for request. To date, we have shipped over 1500 vials of characterized iPSCs to approximately 45 academic institutions and 25 commercial companies across the globe. We have improved and optimized iPSC generation, passaging and cryopreservation protocols. We have derived excellent quality non-integrating iPSC lines while maintaining a low rate of chromosomal abnormalities. Work is now underway to transform our protocols into high-throughput and clinically compatible iPSC generation platform.

W-3207

RESOLVING CELL FATE DECISIONS DURING MOUSE SOMATIC CELL REPROGRAMMING BY SINGLE-CELL RNA-SEQ

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Somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs), which is a highly heterogeneous process. Here we report the cell fate continuum during somatic cell reprogramming at single-cell resolution. We first develop SOT to analyze cell fate continuum from Oct4/Sox2/Klf4 or OSK mediated reprogramming, and show that cells bifurcate into two categories, reprogramming potential (RP) or non-reprogramming (NR). We further show that Klf4 contributes to Cd34+/Fxyd5+/Psca+ keratinocyte-like NR fate, and IFN-γ impedes the final transition to chimera competent pluripotency along the RP cells. We analyze more than 150,000 single cells from both OSK and chemical reprograming, and identify additional NR/RP bifurcation points. Our work reveals a generic bifurcation model for cell fate decisions during somatic cell reprogramming that may be applicable to other systems and inspire further improvements for reprogramming.

TECHNOLOGIES FOR STEM CELL RESEARCH

W-3209

NEW POLYCISTRONIC TALENS GREATLY IMPROVE GENOME EDITING

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Genome editing technologies have become some of the most powerful tools in present day stem cell research. In spite of their rapid acceptance and widespread use, there is still room for improvement. In this communication we present data regarding a new, more efficient, type of Transcription Activator-Like Effector Nuclease (TALEN). Our group has generated polycistronic genes in which classical TALEN coding sequences are linked by 2A elements to different fluorochromes. This structure results in two proteins transcribed from the same plasmid, the second of which (the fluorochrome) can be used as target for selection by FACS. The use of these new TALEN-F genes allows for a rapid enrichment of cells in which both members of the TALEN pair are active and, thus eliminates the need for lengthily selection in culture, and laborious characterization of a large number of clones.

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W-3211

PRECONDITIONING OF DIMETHYLOXALYLGLYCINE ENHANCES THE ANGIOGENIC EFFECT OF CANINE ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS VIA HIF-1 ACTIVATION

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The paracrine function of mesenchymal stem cells (MSCs) has been recently observed during transplantation, due to the poor differentiation ratio. Dimethyloxalylglycine (DMOG) has been used to promote angiogenesis in experimental animal models, but comparable approaches for canine MSCs are not sufficient. In the present study, we assessed whether DMOG improves angiogenesis in canine adipose tissue-derived mesenchymal stem cells (cAT-MSCs). cAT-MSCs were treated with DMOG and their effect on angiogenesis was investigated by cell proliferation assay, western blot, and tube formation assay. Dimethyloxalylglycine preconditioning enhanced the expression of vascular endothelial growth factor (VEGF) among pro-angiogenic factors in cAT-MSCs via hypoxia-inducible factor 1α stabilization. Dimethyloxalylglycine primed-cAT-MSC-conditioned media increased angiogenesis with human umbilical vein endothelial cells. These results suggest that DMOG-conditioned cAT-MSCs augmented the secretion of VEGF, which acted as a prominent pro-angiogenic factor during angiogenesis. In clinical trials, DMOG primed cAT-MSCs might induce more beneficial effects on ischemic diseases.

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W-3213

RAPID, EFFICIENT AND PRECISE GENE KNOCKIN IN STEM CELLS

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The targeting efficiency of knockin sequences in non-expressed genes is generally low in human embryonic stem (hES) and induced pluripotent stem (iPS) cells. To address this challenge, we describe a method that uses either a single-stranded DNA repair oligo or an enzyme-cut transgene donor with asymmetrical arms. These components are electroporated along with a recombinant Cas9 protein and single guide RNA

into hES and iPS cells. Electroporated cells are cultured for 72 hours, then half of the cells are cryopreserved and half cells are collected for genomic PCR and sequenced to predict screening size. An allele-specific PCR was designed to screen transduced clones, and selected clones were sequenced to ensure correct incorporation of the donor DNA. Overall targeting efficiency ranged from 30 to 70% for a repair oligo and 5 to 20% for the double stranded donor. Modified cells were characterized and able to differentiate into specific cell types. The high knockin efficiency achieved, makes the proposed method suitable to engineer isogenic stem cells and reporter systems, which can provide new insights into the molecular mechanism of cell differentiation or facilitate drug screening.

W-3215

ARTIFICIAL INTELLIGENCE IN THE REGENERATIVE MEDICINE LANDSCAPE

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Since the 1950s, information science, benefiting from the invention of the transistor, and life science, benefiting from the elucidation of the structure of DNA, have become increasingly entwined, with research and development in one field inspiring research and development in the other. This symbiotic relationship extends broadly across a landscape that combines the functional activities within each field: R&D, engineering, coding, manufacturing, diagnosis and treatment, regulatory compliance, patient advocacy, marketing, and data analysis. The growing linkages between artificial intelligence and regenerative medicine presents the most recent set of features in this dynamic landscape. We explore these features along three dimensions: the life science pathway from discovery and development to regulatory approval and commercialization of innovative products; the disciplines that comprise artificial intelligence (including machine learning, deep learning, image processing, computational reasoning, natural language processing, and expert systems); and the combined functional activities mentioned above. The manifold formed by these three dimensions enables us to map existing applications of AI to regenerative medicine and to identify potential extensions of these applications as well as opportunities for development of novel applications. Existing applications, for example, include machine learning for pluripotency screening and image processing to study cell line differentiation. Opportunities for innovation include development of natural language-based predictive models for precision-oriented cell-based regenerative therapies, and deep learning for discovery and development of new therapies. Cell- and gene-based therapies will generate unprecedented volumes of data, and we expect that the processing, analysis and comprehension of this data will be substantially facilitated with the AI toolset.

W-3217

IMAGE-BASED ASSESSMENT OF PSC QUALITY DURING EARLY IPSC ESTABLISMENT

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Somatic reprogramming for the generation of induced pluripotent stem cells (iPSC) has rapidly evolved with promising applications in disease modeling and translational applications. Genetically diverse iPSC derived from varying somatic sources is enabled by foot-print free reprogramming methods under different culture conditions. Despite these advances, early iPSC identification and clonal expansion continues to be a tedious and laborious process which needs to be further streamlined for high throughput and automated workflows. Traditional methods of morphological assessment are now complemented with a combination of positive and negative markers albeit with varying levels of success. Previously we had reported the use of CD44 and SSEA1 to distinguish unreprogrammed and partially reprogrammed cells from fully reprogrammed colonies. This and other similar methods have been used to effectively identify and select bona fide iPSC. Here, we extend this approach to monitor subtle differences in early expanding iPSC clones to eliminate unstable clones that have a higher propensity to spontaneously differentiate. Using a panel of surface and intracellular markers (OCT4, TRA-1-60, organelle markers, et al.), correlation of morphology to the increase or decrease of specific markers were measured using global transcriptome analysis and confirmed using immunostaining . Additionally, cellular organization in PSC colonies was dissected using organelle dyes. Combined use of marker expression with cellular organization provides a compelling avenue to explore the subtle and in most cases unobservable morphological changes that can signal early differentiation before it would otherwise become apparent. This could benefit stem cell researchers by providing them with greater confidence when picking freshly reprogrammed clones to expand and invest in.

W-3219

DONOR-FREE MESENCHYMAL STEM CELLS EXPANDED IN A CUSTOM MICROCARRIER SYSTEM

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Mesenchymal stem cells (MSCs) are attractive candidate cytotherapeutics for treatment of inflammatory disorders, cancers, and musculoskeletal diseases. However, the ability to generate clinically relevant quantities of MSCs is impaired by several factors including donor-to-donor variation in cell quality, expensive supplements such as fetal bovine serum (FBS) or human platelet lysates (hPL) in cell culture media, and significant time investments for production of large scale quantities. To address issues with donor variability, we have developed "donor-free" MSCs from a theoretically infinite source of induced pluripotent stem cells (iPS-MSCs). Expansion of the cells on custom gelatin methacrylamide (GelMA) microcarriers permits reproducible and large-scale expansion of MSCs into a potential therapeutic product, while dismissing many of the current limitations with manufacture. Expansion of iPS-MSCs on these microcarriers requires less than half of the standard FBS or hPL used in monolayer culture while maintaining comparable proliferation rates and maintenance of favorable therapeutic attributes. Continuous morphological analysis of cultures permits non-invasive assessment of culture quality and supplementation of FBS or hPL can be applied as needed to maintain the quality of the cells during expansion. The microcarriers can be loaded with growth factors or attachment substrates during synthesis, and can be digested by brief trypsin exposure to facilitate harvest. Herein, we propose a sustainable source of reproducible MSCs and a customizable platform for their large-scale expansion that maintains culture quality and reproducibility while minimizing the cost of media supplementation and harvest.

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W-3221

REACTIVATION OF GENE EXPRESSION AFTER CRISPRI KNOCKDOWN IN PLURIPOTENT AND DIFFERENTIATED CELLS

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CRISPR-interference (CRISPRi) using nuclease-dead Cas9 (dCas9) is a widely employed tool for targeted gene knockdown. Fusion of the KRAB repressor domain to dCas9 (dCas9-KRAB) further improves gene repression by recruiting epigenetic modifiers, and drug-regulated expression of dCas9-KRAB allows for inducible and reversible CRISPRi. However, the long-term effects of epigenetic modification on gene reactivation have not been fully elucidated. In order to study target gene reactivation in pluripotent and differentiated cells, we constructed a novel human Tet-OFF CRISPRi induced pluripotent stem (iPS) cell line that expresses dCas9-KRAB in the absence of doxycycline for constitutive, yet drug-reversible, gene knockdown. As a model, we selected sgRNAs targeting beta-2 microglobulin (B2M), the common subunit of human leukocyte antigen class I (HLA cI), resulting in iPS cells that lack HLA cI presentation. The addition of low-concentration doxycycline completely blocked dCas9-KRAB expression, permitting reactivation of B2M and the return of HLA cI molecules to the surface of iPS cells. In haematopoietic progenitor cells a robust B2M knockdown was similarly achieved, but HLA cI presentation was not recovered upon doxycycline-treatment, despite the lack of dCas9-KRAB. These data suggest an altered capacity for erasing repressive epigenetic marks in pluripotent versus differentiated cells, and sheds new light on the reversibility of CRISPRi using dCas9-KRAB.

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W-3223

DISSOLVABLE POLYGALACTURONIC ACID MICROCARRIERS FOR HMSC GROWTH AND EFFICIENT RECOVERY

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Two-dimensional cultureware and multi-stack vessels are used frequently to scale-up adherent cells. Unfortunately, these lack the ability to monitor and control the cellular environment, and manipulation of multiple vessels can be labor-intensive and risk culture contamination. Cell growth on microcarriers in closed-system bioreactors can minimize the challenges associated with these planar technologies. Bioreactors provide the ability to remotely and non-invasively monitor and control the cell culture environment in real time, and cell growth on microcarriers allows the flexibility to optimize the total surface area, thereby maximizing the cell yield per unit volume. Utilizing both technologies enables efficient scale-up of cells for therapeutic applications. Depending on cell type and density, cell release and separation from microcarriers can be difficult. Effective cell recovery from microcarriers often requires the use of concentrated enzymes, extended treatment times, and continuous centrifugation/filtration cycles, which can negatively affect cell health and recovery yield. To address these challenges, we developed a

calcium-crosslinked polygalacturonic acid (PGA) microcarrier that can be dissolved during the cell harvest process. Microcarrier dissolution requires EDTA to chelate calcium ions and pectinase to degrade the PGA polymer. When a single cell solution is desired, a standard cell culture protease (e.g. trypsin, accutase, dispase) can be directly added to the EDTA/pectinase solution. In contrast, when cell-cell and cell-ECM protein networks are critical for cell pluripotency or differentiation capability, cells are released as sheets as the microcarriers dissolve, and agitation (i.e. shear) during microcarrier dissolution can control the cell aggregate size. Here we demonstrate human mesenchymal stem cell (hMSC) growth on dissolvable microcarriers in spinner flasks and controlled bioreactor systems. Cells maintained their respective phenotype and functionality after recovery from microcarriers. In addition, we show cell passage via bead to bead transfer and discuss best practices for optimization of this type of passage method. As presented here, dissolvable microcarriers in bioreactors enable efficient production and recovery of a high-quality cell product.

W-3225

STRUCTURAL AND MOLECULAR-BASED DIFFERENTIAL FEATURES BY LONG TERM-INDUCED MESENCHYMAL STEM CELLS IN A RAT MODEL OF PARKINSONS DISEASE

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Adult stem cell is a therapeutic source for restoring neuronal function in brain disease. To improve Parkinson's disease (PD) belonging to neurodegenerative disease, stem cell therapy is known to functionally enhance nigrostriatal dopaminergic neurons. Despite these functional recoveries, the long-term state of stem cells in vivo and their relationship with surrounding cells have not been clearly studied. In previous study, we examined a three dimensional structure of intact brain tissue after bone marrow mesenchymal stem cell (MSC) treatment at short-term induction in PD. In this study, we investigated in vivo Proof-of-concept and their microenvironment of MSCs 1 year after transplantation into 6-hydroxydopamine hydrobromide-induced rat PD model at the medial forebrain. One year after surgery, amphetamine-induced rotation test was performed to evaluate the degree of the dopaminergic lesion. For transplantation, MSCs were injected into the femoral vein. One day after the final behavior test, the rats were anaesthetized, and perfused with a mixture of 4% paraformaldehyde, 4% acrylamaide, 0.05% bis-acrylamide, 0.25 VA044 in PBS. Isolated brains were incubated in 4°C for 2 days, and then temperature was increased to 37°C to induce polymerization for 2.5 hours. Hydrogel-embedded brains were plated in 10~60V organ-electrophoresis system, circulating 200 mM sodium borate buffer with 4% SDS. For three dimensional interface of graft-host axis, it was visualized by immunofluorescence to MSC-positive cells (PKH26-labelled), polysialylated neural cell adhesion molecule, and/or neuron-specific class III beta-tubulin or NeuN marker. At this site, we analyzed RNA-Sequencing to confirm the change of gene expression between PD and PD transplanted with MSC group. Using Excel-based Differentially Expressed Gene Analysis tool, we analyzed the categories related to cell migration and neurogenesis between the two groups. Furthermore, GO and Pathway and Network Analysis was mainly performed on the specifically changed genes. Taken together, our results demonstrate distinct pathophysiological and molecular patterns of in vivo responses between PD and PD transplanted with MSCs suggest structural and molecular-based differential features as candidate tool for the evaluation of stem cell during long-term therapy.

Funding Source: This study was funded by NRF-2015R1C1A1A02037693 and NRF-2015M3A9E6029519.

W-3227

INTRODUCING THE CIRM STEM CELL HUB

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The California Institute for Regenerative Medicine (CIRM) and the Center of Excellence in Stem Cell Genomics (CESCG) is spearheading the investigation into how stem cells can be used to treat disease. The CESCG funds projects at universities and research institutes across California. These projects were intended to interrogate a few different broad subjects such as neurobiology, cardiac biology, blood stem cells and therapeutics, and the molecular regulators of stem cells. The Stem Cell Hub is the data warehouse for the data produced by the CESCG. It houses primary data files such as DNA reads in fastq format, as well as many types of files derived from mapping and other analysis of the primary data, and other document files describing protocols. It has a small but flexible system for associating metadata with a file. Any CIRM-genomic associated lab can submit data. Once submitted, data is treated as prepublication human sequence data, and access is only allowed to authorized users. The CIRM Stem Cell Hub contains nearly 60 terabytes of data that covers 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.

Funding Source: California Institute for Regenerative Medicine grant GC1R-06673-C

W-3229

DEVELOPMENT OF A FEEDER-FREE PSC CULTURE SYSTEM ENABLING TRANSLATIONAL and CLINICAL RESEARCH

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Pluripotent stem cell (PSC) culture using the xeno-free Essential 8™ Medium/truncated recombinant human Vitronectin system has been shown to support normal PSC properties and provide a large pool of cells for disease modeling and drug development. As research moves from translational to clinical research, general regulatory guidance from the US Food and Drug Administration (FDA) indicates that, cGMP manufactured, or clinical grade reagents should be used whenever available as ancillary reagents to minimize downstream risk to patients. Thus, we sought to identify regulatory compliant, animal-origin-free alternatives for growth factors contained within the Essential 8™ Medium, producing a qualified ancillary system for PSC expansion. Here we present data to support a seamless transition from the xeno-free Essential 8™ Medium system to the Cell Therapy Systems (CTS™) animal-origin free system. Compatibility is shown with cGMP-manufactured CTS™ passaging and cryopreservation reagents, including CTS™ Versene Solution, CTS™ RevitaCell™ Supplement, and CTS™ PSC Cryomedium. Furthermore, we developed a completely xeno-free workflow for derivation of pluripotent stem cells from human dermal fibroblasts. Upon expansion of newly derived or adapted clones, PSCs are shown to maintain normal PSC properties, including morphology, pluripotency, karyotype, and trilineage differentiation potential. Maintenance of normal PSC properties are also shown to be maintained during expansion up to 370 million cells from a 2-stack Nunc™ EasyFill™ Cell Factory, indicating that this clinical grade ancillary system can support large numbers of viable PSCs. Together these data support that the CTS™ culture medium reagents provide a consistent, feeder-free PSC culture system for translational and clinical research.

W-3231

REVEAL THE FULL COMPLEXITY OF CELLULAR DIVERSITY WITH SINGLE CELL GENOMICS

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Unraveling stem cell diversity and fate requires the ability to identify individual gene expression profiles and cellular subtypes, trace cellular lineage, and predict cellular potential. Biased cellular analysis and pooled samples have limited stem cell research and contributed to the masking of dynamic cellular events that occur during cellular differentiation, cellular transdifferentiation, reprogramming to pluripotency, and disease modeling of stem cell derived 2D and 3D organoid in vitro culture systems. Single cell analysis can provide novel insights into the molecular mechanisms underlying both normal cellular function and disease states, and is crucial in understanding the complex interplay between cell lineages, transcription factors, and signaling pathways. Single cell genomics technologies from 10x Genomics provide comprehensive, scalable solutions for cell characterization and gene expression profiling of hundreds to tens of thousands of cells, without a need for prior knowledge of cell types or markers. Explore our newest single cell technologies such as the Single Cell ATAC Solution and Single Cell Gene Expression Solution which enables simultaneous measurements of cell surface proteins, CRISPR pooled gRNAs, and potentially more “features”. Learn how multi-omic interrogations of your sample and spatially resolved transcriptomic measurements can help uncover cellular heterogeneity at high resolution.

W-3233

HIGH-LEVEL GENE AMPLIFICATION IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) share several unique properties with cancer cells, including high growth rate, self-renewal, and acquisition of recurrent genetic abnormalities that confer a selective growth advantage. In hPSCs, these recurrent

abnormalities can be acquired during routine cell culture, and they present a potential safety concern for regenerative medicine. Additionally, recurrent abnormalities endow the aberrant hPSCs with additional cancer-like traits, namely apoptotic resistance and reduced differentiation potential. At present, gain of the BCL2L1 gene region on chromosome 20q11.21 is the most frequently acquired abnormality. We describe a novel instance of focal, high-level amplification (≥8 total copies) of BCL2L1 in an hPSC line at high passage number. High-level amplification of a recurrently acquired abnormality is unreported in hPSCs, although it is prevalent in cancer and correlates with poor prognosis and drug resistance. We performed extensive cytogenetic characterization of this hPSC line, including chromosomal microarray that revealed a stepwise amplification pattern of increasing copy number gains across the 888 kilobase region. Stepwise amplification typically results from constant selection pressure, in which the repeatedly gained amplicon narrows to only the driver gene (in this case, BCL2L1) at the region's apex. Such amplification patterns are used for potential driver gene identification in cancer. These findings demonstrate the same mapping utility of amplification in hPSCs, since BCL2L1 has previously been confirmed as a driver gene through other methods. Therefore, we apply this mapping strategy to another recurrent abnormality with a known minimal overlapping region: gain of chromosome 1q, refined to cytoband 1q32.1. We performed microarray testing on hPSC cultures with 1q32.1 gain; preliminary findings identified the smallest measured size of the recurrent region at 1.9 megabases and containing only 31 genes. The emergence of high-level gene amplification in hPSCs is concerning as it is an additional parallel with cancer. This strategy provides a new approach to map driver genes, thereby enabling targeted screening and providing insight into the functional consequences of recurrent abnormalities in hPSCs.

W-3235

SCRAPER-FREE DETACHMENT METHOD USING EDTA WITHOUT TRYPSIN FOR HUMAN INDUCED PLURIPOTENT STEM CELLS CULTURED ON LAMININ-511E8

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Recombinant E8 fragment of laminin-511 (LM511E8) is used in the culturing of human pluripotent stem cells (hPSCs) because it sustains long-term single cell passaging, preserves pluripotency and maintains cells in an undifferentiated state. LM511E8 is a truncated form of laminin-511, and its ability to offer these features comes from its binding activity with integrin α6β1, an isoform predominantly expressed on hPSCs. However, because of their strong interaction with each other, trypsinization followed by scraping is required to harvest the cultured cells. Nevertheless, cell scraping causes mechanical damage to the cells, reducing cell viability. In addition, cell scraping cannot be used when cells are cultured on multi-layer flasks or in automated cell culture systems. In this study we aim to develop a scraper-free cell detachment method for human induced pluripotent stem cells (hiPSCs) cultured on LM511E8. By referring to the protocols available to date, we examined whether incubation of hiPSCs with 5 mM EDTA/PBS(-) at 37°C for 15 min enabled to detach the cells with high efficiency. The inclusion of trypsin to EDTA/PBS(-) was also tested to see if there were any additional gain to cell detachment efficiency. We found that more than 95% of hiPSCs were detached without compromising cell viability when they were incubated with 5 mM EDTA/PBS(-) alone. Surprisingly, the addition of trypsin decreased the detachment efficiency. This decrease was rescued when enough trypsin inhibitor was added to neutralize the enzyme, therefore the lowered detachment rate must be a result of its proteolytic activity, suggesting an ill-defined mechanism operating in hiPSCs to render them less susceptible for cell detachment by depletion of divalent cations.

W-3237

SELECTION OF CARDIOMYOCYTE DIFFERENTIATION POTENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS BY TANTALUM OXIDE NANODOT ARRAYS

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Human iPSC-derived cardiomyocyte is a high potential source for disease modeling, high throughput toxicity screening, and cell-based therapies for cardiac disorders. However, not all iPSC lines can differentiate into cardiomyocytes with the consistent efficiency. In several reports, Nanotopography

modulates cell fate in relating to the diameter of the nanodots. In this study, we demonstrated how various nano-sized surface modulate the gene profile of iPSC differentiation and attempt to evaluate the cardiomyocyte differentiation potency of iPSCs by using Tantalum Oxide (TO) nanodot arrays. A series of nanodot arrays were fabricated in different dot diameter ranging from 10 to 200 nm. Four iPSC lines were cultured with EB medium on these various nanodot arrays or on the flat surface (including matrigel/ gelatin-coated flat surface) for 7 days. Pluripotent and cardiac-related genes were examined by quantitative RT-PCR (qPCR). Furthermore, differentiation efficiency of iPSC-derived cardiomyocytes was confirmed by flow cytometry. The qPCR results showed that gene expression of iPSCs cultured on the TO nanodot surface was significantly enhanced compared with those on flat surface. Flow cytometry analysis indicated that the highest express level of two cardiac-related genes, NKX2.5 and GATA4, of iPSCs on 200 nm nanodot surface had positive correlation to the high differentiation efficiency of iPSC-derived cardiomyocytes. In conclusion, this 200nm nanodot arrays can be applied as a selective platform to screen iPSC lines with high efficiency of cardiomyocyte differentiation. Furthermore, we hope that this platform can provide shortcut to select good iPSC lines for differentiation to other cell types and overwhelm time consuming of conventional protocol.

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W-3239

CONTRAST AGENT FREE ACOUSTOFLUIDIC GENE DELIVERY TO HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Advances in gene editing are inspiring innovative regenerative medicine approaches and therapeutic interventions where physicians are able to correct disease-causing mutations in patients' own cells to treat genetic diseases. One of the critical limitations that precludes the clinical translation of these novel treatments is the development of scalable technologies that can engineer cells efficiently, safely, and economically. To address this challenge, we are developing and applying a microfluidic method to deliver biomolecular cargo intracellularly based on the permeabilization of cell membranes using acoustic induced shearing against the sidewall of a glass capillary. This acoustofluidic-mediated approach achieves rapid and efficient intracellular delivery of an enhanced green fluorescent protein (eGFP)-expression plasmid to Jurkat cells at a throughput of 12 million cells/hour/channel, showing 62% eGFP-expression after 72 h post-acoustic treatment. Our data indicate the working mechanism of acoustofluidic delivery includes cytosolic diffusion across the plasma membrane, endocytic transport, and nuclear membrane rupture. Following these observations, we tested delivery to peripheral blood mononuclear cells and CD34+ hematopoietic stem and progenitor cells and showed positive eGFP-expression in both cell types. Collectively, these results demonstrate significant potential for this device to be utilized for mechanistic studies of cell membrane repair that will inform strategies for manufacturing gene-modified stem cell therapies and cancer immunotherapies.

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W-3241

CHEMICALLY DEFINED YEAST ALBUMIN SUPPORTS ROBUST EXPANSION AND VIABILITY OF DIFFERENT CELL LINES IN CULTURE

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Mammalian cell culture has played an important role in the progression of the life sciences over many decades. More recently, it has played a key role in the application of biomedical research for the development of new therapeutic strategies. Cell culture has been an important element in the discovery of umpteen biological factors vital for the overall understanding of

the whole animal at cellular and molecular level. Last decade has seen an increase in the importance of cell culture in the advancement of stem cell technology, drug discovery, cellular therapy and vaccine production. Human serum albumin (HSA) has long been an indispensable element of cell culture methodology, as a supplier of hormones, growth and attachment factor. Its unique intrinsic biochemical and biophysical properties have been exploited in various fields of biologics. Current desire for high quality and animal-free components has led to the development of recombinant versions of albumin. Here, we introduce our in house chemically defined yeast recombinant albumin, namely deAlbumin. We employed a patent-pending process to produce high purity of recombinant human serum albumin formulated with a pool of defined chemicals found in plasma derived albumin which shows most robust performance, which delivers a secure supply of batch-to-batch consistent materials. Moreover, we provide an established path through the regulatory approval process, and customer assurance to facilitate the safe and successful use of our product. Absence of any animal-derived component makes deAlbumin an ideal component for cell cultures. In addition to the above-mentioned cell lines, upon request, customization of deAlbumin in different cell types is being offered.

Llate-breaking abstracts

W-4001

SELECTIVE EXPANSION OF MYOGENIC STEM CELLS FOR MUSCLE REGENERATION

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Traumatic injury of skeletal muscle often leads to muscle loss and dysfunction. Activation of resident stem cells or transplantation of myogenic stem cells can be employed to regenerate muscle. However, the limited availability of autologous myogenic stem cells and the lack of an effective method to expand satellite these cells for therapeutic use are major barriers for effective therapies. Here we discovered a chemical cocktail which that can selectively induce and expand induced a robust expansion of myogenic stem cells (CiMSCs) with high efficiency from both easily obtainable dermal fibroblast population and skeletal muscle cells-like cells. Further Detailed in vitro analysis showed illustrates that these selective expanded chemically-induced myogenic stem cells (CiMSCs) are were mainly dominantly originated derived from sparse Pax7+ positive slow-adhesion cells in fibroblast-like cell populations. These . We then show that the CiMSCs are were highly engraftable to repair the cardiotoxin-injured tibialis anterior (TA) muscle upon transplantation. Furthermore, a novel injectable drug-loaded nanoparticle system was developed which enables to enable the controlled and sustained release of the chemical cocktails. Histological analysis and lineage-tracing unveil demonstrated that the injection of drug- loaded nanoparticles into injured muscle can significantly expanded localized resident satellite cells and promoted in situ muscle regeneration. This work These findings will lead to the development of novel thus presents novel chemical induced in vitro and in situ stem cell engineering approaches for effective skeletal muscle regeneration.

W-4003

SUPER-ACTIVATED PLATELET LYSATE AND ITS APPLICATION IN TISSUE REPAIR

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Platelets contain a variety of growth factors, cytokines and attachment factors that have a major role in stem cell proliferation in vitro and in tissue repair in vivo. For many years platelet-rich plasma (PRP) has been used in the treatment of Orthopedic disorders, such as osteoarthritis, bone nonunion and osteonecrosis. However there are many drawbacks of PRP, such as platelets insufficient activation, white blood cell contamination and difficulty in quality standardization. We have obtained super-activated platelet lysate derived multiple growth factor solution (sPL) by platelet enrichment, activation induction and multiple freeze/thaw processes of the leukocyte-depleted PRP to overcome these problems. Our results have shown that the growth factors are effectively released into sPL. The level of PDGF-AB, TGF-β1, bFGF and VEGF in sPL is significantly higher in comparison with PRP. While the level of pro-inflammatory molecules such as TNF-α, IL-1 and IL-6 is much lower compared to leukocyte containing PL. Several major growth factors are specially activated and standardized in order to meet certain needs of treatment. For preclinical and clinical application, sPL has been manufactured in GMP-compliant clean room. Quality tests for the total protein content, the level of growth factors, microbial contamination and endotoxin are performed before sample releasing. In vitro studies have proven that sPL can promote expansion and migration of hUC-MSCs and hFB. IL-1β-induced chondrocytes apoptosis is declined when culture media contain sPL. Here we also demonstrate the safety and efficacy of sPL in treating knee osteoarthritis, wound and nonunion of rabbit. Our data suggest that sPL has huge potentials as a regenerative medicine.

W-4005

GROWTH FACTOR-FREE OSTEOCONDUCTIVE ADHESIVE HYDROGEL WITH THE POTENTIAL TO SUPPRESS OSTEOCLAST ACTIVITY

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The ultimate goal of bone tissue engineering is the regeneration of a construct that matches the physical and biological properties of the natural bone tissue. Bone regeneration using mesenchymal stem cells (MSC) is an advantageous therapeutic option. Human Gingival mesenchymal stem cells (hGMSCs) are of special interest as they are easily accessible in the oral cavity and readily found in discarded dental biological tissue samples. Osteoconductive hydrogels have been developed by incorporating various growth factors (GFs), bioactive particles, or without the use of any GFs. Whitlockite is one of the important inorganic phases composing up to 20% weight of the bone structure. It has been shown that WH microenvironment encourages cellular proliferation/osteogenic differentiation better than hydroxyapatite (HAp). Additionally, WH particles have proved to down-regulated osteoclast-specific genes. To address the clinical need for a smart biomaterial for bone regeneration, we propose to engineer an osteoconductive hydrogel based on Alginate and WH microparticle (WHMPs) with tunable physical properties and ability to direct hGMSCs toward osteogenesis. Our in vitro studies showed that WHMPs can induce osteogenesis of hGMSCs significantly better than HAMPs by continuous supplying of Ca2+, PO43-, and Mg2+ along with the increased protein adsorption. The ICP and mechanical properties studies suggest that the Ca2+ released from WHMPs can act as an in situ cross-linking agent that further reinforces the hydrogel structure. Our mechanistic analysis confirmed that the higher elasticity in the Alg-WHMPs can activate P38MAPK pathway which modulates several osteogenic transcription factors in the encapsulated GMSCs. The results of co-culture studies with osteoclasts demonstrate that Alg hydrogel containing WHMPs encapsulating hGMSCs suppressed the activity of the osteoclasts. The higher amount of released Mg2+ ions from WHMPs along with higher secretion of osteoprotegerin (OPG) from the hGMSCs resulted in down-regulation of osteoclasts proliferation and activity. Altogether, the results of the current study demonstrate that the developed growth factor free hydrogel delivery system containing WHMPs actively contributes to osteogenesis of the encapsulated hGMSCs while suppresses the osteoclast activity

W-4007

O-CYCLIC PHYTOSPHINGOSINE-1-PHOSPHATE STIMULATES THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELLS THROUGH MTOR-DEPENDENT HIF1α STIMULATION

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O-cyclic phytosphingosine-1-phosphate (cP1P) is a novel sphingosine metabolite derived from chemically synthesized phytosphingosine-1-phosphate. cP1P has a structural similarity to sphingosine-1-phosphate (S1P), but the its regulatory effect on stem cell biology has been not reported yet. We investigated the effect of cP1P on therapeutic potential of mesenchymal stem cells (MSCs) and its regulatory mechanism. In this study, cP1P suppressed mitochondrial dysfunction and apoptosis of MSC under hypoxia. Metabolic data revealed that cP1P stimulated glycolysis via up-regulation glycolysis-related genes. cP1P-induced hypoxia-inducible factor 1 alpha (HIF1α) is plays a key role in glycolytic reprogramming and transplantation efficacy of MSC. Intracellular calcium-dependent PKCα/ mammalian target of rapamycin (mTOR) signaling pathway triggered by cP1P regulated HIF1α translation via S6K1, critical for HIF1 activation. Furthermore, cP1P-activated mTOR pathway induced bicaudal D homolog 1 expression leading to HIF1α nuclear translocation. In conclusion, cP1P-activated mTOR signaling enhances therapeutic potential of MSC through HIF1α translation and nuclear translocation.

W-4009

SINGLE-CELL RNA-SEQ HIGHLIGHTS HETEROGENEITY IN HUMAN PRIMARY WHARTON' S JELLY MESENCHYMAL STEM/STROMAL CELLS CULTURED IN VITRO

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Mesenchymal Stem/Stromal cells (MSCs) are self-renewing, multipotent cells with immunomodulation function, displaying highly promising applications in regenerative medicine and cell immunotherapy. However, MSCs derived from different donors, different tissues, or even from the same tissue in a population with similar genetic background still exhibited functional heterogeneity, which may finally influence their clinical applications. The precise underpinning molecular mechanisms remain to be elucidated. Here, we investigated gene-expression profile in human primary Wharton's Jelly derived mesenchymal stem cells (WJ-MSCs) isolated from three human umbilical cord samples by single-cell RNA-seq. Total 6, 878 cells were obtained, average 2, 293 cells for each sample, with 209, 769 mean reads, 38, 983 median unique molecular identifier (UMI) counts and 6, 279 median genes per cell. After filtering out low-quality cells, normalization, and scaling, we identified 1,129 highly variable genes (HVGs) based on expression and dispersion. GO enrichment analysis showed that these HVGs are significantly enriched in extracellular region with binding function, involved in developmental process, signal transduction, cellular component morphogenesis, cell communication, cell proliferation, etc. biological process, indicating that different single cell may exhibit different response to microenvironmental change. Meanwhile, pathway analysis revealed that these highly variable genes expressed among cells are associated with functional characteristics of MSCs, such as inflammation mediated by chemokine and cytokine signaling pathway, integrin signaling pathway, p53 pathway, TGF-beta signaling pathway, and angiogenesis being overrepresented. After regressing out the cell cycle effect, several different subpopulations were identified by dimensional reduction and clustering. These results together implicated that genes highly varied among WJ-MSCs culture in vitro play an important role in individual cell response to the extracellular environment, which could eventually impact on population differentiation behavior and immunomodulation potency.

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W-4011

REAL-TIME MONITORING SYSTEM FOR ANTI-CANCER DRUGS: EFFECT OF ANDROGRAPHOLIDE, CISPLANTIN, 5-FU AND CANNABIDOIL ON CULTURED HUMAN TONGUE CANCER CELLS

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Squamous cell cancers of the head and neck are often considered together in oncology research. Because of their similarities the cancer types, in incidence, pathological features and prognosis. It is important to find a treatment for oral cancer. Andrographolide (Andro) is an active diterpenoid compound extracted from andrographis paniculata. It shows some pharmacological activities, such as anti-inflammatory, and anti-cancer effect. And best of all, it is also known as a nearly non-toxic compound. Cannabidiol (CBD) which is extracted from marijuana. But does not appear to have any psychotropic effects which caused by Δ-THC. CBD has been reported that it also has the ability of anti-cancer. The purpose of this study is to investigate the cytotoxic effect of both Andro and CBD on cellular morphology, proliferation, and migration on SCC-25. In addition, we use these two extracts to compare the effects of current anticancer drugs cisplantin and 5-fluorouracil (5FU). We use the electric cell substrate impedance sensing (ECIS) system to monitor the micro-motion such as migration, proliferation and cytotoxic effect. Also, we used the Alamarblue, qPCR and the western blot to observe the changes of morphology, viability and apoptosis in SCC-25. Here, we report the chemotherapeutic effects of these extracts in comparison to cisplantin and 5FU through an impedance-based measurement and biochemical assays. We monitored the cytotoxic effect of Andro, cisplantin, CBD and 5FU on wound healing tests of SCC-25 with ECIS. Our results demonstrated a dose-dependent decrease from 10 μM to 300 μM concentrations. From the ECIS wound healing assay we showed that 30 μM CBD and 56 μM Andro can effectively inhibit the migratory rate of SCC-25. From the biochemical assay, both CBD and Andro expressed apoptotic markers and protein caspase-3, BAX, and PARP at a higher level in comparison to cisplantin and 5FU, all drugs induced a decrease in Bcl-2 which is anti-apoptotic. In conclusions, CBD and Andro presented exceptional results in inhibiting cell migration and motility. Moreover CBD and Andro both expressed high levels of apoptotic markers and protein in comparison to cisplantin and 5FU. Andro and CBD have the potential for curing tongue cancer. ECIS is a powerful technique to identify potential therapeutic agents.

W-4013

DOWN REGULATION OF GLI1 EXPRESSION IN HUMAN PLURIPOTENT STEM CELLS DIFFERENTIALLY AFFECTS LINEAGE COMMITMENTS

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GLI1 is one of three GLI family transcription factors that mediate the Sonic Hedgehog (SHH) signal transduction pathway. The pathway plays an important role in normal development and cell differentiation. GLI1 is also a human oncogene with gene targets that sustain proliferation, inhibit apoptosis, promote angiogenesis, and promote tumor cell migration. GLI1 is highly expressed in embryonic stem cells (ESCs), neural stem cells (NSCs), and mesenchymal stem cells (MSCs). Over- expression of SHH and GLI1 in stem cells enhances production of neural progenitor and dopaminergic neurons. GLI1 can bind the promotor of Nanog and activate its transcription for regulating self-renewal of NSCs. However, Nanog binds GLI proteins in ESCs and represses GLI1-mediated transcriptional activation. The expression profiles of GLI1 during differentiation and its function in human ES cells are not yet clear. To determine the impact of GLI1 on stem cell differentiation, we utilized the CRISPR/Cas9 genomic editing system to produce H1 hES cell clones with 70% down-regulated Gli1 expression. For homozygous editing we used electroporation to introduce the donor plasmid, in which the puro gene was replaced with BSD, into the heterozygous puro resistant H1 hES cell clones. Homozygous clone selection was achieved using blasticidin. The roles of the SHH pathway in the differentiation of human ESCs were investigated by comparing the results of RNAseq, qPCR, western blot, and immunocytochemistry analysis. Our data demonstrated that GLI1 down regulation promoted mesodermal and neural differentiation while inhibiting the endodermal commitment during directed and spontaneous differentiation experiments. Additionally, early stage of hematopoietic differentiation was skewed toward the mesodermal component (mesengioblast). The pluripotency marker expression was not affected. These results indicate that SHH pathway has lineage specific effects on the differentiation of embryonic stem cells.

W-4015

FUNCTIONAL RELEVANCE OF THE SONIC HEDGEHOG RECEPTORSOME FOR NEUROEPITHELIAL PATTERNING AND FOREBRAIN SPECIFICATION

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The mammalian forebrain arises from a single-layered sheet of neural stem cells forming the neuroepithelium. Neuroepithelial cells receive inductive signals from a set of morphogens to form the neural tube that is further subdivided into the various parts of the embryonic central nervous system, including the forebrain. Secreted by the prechordal plate (PrCP), the morphogen sonic hedgehog (SHH) targets neuroepithelial cells of the overlying forebrain organizer, the rostral diencephalon ventral midline (RDVM), to establish the ventral midline identity of the future forebrain. Besides the canonical SHH receptor patched 1 (PTCH1) several additional cell surface proteins have been identified as being essential for SHH signal reception in the neuroepithelium. Jointly, they are referred to as the SHH receptorsome and include the receptors LRP2, CDON, BOC, and GAS1. How exactly these proteins modulate SHH signal reception in the neuroepithelium remains speculative. Using mouse models with single or combined receptor deficiencies as well as iPSC-based in vitro models of the neuroepithelium, we elucidate the interaction of SHH co-receptors during forebrain specification. While mice deficient for Lrp2 or Gas1 show distinct craniofacial and forebrain malformations reminiscent of partial Shh deficiency, combined loss of both receptors results in an aggravated phenotype resembling complete loss of SHH activity. In the embryo, Lrp2 deficiency delays SHH signal reception and mislocalizes SHH expression from the immediate midline to more lateral regions of the RDVM. By contrast, SHH expression in the RDVM of Gas1 mutants is induced normally but lost with further development. Finally, SHH transcript and protein are completely absent in the RDVM of mice with dual receptor deficiencies. In iPSC-derived neural progenitor cells (NPCs), loss of LRP2 impairs SHH binding and uptake whereas GAS1 deficiency prevents induction of SHH target genes. Taken together, our data show that LRP2 and GAS1 perform distinct but complementary functions in SHH reception, stability, and signal transduction in vivo and cultured NPCs. Further clarifying these complex interactions will advance our understanding of the regulatory processes that modulate SHH activities in neuroepithelial development and forebrain formation.

W-4017

ENGINEERING STEM CELL SPHEROIDS TO RESCUE MUSCULAR DENERVATION ATROPHY

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Muscular denervation occurs in trauma and motor neuron disease and can cause significant morbidities, but there is currently no effective therapy nor clear mechanistic understanding. Nerve regeneration still does not rescue function if the muscle becomes atrophied and unreceptive to reinnervation. Appropriate recapitulation of regenerative niche growth factors (GFs) at the muscle could prevent atrophy and prolong the regenerative period for functional recovery. Harnessing cells, as

environmentally-responsive GF reservoirs, has advantages over synthetic manipulation of complex, incompletely-understood paracrine programs. Neural crest stem cells (NCSCs) are novel and accessible, and may be uniquely advantageous by being the natural precursors of the peripheral nervous (PN) system, and by playing key roles in early muscle formation and maintenance of skeletal muscle progenitors. Here we investigate whether three-dimensional spheroidal culture, which in other cells have promoted survival, preserved phenotype and enhanced functionality, and may promote neuromuscular junction (NMJ) assembly, could be used to optimize intramuscular NCSC transplantation for rescuing muscular atrophy. A streamlined protocol derives functional p75+ NCSCs from human iPSCs generated without genome integration of reprogramming factors. We showed for the first time that NCSCs can be formed into and maintained as spheroids whose sizes are controlled by number of cells during seeding, and in which form enhance secretion of GFs implicated in neuromuscular function– an advantage for paracrine therapy to prevent muscle atrophy. NCSC spheroids injected intramuscularly into a nude rat model of PN injury, in turn, have significant effect on functional recovery after 4 weeks via sciatic function index ($p<0.02$), and electrophysiology and muscle wet weight. Mesenchymal SC spheroid injection, in contrast, had insignificant effect, suggesting cell type specificity. As a possible mechanism of benefit, treated denervated muscle had higher relative NMJ area than PBS-injected controls. Here we demonstrated that biophysical effects of spheroidal culture on NCSCs could provide a distinct advantage for PN injury therapy, and continue engineering therapeutic potential for NCSCs uniquely relevant to nerve and muscle regeneration.

W-4019

STEMNET: A GENE NETWORK MODELING APPROACH TO ENGINEERING OF HUMAN STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS

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The differentiation of stem cells to hepatocyte-like cells (HLC) offers the perspective of unlimited supply of human hepatocytes. In our previous work we demonstrated that the gene expression profile of HLCs includes prominent features of intestine, fibroblast and stemness that negatively affect the degree of differentiation in comparison to primary human hepatocytes. However, due to the limitations of bulk transcriptomic analysis it remains unclear whether HLCs represent homogenous populations of cells with hybrid tissue identities or include subpopulations that arise from uncontrolled signaling dynamics in the differentiation program. We now employ an approach of single cell transcriptomics, epigenetics and phospho-proteomics combined with advanced statistical analysis to identify key components of non-liver associated differentiation that currently prevent hepatic maturation. In the BMBF network StemNet we will make use of our insights to improve terminal differentiation of HLCs through targeted interventions. Presenters: Patrick Nell, Graduate Student. David Feuerborn, Graduate Student

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W-4021

SINGLE-CELL IMAGING REVEALS UNEXPECTED HETEROGENEITY OF TELOMERASE REVERSE TRANSCRIPTASE EXPRESSION ACROSS HUMAN CANCER CELL LINES COMPARED TO IPSCS

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Telomeres, protective structures found at the ends of eukaryotic chromosomes, are important for maintaining genomic stability. In stem cells and early human development, chromosomal telomere shortening that occurs due to the “end-replication problem” during cell proliferation is normally compensated by telomerase. After development, telomerase is inactivated in somatic cells, leading to progressive telomere shortening until a critical length triggers cell senescence. However, telomerase is pathologically reactivated in approximately 80-90% of malignant human cancers, where it is considered an early cancer progression event and enables immortalization. Telomerase, which is a ribonucleoprotein enzyme, requires telomerase reverse transcriptase (TERT) for its activity; increased TERT expression is associated with poorer patient prognoses for several cancer

types. It is unclear how TERT becomes reactivated in cancer cells, and how this pathological reactivation is different from its normal activation in stem cells. Here we analyzed single-cell TERT expression across 10 human cancer cell lines and a human induced pluripotent stem cell (iPSC) line using single-molecule RNA fluorescent in situ hybridization (FISH). These cancer lines were previously classified as having monoallelic or biallelic TERT expression, but we found this classification to be oversimplified. Cancer lines exhibited substantial cell-to-cell variation in both number of transcription sites and ratio of transcription sites to gene copies (assessed using TERT DNA FISH) compared to iPSCs, which typically had 2 actively transcribing gene copies. The variance in the number of active transcription sites increased with the number of transcription sites, suggesting transcriptional bursting. While iPSCs were TERT diploids, as expected, several cancer lines had amplified TERT gene copies, which correlated with an increased number of transcription sites. iPSCs maintained longer telomeres than those of the telomerase-expressing cancer lines, which had heterogeneous lengths with little correlation to TERT expression levels. These data demonstrate an unappreciated heterogeneity in TERT expression across human cancers compared to iPSCs, which could help guide future cancer modeling and targeted therapeutic efforts.

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W-4023

A NOVEL HIGH-THROUGHPUT PLATFORM FOR HEAD AND NECK CANCER ORGANOIDS DRUG SCREENING

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Head and neck (HN) cancer is a broad category of tumor types arising from various anatomic structures including the craniofacial bones, soft tissues, salivary glands, skin, and mucosal membranes. Treatment often involves an intensive combination of surgery, radiotherapy and chemotherapy. Despite this, tumour recurrence rates remain high and survival rates are relatively poor. Here, we describe a novel high throughput drug screening platform combining the OrganoPlate®, a microfluidic based 3D-culture plate, and HN cancer-derived organoids. MIMETAS develops Organ-on-a-Chip-based models for evaluation of new medicines. Our unique microfluidic technology enables testing of compounds on miniaturized 3D organ models in high-throughput. These models are expected to show better predictivity as compared to laboratory animals and conventional 2D cell culture models, without compromising throughput or ease of use. Hereby we show the establishment of HN cancer-derived organoids in 2-lane OrganoPlate®, and its usefulness for phenotypic drug screenings. The aim of the study is to evaluate the 2-lane OrganoPlate® as a platform for growing HN cancer organoids and drug screening. Organoid lines (T2, T3 and T4) were embedded in the 2-lane OrganoPlate® as single cells in an Extracellular Matrix gel. At day 3, cultures were treated with Cisplatin or Carboplatin for 120 hours. Drug response was evaluated by assessment of morphology (phase contrast), Cell Viability (Alamar blue) and proliferation (EdU incorporation). Organoids cultures grow well under perfusion in the 2-lane OrganoPlate® and different sensitivity to cisplatin is captured by the used readouts. The high-throughput, microfluidic 2-lane OrganoPlate® platform offers an attractive method for growing HN cancer-derived organoids, supporting development of individualized tumour models for phenotypic drug screenings.

W-4025

ROBUST BONE REGENERATION THROUGH ENDOCHONDRAL OSSIFICATION OF HUMAN MESENCHYMAL PROGENITOR CELLS WITHIN THEIR OWN EXTRACELLULAR MATRIX

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Mesenchymal stem cells (MSCs) embedded in their secreted extracellular matrix (mECM) constitute an exogenous scaffold-free construct capable of generating different types of tissues. Whether MSC-mECM constructs can recapitulate endochondral ossification (ECO), a developmental process during in vivo skeletogenesis, remains unknown. In this study, MSC-mECM constructs are shown to result in robust bone formation both in vitro and in vivo through the process of endochondral ossification when sequentially exposed to chondrogenic and osteogenic cues. Of interest, a novel trypsin pre-treatment was introduced to change cell morphology, which allowed MSC-mECM constructs to undergo the N-cadherin-mediated developmental condensation process and subsequent chondrogenesis. Furthermore, bone formation by MSC-mECM constructs was significantly enhanced by the ECO protocol, as compared to conventional in vitro culture in osteogenic medium alone, designed to promote direct bone formation as seen in intramembranous ossification (IMO). The developmentally

informed method reported in this study represents a robust and efficacious approach for stem-cell based bone generation, which is superior to the conventional osteogenic induction procedure.

W-4027

PERIODONTAL LIGAMENT STEM CELLS CONTRIBUTE PERIODONTAL REGENERATION VIA IMMUNOMODULATION IN LIGATURE INDUCED PERIODONTITIS

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Periodontal ligament stem cells (PDLSCs) were recently identified as mesenchymal stem cells (MSCs). However, it is uncovered whether PDLSCs could regenerate periodontal tissue via modulation of host immune responses in periodontitis bearing rats. In this study, we investigated rat PDLSCs (rPDLSCs) mediated periodontal regeneration, related to host immune modulations in ligature-induced periodontitis bearing rats. Characteristics of rPDLSCs were verified as expression of MSC markers, colony forming abilities, adipogenic, osteogenic and chondrogenic differentiation potentials. A 3-0 silk was tied to induce periodontitis around the maxillary second molars and rPDLSCs were injected onto palatal side of the maxillary second molars of rats. No ligatured and no cell treated rats were used as negative control. Rats were sacrificed to harvest tissues for flow cytometry analysis and confocal microscopy on 1 day, for relative gene expression analysis on 7 days, for micro computed tomography (micro-CT), histological and histometric analyses at 8 weeks after rPDLSCs injection. The transplanted rPDLSCs were detected in draining lymph nodes (dLNs) of around oral cavity in ligature-induced periodontitis bearing rats. Micro-CT, histological and histometric analyses revealed that periodontal tissue regeneration was enhanced by the rPDLSCs transplantation in ligature-induced periodontitis bearing rats. The transplanted rPDLSCs appeared to function efficiently in the local periodontal tissue of ligature-induced periodontitis bearing rats. Flow cytometry profiles revealed that transplanted rPDLSCs could decrease CD4+CD25+ T cells of gingiva and increase CD4+PD-1+ T cells of dLNs in ligature-induced periodontitis bearing rats. Relative gene expression analyses showed that rPDLSCs transplantation could down-regulate the levels of IL6, IL17α, Cxcl1 and Ccl2 in gingiva of ligature-induced periodontitis bearing rats. Taken together, our results suggested that rPDLSCs transplantation could have a therapeutic potential for periodontal tissue regeneration, via modulation of CD4+ T cells in ligature-induced periodontitis bearing rats.

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W-4029

EARLY TISSUE GROWTH AND CELL FATE DETERMINATION USING GFP TRANSDUCED MESENCHYMAL STEM CELLS ON CELLSPAN ESOPHAGEAL IMPLANTS IN A PORCINE MODEL

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Esophageal disease may require resection and replacement of the diseased tissue, often with poor clinical outcomes. Biostage's Cellspan™ Esophageal Implant (CEI) supports regeneration of the esophagus using autologous adipose derived mesenchymal stem cells (AD-MSCs) seeded on a polyurethane scaffold. The timeline and mechanism of anastomotic growth as well as the fate of the implanted AD-MSCs is unclear. To address this, we have transduced autologous porcine AD-MSCs with green fluorescent protein (GFP) prior to seeding and implantation, and assessed tissue regrowth at 14, 21, and 28 days post implantation. Here, we describe GFP transduction, CEI production, cell fate and tissue regrowth determined by pathological and gross observation using short term study. Briefly, pAD-MSCs from 9 adult pigs were transduced with lentiviral GFP constructs. Cells were expanded and GFP expression confirmed. Cells were seeded on scaffolds and grown in bioreactors for 6 days. Glucose consumption was monitored every 2 days. On day 6 QC segments were analyzed for cell viability, penetration, and DNA content. Expression of VEGFA, MMP2, IL-6, and IL-8 was confirmed by ELISA. CEIs made using GFP transduced MSCs displayed similar characteristics to CEIs made using non-transduced MSCs. A full-circumferential resection of 5cm was performed in the esophagus and CEIs were implanted into each pig. All animals had esophageal lumen continuity at time of necropsy as early as 14 days, with early epithelial regrowth from both outer edges toward the center of the implant zone. Microscopy revealed fibrovascular tissue at the implant site and neovascularization on the adventitial side, with no discernable differences in tissue reorganization between 14 and 28 days. The majority of GFP+ cells were on the abluminal surface of the esophagus, few GFP+ debris and cells on the mucosal side, and little to no cells within the tissue. GFP+ cells were also found surrounding capillaries at sites of angiogenesis. No GFP+ cells were detected in the lymph nodes or on extruded scaffolds. Morphometric analysis of the implant zone indicates increased epithelial regrowth at day 28 compared to day 14. In conclusion, these findings support fibrotic luminal continuity by day 14, with neovascularization and epithelialization beginning as early as day 14 post-implantation.

W-4031

MODELING HUMAN HEPATO-BILIARY-PANCREATIC ORGANOGENESIS FROM THE FOREGUT-MIDGUT BOUNDARY

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Organogenesis is a complex and inter-connected process, orchestrated by multiple boundary tissue interactions. However, it is currently unclear how individual, neighboring components coordinate to establish an integral multi-organ structure. The hepato-biliary-pancreatic (HBP) anlage, which is demarcated by HHEX (Hematopoietically-expressed homeobox protein) and PDX1 (Pancreatic and duodenal homeobox 1) expression is first specified at the boundary between the foregut-midgut. Here, we leverage a three-dimensional differentiation approach using human pluripotent stem cells (PSC) to specify gut spheroids with distinct regional identities comprised of both endoderm and mesoderm. We show that antero-posterior interactions recapitulate the foregut (marked by SOX2, SRY-Box 2) and the midgut (marked by CDX2, Caudal type homeobox 2) boundary in vitro, modeling the inter-coordinated specification and invagination of the human hepato-biliary-pancreatic system. The boundary interactions between anterior and posterior gut spheroids enables autonomous emergence of HBP organ domains specified at the foregut-midgut boundary organoids in the absence of extrinsic factor supply. Whereas transplant-derived tissues were dominated by midgut derivatives, long-term culture develop into a segregated HBP anlage, followed by the recapitulation of early morphogenetic events including the invagination and branching of three different and inter-connected organ structures. Together, we demonstrate that the experimental multi-organ integrated model can be established by the juxta-positioning of foregut, midgut tissues, and potentially serves as a tractable, manipulatable and easily-accessible model for the study of complicated endoderm organogenesis and disease in human.

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W-4033

REGENERATIVE POTENTIAL OF LIMBAL EPITHELIAL STEM CELL SHEET ACCORDING TO DONOR'S AGE ON AMNIOTIC MEMBRANE SCAFFOLDS

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In patients with bilateral limbal stem cell deficiency (LSCD), transplantation of cultivated limbal epithelial stem cell sheets from limbal explants can restore the structural and functional integrity of the corneal surface. Here we comparatively investigated the optimized conditions on human amniotic membrane scaffolds (HAMS) with xenofree medium for clinical application and the efficiency of limbal epithelial sheet from donors of different age. We evaluated limbal epithelial stem cell sheet size, expression of stem/progenitor cell markers p63α and ABCG2, colony formation efficiency (CFE), and differentiation marker CK12. Human limbal explants from cadevaric donors of different age (60 years) were set on human amniotic membrane with xenofree medium for clinical application. The outgrowth sheet size, cell yield, ABCG2 efflux activity, p63α and Ki67 expressions, and CFE were increased in limbal epithelial sheets on HAMS. In terms of stemness, outgrowth cells from aged donors (>60 years) expressed less expression of stem/progenitor cell markers p63α and ABCG2 and low CFE compared to other two groups. Our results imply that donor less than 60 years are a better source for limbal epithelial stem cell sheet generation on HAMS with high regeneration potential.

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W-4035

A SNAPSHOT OF BORTEZOMIB-INDUCED NEUROTOXICITY IN HUMAN PLURIPOTENT STEM CELL-DERIVED SENSORY NEURONS REVEALS EXTENSIVE CHANGES IN MICROTUBULE DYNAMICS

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The neurotoxic effects of the chemotherapeutic agent Bortezomib are well documented, yet the mechanistic underpinnings that govern these cellular processes remain incompletely understood. In this study, system-wide proteomic changes were identified in patient-derived induced pluripotent stem cell sensory neurons (iSNs) exposed to a clinically relevant dose of Bortezomib. Our disease-in-a-dish model involved the differentiation of iPSCs created from 3 disease-unaffected individuals using an established, directed approach with typical iSN yields of 60-70%. At 21 days post-induction, iSNs were treated with 100nM Bortezomib, DMSO, or left untreated for 48h. Label-free mass spectrometry facilitated the identification of approximately 2800 differentially expressed proteins. A significant proportion of these proteins affect the cellular processes of microtubule dynamics, cytoskeletal and cytoplasmic organization, and molecular transport, and pathway analysis revealed an enrichment of proteins in signaling pathways attributable to the unfolded protein response. Expression changes in microtubule-associated proteins suggest a multifaceted relationship exists between bortezomib-induced neurotoxicity and microtubule cytoskeletal architecture, and MAP2 was prioritized as a topmost influential candidate. We observed a significant reduction in the overall levels of MAP2c in somata without discernable changes in neurites. As MAP2 affects cellular processes including axonogenesis, neurite extension and branching, and neurite morphology, its altered patterns of expression are suggestive of a central role in mitigating neurotoxicity.

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W-4037

ENHANCING PRE-CLINICAL IN VITRO CARDIOTOXICITY ASSAYS WITH BIOENGINEERING STRATEGIES IN A HIGH-THROUGHPT MANNER

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Stem-cell based in vitro cardiotoxicity assays hold great promise for mitigating the cost of drug development. These assays have the potential to be more predictive of drug arrhythmogenicity than contemporary screening methods which rely on singe ion channel recordings. However, stem cell assays have several important shortcomings when detecting and classifying the risk profile of several known compounds. One likely source of this error is the maturity of the cells; most phenotypes are indicative of a fetal developmental stage. To enhance the predictive power of stem-cell based assays, we have implemented bioengineering techniques to control the cell's culture surface. We utilized standard photolithography techniques to create nanoscale biomimetic grooves that mimic the shape and structure of collagen on a polymer-coated glass layer. The fabrication technique allows for generation of this pattern on standard formats and permits use of high-NA fluorescence microscopy. Using this approach we demonstrate that biomimetic engineering enhances several structural and biochemical phenotypes including sarcomere spacing, myofibril alignment, and sarcomere width. We extended this technique to pattern the surface of micro-electrode arrays, and demonstrate that these ECM-based cues enhance the electrophysiological response of cardiomyocytes to various drugs of known action that fail to elicit in vivo responses to drugs in vitro and recapitulated physiological IC50s when compared to traditional assays.

W-4039

DEVELOPMENT OF NEW TYPES OF ALLOGENIC GRAFT MATERIAL USING DEMINERALLIZED BONE MATRIX(DBM) CONTAINING HUMAN UMBILICAL CORD MESENCHYMAL STEM CELL CONDTIONED MEDIA

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In several decades, Allogenic demineralized bone matrix (DBM) has been used widely for bone formation and bone fusion because of their osteoinductive and osteoconductive properties. Despite DBM has advantages for osteogenesis, due to insufficient bone-inducing abilities, a variety of biomaterials containing rhBMP-2 have been developed. The BMP-2 has an excellent bone regeneration ability, however it has also reported various side effects in many clinical trials (ectopic bone formation and inappropriate adipogenesis etc.). Therefore, it is necessary to development a new bone graft substitutes which has an excellent bone regeneration effect with minimized side effects. Human umbilical cord mesenchymal stem cell(hUC-MSC) conditioned media (CM) has been shown to be a novel treatment for an efficacy and safety because CM contains several major growth factors derived from human. Additionally, there are various kinds of cytokines which involved highly in bone regeneration (such as TGF-beta, PDGF, EGF etc.). In this study, we developed a new types of bone graft material for osteogenesis using osteoconductive and osteoinductive properties. We produced DBM-CM which is a gel-type mixture of DBM (DBM: Cancellous bone: Caboxy Methyl Cellulose=15 % :15 % :70 %) and hUC-MSC CM. Bone forming potential of DBM-CM was simultaneously examined in vitro and in vivo experiments by using osteoblast-like cell SaOS-2 and implanting intra-muscularly in SD-rats respectively. We performed in vitro experiments of cytotoxicity and calcium matrix deposition that we co-cultured SaOS-2 with DBM or DBM-CM respectively. In our data, DBM-CM has been showed non-cytotoxicity toward SaOS-2 cells and induced mineralized nodule production compared with DBM and Control group. DBM-CM significantly increased of new bone formation and bone augmentation in the SD-rat after implantation. The bone augmentation was measured with H&E staining and Micro CT after 4-weeks and 8-weeks. In addition, various kinds of in vivo safety test have shown that our newly developed bone graft materials safer than our former graft materials. To the best of our knowledge, DBM-CM is the first investigation of bone graft materials using CM which has improved biocompatibility and bone formation efficiency.

W-4041

CONSORTIA FACTORS INDUCE DIFFERENTIATION OF ADIPOSE STEM CELLS TO HAIR FOLLICLE STEM CELLS

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Human adipose stem cells (ASCs) can differentiate to lineages of mesenchymal tissues. However, recent evidence suggests that these cells can also differentiate to non-mesenchymal cell lineages. Our objective is to evaluate a set of consortia factors (CFx) derived from human ASCs interactions with specific plant extracts in the in vitro differentiation of ASCs to hair follicle stem cells (HFSCs). ASCs were obtained from patient lipoaspirates and isolated from human donors after enzymatic treatment. These cells were expanded and treated with human recombinant differentiation factors and selective plant extracts to produce CFx. The CFx was then used to induce differentiation of ASCs to HFSCs. The CFx was analyzed by cytokine multiplex immunoassay and liquid chromatography mass spectroscopy (LCMS). The expression of cytokeratin 15 was analyzed by immunocytochemistry. The pattern and levels of secretory factors released by ASCs treated with CFx was significantly different compared to ASCs that grew in conventional hair differentiation media (controls). Higher expression of cytokeratin 15 was found in ASCs treated with CFx in comparison to controls. These results demonstrate for the first time that CFx enhance the differentiation of ASCs to HFSCs in vitro and may be benefit in hair regrowth.

W-4043

A HUMAN STEM CELL DERIVED MODEL OF WHITE ADIPOSE TISSUE FOR TYPE 2 DIABETES AND CARDIOMETABOLIC DRUG DISCOVERY.

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Type 2 Diabetes (T2D) and obesity are complex metabolic disorders affecting hundreds of millions of individuals globally. Whilst these diseases are characterised by multi-organ dysfunction, a wealth of evidence including human genetics implicates a role for white adipose tissue (WAT) in their pathophysiology. Insulin resistant WAT is dysfunctional, with corresponding WAT-secreted factors negatively influencing other metabolic organs such as the liver. Understanding this dysfunction is paramount to uncovering new disease biology and novel therapeutic targets, however this discovery is hampered by limited access to-, and functional and genetic heterogeneity of-, primary human WAT. To overcome this issue, we have generated a human WAT-like cell model via directed differentiation of human mesenchymal stem cells (hMSCs). To optimise this strategy, we studied the differentiation capacity of hMSCs from varying origins (umbilical cord, bone marrow,

and adipose tissue (AMSCs)). Differentiation efficiency was quantified by evaluating lipid accumulation via Oil red O staining, gene expression via qPCR, and protein expression via immunocytochemistry (ICC). Approximately 90% AMSCs were positive for Fatty acid binding protein 4 (FABP4) and lipids (n=3) after 16 days of adipogenic differentiation. They also expressed other adipose maturity gene markers such as Adiponectin (ADIPOQ) and Lipoprotein lipase (LPL). Upon functional characterization, AMSCs showed adipocyte-like function including lipolysis upon forskolin stimulation (via enzymatic colorimetric assay) and glucose uptake (quantified by detection of 2-deoxyglucose-6-phosphate). AMSCs were sensitive to insulin, decreasing lipolysis and increasing glucose uptake. These cells were also sensitive to different nutrients, such as fatty acids, which increased FABP4 and LPL expression, and low glucose, which increased leptin (LEP) expression. In summary, we have generated human WAT-like cells from AMSCs which exhibit characteristics of human primary adipocytes, and after further validation, will facilitate human disease modelling and target discovery studies for T2D and obesity.

W-4045

CADHERIN-11 AND N-CADHERIN REGULATE HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION

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The acquisition of specific cell fate is one of the core aims of tissue engineering and regenerative medicine. The influence of the cell-material interface on cell fate has been an area of significant research, but comparatively little is currently known about cell-cell interaction. Furthermore, while there is significant evidence that three-dimensional (3D) cultures positively influence fate decisions, the mechanisms underlying this are not known. Human mesenchymal stem cells (hMSCs) from bone marrow have the ability to differentiate into three lineages in vitro and are an example of a cell type that has been shown to differentiate more effectively in 3D culture. Here we study 3D spheroids of hMSCs in vitro, in which we study cadherin expression and signaling during proliferation and differentiation into different lineages. We make a comparison to cells cultured in 2D. We observe that proliferating hMSCs in 2D express N-cadherin and undergo a switch to cadherin-11 over time, which was not evident in the 3D cultures. This underlines a hypothesis for the mechanism regulating the differentiation of cells in 3D compared to 2D cultures. Furthermore, the importance of the cadherins in regulating the differentiation of hMSCs is evident by their knockdown. Knocking down cadherin-11 impeded hMSC differentiation in 2D, but cells continued to differentiate in 3D.

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W-4047

AFRICAN SPINY MOUSE (ACOMYS) REGENERATION FOLLOWING ACUTE, CHRONIC, AND VOLUMETRIC MUSCLE LOSS INJURIES

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Regeneration is the perfect regrowth and repair of damaged tissue. It is nature's ultimate solution to wound healing. The African spiny mouse (Acomys) is the only mammal in the world capable of scar-free skin regeneration as an adult. In order to study ear skin regeneration, we punched holes in the ears of Acomys as well as normal mice (Mus), which serve as non-regenerating controls. We observed that Mus simply scarred, whereas Acomys was able to regenerate hair, adipocytes, cartilage, and, most interestingly, skeletal muscle. We sought to further characterize Acomys's ability to regenerate different types of skeletal muscle. The Tibialis Anterior (TA) leg muscles of the mice were injected with cardiotoxin and found that regeneration occurs much faster in Acomys. Next, we sought to determine the extent to which Acomys is able to regenerate in response to repeated injury. Amazingly, even after chronic insult Acomys was still able to regenerate its muscle perfectly. We then looked to see whether Acomys could recover from volumetric muscle loss (VML) in which a portion of the muscle is removed. VML injuries are common in gunshot or car accident victims. To simulate VML, hole punches were made in the TA muscles of the mice. Preliminary data suggests that Acomys shows improved regeneration following VML injury. The results of continued study of Acomys could prove integral in gaining a comprehensive understanding of the regenerative process. Findings could ultimately improve the healthcare field by allowing for the regeneration of muscle and other tissue types.

W-4049

SURVIVAL OF EMBRYONIC CHICK DORSAL ROOT GANGLION NEURONS FOLLOWING CULTURE WITH HUMAN DENTAL PULP STEM CELLS CONDITIONED MEDIUM

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The dorsal root ganglion (DRG) consists of heterogeneous population of sensory neurons that function to relay sensory stimuli to the central nervous system (CNS). The sensory neurons have been defined as the fully-differentiated cells which less proliferation and self-renewal capacity. These characteristics influence the ability of survival of sensory neurons. Moreover, the culture of DRG neurons require the neurotrophic factors to support the survival capacity. Therefore, we investigate the cell source which provides the neurotrophic factors. Previous studies reported that the human dental pulp stem cells (hDPSCs) express a wide variety of neurogenic-associated markers and produce several types of neurotrophic factors including brain-derived neurotrophic factor (BDNF), glial -derived neurotrophic factor (GDNF) and nerve growth factor (NGF) which associated with survival capacity of neurons. The conditioned medium that collected from the hDPSCs could provide the survival capacity of neurons. Therefore, this study aims to investigate the survival of embryonic chick dorsal root ganglion neurons following culture with human dental pulp stem cells conditioned medium (hDPSCs-CM). The embryonic chick dorsal root ganglion neurons at embryonic day 8 (E8) were isolated and cultured for 4 weeks with 1:1 ratio of hDPSCs-CM/DMEM high glucose 10% FBS 1% Penicillin/Streptomycin compared with control medium (DMEM high glucose 10% FBS 1% Penicillin/Streptomycin). The immunocytochemistry (ICC) staining with neurofilament-low type (NF-L) and DAPI (nuclei staining) showed the survival rate with 67.21% of the hDPSCs-CM group compared with the control group (63.48%). The preliminary results indicated that the human dental pulp stem cells conditioned medium to support the survival of chick embryonic sensory neurons.

W-4051

DOWN-REGULATION OF NEURAL STEM CELL SPECIFIC MICRORNA INDUCES APOPTOSIS IN NEURAL STEM CELLS AND ASTROCYTES

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Neural stem cells (NSCs) have been defined as stem cells with the ability to self-renew and generate all cell types of the nervous system. It is important to understand an underlying mechanism by which NSCs proliferate and differentiate for efficient modulation of in vivo neurogenesis. MicroRNAs (miRNAs) are small noncoding RNAs controlling gene expression concerned in post-transcriptional control by blocking mRNA translation or degrading mRNA. miRNAs play a role as control mechanisms in accordance with matching target mRNAs. Recent studies have discussed the biological mechanism of miRNAs regulation in NSCs and neurogenesis. In this study, we screened out NSC specific miRNAs using miRNome-wide analysis. Then, we have induced downregulation by sponge against the specific miRNA to evaluate the functional role of the miRNA in NSCs and NSC-derived astrocytes. We found down-regulation of NSCs specific miRNA induces apoptosis in NSC and NSC-derived astrocytes. In our study, we have identified the new NSC specific miRNA and found its role as apoptosis regulator in NSCs and astrocytes. Overall our finding can provide us insight of potential roles of NSC specific miRNAs in brain neurogenesis and possible usage of the miRNAs for biomarker of neurodegenerative disease.

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W-4053

MOTOR FUNCTION RECOVERY IN HEMIPLEGIC MICE AFTER NEURAL STEM / PROGENITOR CELLS TRANSPLANTATION

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Neural cell transplantation is thought to be one of the promising strategies for treating brain damage. The aim of this study was to investigate whether neuron transplantation and signal activation through in paracrine manners would be associated with motor function recovery in hemiplegic mice. Hemiplegic mice were generated by cryoinjury of the motor cortex We dissected the brains sequentially after the injury and then analyzed the dissected brain. We examined whether endogenous Reelin was expressed in the damaged motor cortex. At day -6 (1 day after the cryoinjury), Reelin and Gfap were weakly expressed and Nfm was not significantly expressed in the damaged motor cortex. Reelin and Gfap expressing cells were observed in

moderate amounts at day 0 and such the cells increased at day 7. Expression of Reelin in the damaged motor cortex elicited gradual reduction at day 14. At day 28, the expression of Reelin decreased somewhat whereas Gfap positive cells sustained moderate abundance. It is possible that Gfap expressing cells were somehow associated with Reelin producing cells in the damaged cortex. Motor cortex without injury scarcely had Reelin and Gfap expressions. We then examined the downstream signaling molecules of Reelin in order to elucidate the molecular mechanisms governing the histological regeneration and functional recovery of the hemiplegic mice after transplantation of neural stem/progenitor cells (NSPCs) from wild-type mice or yotari mice (yot/yot genotype) having nonfunctional Dab1 by a mutation of its gene. Neurospheres were induced from the neonatal brain of WT/WT mice and yot/yot mice at day -7. At day 0, the neurospheres were disaggregated and the resulting single cells as NSPCs were transplanted to the brain in the striatum under the damaged motor cortex. The motor functions of the hemiplegic mice having WT/WT NSPC transplantation improved significantly compared with those of yot/yot NSPC transplanted mice. The grafts derived from WT/WT mice migrated from the striatum and reached the injured cortex 14 days after transplantation. On the other hand, majority of the grafts from yotari mice didn't migrate and thus remained at the striatum. Our findings support involvement of Reelin signaling pathway for regeneration of motor cortex and subsequent functional recovery in adult hemiplegic mice as well.

W-4055

DIRECT DIFFERENTIATION OF NEURON-LIKE CELLS FROM TONSILLAR-BIOPSY DERIVED MULTIPOTENT STEM CELLS

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Parkinson's disease (PD) is a devastating aging-related neurological disorder affecting about 1 million people in the US and more than 6 million worldwide. Despite considerable efforts, there is currently no cure for PD. Adult multipotent stem cells (MSCs) hold great promise for the treatment of PD. These adult stem cells possess major advantages over embryonic stem (ES) cells or induced pluripotent stem (iPS) cells as they are derived from human tissues at any age, do not form teratoma tumors, and possess natural ability to differentiate and secrete factors to promote tissue healing without genetic manipulation. MSCs are present in tissues throughout the body and are capable of proliferation and differentiation to promote tissue regeneration and cellular replacement in disease conditions. Previous reports indicate that MSCs derived from bone marrow (BM-MSCs) can differentiate toward neural stem cells. However, unlike with embryonic stem cells, it is not clear whether BM-MSCs can develop into mature neurons. Moreover, they rapidly lose proliferative and differentiation capacity, creating a critical barrier for their use in clinical cellular therapy. We recently reported the isolation of MSCs and the generation of 14 cell lines from small biopsies of human palatine tonsils (T-MSCs). Our T-MSCs have been shown to exhibit equivalent multipotency and marker expression to those of BM-MSCs. We show that millions of MSCs can be harvested from a sample as little as 0.6 g, which can be collected in an outpatient setting without the need for general anesthesia or hospitalization. Importantly, these cells exhibit delayed senescence in culture, which could serve to bypass the clinical barrier seen with BM-MSCs. In this study, we demonstrate for the first time that tonsillar-biopsy derived stem cells have a potential to differentiate into post-mitotic neuron-like cells without genetic manipulation. Given that immature neural cells have shown a superior therapeutic potential compared to undifferentiated MSCs,our study has the potential for novel cell therapies of PD and other neurodegenerative diseases.

W-4057

HUMAN STEM CELL-DERIVED MOTOR NEURONS AS A POTENTIAL REPLACEMENT OF DEGENERATING SPINAL CORD NEURONS IN A NON-HUMAN PRIMATE MODEL OF CONUS MEDULLARIS/CAUDA EQUINA INJURY

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Spinal cord injury result in a variety of neurodegenerative reactions, including motor neuron degeneration and death. Currently there are no treatments to reverse associated neurological deficits. The present study investigated the feasibility of using human embryonic stem cell-derived motor neurons as a potential replacement of degenerating spinal cord neurons in a non-human primate model of conus medullaris/cauda equina form of spinal cord injury. Female rhesus macaques (n=8) were subjected to an L6-S3 ventral root avulsion (VRA) injury and replantation of the L6 and L7 ventral roots into the spinal cord. Approximately 250,000 cells were injected into the L5 spinal cord segment. An immunossupression protocol was developed and included anti-thymocyte globulin, tacrolimus, prednisone, and anti-CD40. Spinal cord tissues were analyzed at 24 hours after surgery (n=1), 2 months post-surgery (n=3), and 7 months post-surgery (n=4). Morphological studies identified the injection site and survival of human cells in all animals. At 2 months post-surgery some transplanted cells formed rosettes of neural progenitors expressing SOX2, and NESTIN. Subsets of cells also showed labeling for motor neuron and oligodendrocyte progenitors, such as OLIG2, but no markers for astrocytes (GFAP) or microglial cells (IBA1) were identified. At 7 months post-surgery no rosettes, astrocytes, or microglial cells were identified, although oligodendrocyte progenitors were readily identified. STEM 121 and βIII-tubulin markers confirmed a neuronal phenotype among human cells at both 2 and 7 months post-surgery, and showed elongated fiber tracts in the grey and white matter. A small group of transplanted cells showed Ki-67 labeling, but no tumor formation was detected. The integration of human cells in the primate spinal cord was confirmed using pre-embedding immuno-gold labeling for analysis of STEM121 labeling in the electron microscope. Functional analysis showed a VRA-induced left leg weakness, but preserved capability to use the affected limb for climbing, balancing, and stepping. Cystometrogram and EMG recordings showed preserved micturation reflexes. We concluded that our new immunosuppression protocol was successful with human cell survival and formation of neural circuits in the primate spinal cord.

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W-4059

APOE4 ALTERS TRANSCRIPTIONAL PROFILES IN MICROGLIA FROM MOUSE MODELS AND HUMAN IPSCS

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The strongest genetic risk factor for sporadic Alzheimer's disease (AD) is the E4 allele of the cholesterol transporter apolipoprotein E (APOE4). There are multiple mechanisms through which APOE4 may be increasing AD risk, with its actions on neuroinflammation and glial function being a central pathway. For example, APOE4 significantly alters the function of microglia, the major immune cell type of the brain, whose role in AD pathogenesis is well established. Recent findings have shown a distinct transcriptional signature of microglia in AD and other neurodegenerative diseases. However, the extent to which APOE4 may affect microglial transcription, both in healthy brain and in the context of AD is currently unknown. To address this knowledge gap, we assessed the effects of APOE4 on microglial transcription profiles using both mouse models of AD and human induced pluripotent stem cells (iPSCs). CD11b+ microglia were isolated from whole brain of mice carrying either human APOE4, or human APOE3, the neutral risk allele. These mice were either on a wildtype C57BL6/J background or crossed with the 5xFAD mouse, which carries 5 familial AD mutations, thus resulting in 4 groups: E3WT, E4WT, E3FAD, and E4FAD. Additionally, iPSCs from healthy, non-demented APOE3 and APOE4 carriers were differentiated into microglia. Both microglia isolated from whole mouse brain and differentiated from human iPSCs were analyzed by RNAseq. Our findings demonstrate that APOE4 is associated with a specific transcriptional signature in microglia, both in the context of AD as well as in healthy, non-AD backgrounds. Identifying how APOE4 affects microglial transcription will be critical to understanding how this genetic risk factor alters microglia function to drive neurodegeneration.

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W-4061

MODELING SYNGAP1 TRUNCATING MUTATIONS IN NEURODEVELOPMENTAL DISEASE USING IPSC-DERIVED NEURONS

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The synaptic Ras-GTPase activating protein, SYNGAP1, is one of the most abundant proteins at the postsynaptic site of excitatory neurons. SYNGAP1 plays a critical role in the organization of a complex protein-protein interaction network at the synapse with profound impact on the scaffold and structural functions that shape dendritic spines and neuronal connections. De novo mutations in SYNGAP1 are autosomal dominant are highly prevalent in intellectual disability and have been associated with autism spectrum disorders. Mouse models have showed that mutations in SYNGAP1 alter neuronal morphology and synaptic function. However the role and mechanisms altered by mutations present in human neurons remains to be explored. To study the mechanisms altered by mutations in SynGAP1 in patients, we generated Induced pluripotent stem cells (iPSC) from haploinsufficient patient samples (+/-) and differentiated iPSC to induced excitatory neurons (iN). Mutation-corrected cell line (+/+) were generated by CRISPR-Cas9 and differentiated to iN. We analyzed the expression of SynGAP1 protein, the morphology and electrical activity from patient and control cell lines. Expression of SynGAP1 protein is restored in CRISPR-corrected neurons. Patient derived neurons present abnormal dendritic spines morphology together with alterations in bursts and spikes frequencies in Multielectrode arrays recordings. Here we show that the study of patient-derived neurons can be used to study the role of mutations in components of the postsynaptic synapse and help to understand their role in neurodevelopmental disease.

W-4063

STUDY ON TRANSLATIONAL RESEARCH TRENDS IN SPINAL CORD INJURY FROM CLINICAL TRIAL REGISTRY; DISCUSSION ON THE DIFFERENCES BETWEEN THE NUMBER OF RESEARCH ARTICLES AND CLINICAL TRIALS

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We have previously analyzed and reported trends in regenerative medicine clinical research using the clinical trial registry in four disease areas. Here, we report on the analysis concerning spinal cord injury (SCI). Articles containing “SCI” and “stem cell” as MeSH terms were extracted using PubMed and analyzed according to country and the number of articles. Based on the results, the United States was at the top followed by China, South Korea, Japan, and Canada. However, when the number of clinical trials recorded in ClinicalTrials.gov (CTG) and/or the International Clinical Trial Registry Platform (ICTRP) were analyzed by country, the countries occupying the top two positions were reversed, with China at the top followed by the United States. India and Brazil also advanced to a higher rank on the list, indicating that the number of articles did not reflect the actual number of clinical trials reported in CTG and the ICTRP. This discrepancy is presumed to be due to differences in translation by country. The contributing factors are likely to be the number of patients with the disease, the difficulty of obtaining cell materials, ethics, policy, regulation, funding, development companies, time lag to clinical trial stage, and difficulty of patient recruitment. Among these factors, the analysis was conducted by focusing on “cell material.” In the ladder chart analysis, most of the cells used originated from the bone marrow (BM), followed by adipose tissue (AD), and umbilical cord (UC). This reflects the ease of obtaining available cell materials. On further analysis by country, some specific characteristics were observed: various cell materials including embryonic stem cells (ESCs) and neural stem cells (NSCs) in the United States; UC in China; AD in South Korea; only BM in Japan, India, and Brazil; UC and AD in Spain; and BM and NSCs in Iran. Therefore, the results indicate that while the translation focus in the United States, using ESCs and NSCs, is relatively on a higher difficulty level, China has promoted translation using UC. Furthermore, we predicted the study focus of future reports on translational research trends from each country.

Funding Source: This study was supported by the Highway Program for Realization of Regenerative Medicine of The Japan Agency for Medical Research and Development (AMED) under Grant Number JP18bm0504009

W-4065

SPONTANEOUS CELL CYCLE REENTRY AND THE SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE IN A 3D MODEL OF C9ORF72 FRONTOTEMPORAL DEGENERATION-AMYOTROPHIC LATERAL SCLEROSIS

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Frontotemporal degeneration (FTD) is the second most common presenile dementia of individuals under the age of 65. Even though clinically distinct from each other, it has been known that a link between FTD and ALS exists, resulting in some patients developing an overlap syndrome with features of both disorders. One of the most recent advances of FTD-ALS was the discovery that a large percentage of sporadic and familial ALS and FTD and familial FTD-ALS carry a hexanucleotide repeat expansion (GGGGCC)n in the first intron of C9ORF72. However, few cellular models exist that faithfully delineate the mechanistic and cellular responses associated with either disease or the impact of the C9ORF72. Here we used inducible pluripotent stem cell (iPS) populations derived from control and C9ORF72 (C9+) patients, and differentiated these cells into neuronal-glial co-cultures using two- and three-dimensional (2D and 3D) culturing techniques. We hypothesized that an in vitro 3D culture system would provide a powerful in vitro model for ALS/FTD, one which more closely mimics native brain morphology and pathology than standard two-dimensional cultures. Twelve weeks after neuronal differentiation, the C9+ cells spontaneously expressed cyclin D1 protein compared to control lines in the 3D culture systems, suggestive of aberrant reengagement of cell cycle processes. Further analysis of cell cycle-associated transcripts revealed a significant increase in cyclin dependent kinase inhibitor 2A (CDKN2A), cyclin dependent kinase inhibitor 2B (CDKN2B) and a significant decrease in aurora kinase B (AURKB) gene expression in the C9+ lines. Computational analyses of these gene expression patterns suggest that multiple cellular senescence signaling pathways may be activated in C9+ lines associated with an aberrant reentry into the cell cycle. Subsequent analysis of the culture supernatants from the C9+ lines indicated significant levels of CXCL8, CXCL1, IL13, IP10, CX3CL1 and reactive oxygen species, which are components of the senescence-associated secretory phenotype. Taken together, the results from our in vitro 3D assay system suggest spontaneous neuronal cell cycle reentry and senescence-associated secretory phenotype could be an underlying component of ALS and FTD in C9ORF72.

Funding Source: Fiske Drug Discovery Fund (J.S.L.), the Owens Family Foundation (E.F., J.S.L., G.B.), NIH RF1 AG51085 (G.S.B.), NIH R01 GB10683 (E.R.S.), Hartwell Foundation (E.F.) and the Cure Alzheimer's Fund (G.B., J.S.L, E.R.S.).

W-4067

GENOME-WIDE CRISPR SCREEN FOR ZIKA VIRUS RESISTANCE IN HUMAN IPSC-DERIVED NEURAL CELLS

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The brain was once thought to be largely isolated from the immune system. This view is changing, as recent data suggest that peripheral and resident immune cells play complex roles in brain disorders. We devised human models of microglia-neuron interactions, and are using these models to understand how inflammatory triggers affect brain function. We worked to recreate microglial ontogeny in the dish, from human pluripotent stem cells, generating primitive macrophages resembling early microglia. Using novel tissue-engineering approaches including 3D co-cultures and cerebral organoids, we showed that their transcriptional profile and physiological behavior could approximate different stages of development, leading to their ability to dynamically survey the neuro-glial environment, and respond to injury or immune stimulation. We have focused on the role of microglia in the early dissemination of the Zika virus to the fetal nervous system, and performed an unbiased CRISPR screen for host factors necessary for lethal infection of neural stem cells. Zika virus (ZIKV) is a neurotropic and neurovirulent arbovirus that has severe detrimental impact on the developing human fetal brain. To date little is known about the factors required for ZIKV infection of human neural cells. We comprehensively identified ZIKV host genes in human pluripotent stem cell-derived neural progenitors (NPs) using a genome-wide CRISPR/Cas9 knockout screen. Mutations of host factors involved in heparan sulfation, endocytosis, endoplasmic reticulum processing, Golgi function and interferon activity conferred resilience towards ZIKV of the Uganda strain, and a current endemic American isolate. ZIKV host genes identified in human NPs also provided low level of protection, when targeted in isogenic human astrocytes, against ZIKV and Dengue. This result emphasizes the need to study viral mechanisms in biologically relevant cells. Our findings illuminate host-dependent mechanisms for ZIKV infection in the highly vulnerable human neural stem cells, and indicate molecular targets for potential therapeutic intervention.

Funding Source: NIH, Simons Foundation, International Rett Syndrome Foundation, Brain and Behavior Research Foundation, Canada First Research Excellence Fund, European Leukodystrophy Association, Canada Research Chairs Program, HHMI and ACS.

W-4069

EXOSOMES: A NEW BIOMARKER FOR NEURODEGENERATIVE DISEASES.

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Exosomes can pass the blood-brain barrier. This property makes exosome research attractive for areas such as biomarker discovery for neurodegenerative diseases and also drug delivery. To study these fields, efficient methods for isolating exosomes from small volumes of biofluids such as serum, plasma and cerebrospinal fluid (CSF) need to be identified. Three different exosome isolation systems were compared using serum, plasma and CSF as starting samples in order to identify the best approach. Among other factors, yield, purity as well as structural integrity of the generated samples have been analysed as part of this comparison. Analyses such as NTA, exosome protein to particle ratio, WB and TEM were used to generate comparative data.

POSTER I - EVEN
19:30 – 20:30

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-2002

IMMUNOMODULATORY PROPERTIES OF CANINE PLACENTA-DERIVED MESENCHYMAL STEM CELLS: POTENTIAL APPLICATION FOR INFLAMMATORY BRAIN DISEASE

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Autoimmune diseases of the central nervous system (CNS) are characterized by infiltration of reactive immune cell subsets into the brain and spinal cord. The pathophysiology of naturally-occurring diseases in veterinary species may better recapitulate human diseases and serve as superior models for evaluation of therapeutics. Dogs specifically suffer from inflammatory brain disease (IBD). Mesenchymal stem cells (MSCs) could be a promising therapy for autoimmune CNS diseases based on their ability to inhibit T-cell proliferation, alter B-cell function and inhibit dendritic cell maturation and differentiation. In this study we evaluated the immunomodulatory attributes of canine adipose-derived MSCs (ASCs) and placenta-derived MSCs (PMSCs) to provide potency information to determine the optimal MSC source to treat IBD. We also compared mechanisms of immunoregulation by canine and human PMSCs to demonstrate that the canine is a useful model to evaluate cell therapy for IBD. Our study emphasized immunoregulation of MSCs by showing secretion profiles of anti-inflammatory cytokines and their capacity to inhibit lymphocyte proliferation in vitro. MSCs were activated directly with interferon gamma and tumor necrosis factor alpha or indirectly by co-culture of MSCs with mitogen induced peripheral blood mononuclear cells (PBMCs). Activated canine ASCs and PMSCs secrete high concentrations of indoleamine 2,3 dioxygenase (IDO) and prostaglandin E2 (PGE2) after both direct and indirect stimulation. ASCs and PMSCs inhibit PBMC proliferation when co-cultured in contact with stimulated PBMCs. However, PMSCs inhibited PBMC proliferation significantly more than ASCs. Blocking studies revealed PGE2 is critical for ASC inhibition of PBMC proliferation. ASCs inhibit lymphocyte proliferation via cell cycle arrest in G0/G1, while PMSCs induce lymphocyte apoptosis. Our results have demonstrated that ASCs and PMSCs are both potential targets for cell therapies for IBD; however, PMSCs more potently inhibited lymphocyte proliferation by inducing apoptosis. These data suggest that the mechanism by which ASCs and PMSCs downregulate PBMC proliferation differs. These findings provide critical preclinical data assessing PMSCs for treatment of neurological diseases moving toward human clinical trials.

W-2004

SUSPENSION HYALURONAN INDUCES A MITOCHONDRIAL FUNCTIONAL SWITCH IN FAST-PROLIFERATIVE HUMAN MESENCHYMAL STEM CELLS

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Hyaluronan preserves the proliferation and differentiation potential of mesenchymal stem cells. Supplementation of low concentration hyaluronan (SHA) in medium suspension for culturing stem cells increases their proliferative rate, whereas coated hyaluronan (CHA) on the tissue culture surface maintains cells in a slow-proliferative mode. We have previously demonstrated that in CHA, stem cells' metabolic status during a reduced proliferative state is influenced by upregulating mitochondrial biogenesis and function. However, the effect of SHA on stem cells' mitochondrial energetic metabolism remained unknown. In this study, we demonstrated the effect that low concentration SHA at 0.001 mg/ml (SHA0.001) and high concentration SHA at 5 mg/ml (SHA5) exerts on stem cells' mitochondrial function compared to CHA and non-coated tissue culture surface (control). Fast-proliferative human placenta-derived mesenchymal stem cells (PDMSCs) cultured on SHA0.001, when compared to slow-proliferative PDMSCs cultured on CHA at 5.0 (CHA5) or 30 μg/cm2 (CHA30), was found to have lower mitochondrial mass, lower mitochondrial DNA copy number, and lower oxygen consumption rate. The reduced mitochondrial biogenesis observed in SHA0.001 was accompanied by a 2-times higher ATP content and lactate production, suggesting that hyaluronan-induced fast-proliferative PDMSCs may rely less on mitochondrial function as an energy source and induce a mitochondrial functional switch to non-mitochondrial pathways. Both, PDMSCs cultured on CHA and SHA had a decrease in reactive oxygen species levels. Results from this study clarify our understandings on the influence of hyaluronan in stem cells and provide important insights into the effect of distinct supplementation method used during cell therapies.

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ADIPOSE AND CONNECTIVE TISSUE

W-2006

ENGRAFTMENT POTENTIAL OF MATERNAL ADIPOSE-DERIVED STEM CELLS FOR FETAL TRANSPLANTATION

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In utero stem cell transplantation is a promising medical tool for many genetic disorders, but graft rejection caused by maternal T cells as the main barrier to engraftment limits clinical outcomes. As maternal T cells are trafficking in the fetus during pregnancy, we hypothesized that using maternal adipose-derived stem cells (ADSCs) for in utero transplantation can develop maternal-fetal chimerism in the undeveloped fetal immune system, thus lowering the risk of graft rejection. Herein, fetus brain engraftment using maternal or allogeneic grafts was examined via in utero stem cell transplantation. ADSCs were purified using the mesenchymal stem cell markers PDGFRα and Sca-1 via fluorescence-activated cell sorting. Neuronal differentiation of ADSCs was induced by three-dimensional aggregation culture, and mature neuronal markers were detected. Fetal brain grafts grew for at least 1 month after in utero allogenic ADSC transplantation. Furthermore, maternal ADSCs reduced immune cell infiltration and suppressed the innate immune response, preventing the infiltration of CD8-positive, CD45-positive lymphocytes into the graft. Thus, in utero maternal ADSC transplantation is beneficial for the treatment of congenital CNS diseases because of the ability of the cells to differentiate into neuronal lineages and reduce immune responses.

W-2008

COMPARATIVE ANALYSIS OF HUMAN CARTILAGE DERIVED MESENCHYMAL STEM/ PROGENITOR CELLS FROM OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS PATIENTS

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Arthritis is one of the leading causes of disability around the world affecting different age groups. It has recently been evident that articular cartilage harbours a viable pool of stem/ progenitor cells and has led to reinvent the potentiality of these cells to stimulate endogenous reparative mechanisms to

regenerate articular cartilage. The objective of this study was to compare stem/progenitor cells in the context of osteoarthritis (OA) and rheumatoid arthritis (RA) cartilage tissue, since there is very limited evidence in the literature. Mesenchymal stem/progenitor cells (MPCs) were isolated from OA and RA cartilage samples and compared based on their morphology, viability, growth kinetics, senescence associated β-galactosidase (SA β-gal) activity, colony forming ability, cytogenetic stability, alkaline-phosphatase (ALP) activity and sterility. Phenotypic characteristics of MPCs were analysed using markers, such as CD73, CD90, CD105, CD146, CD166, CD34, CD45 and HLA-DR. The isolated MPCs were plastic adherent and displayed a spindle-shaped fibroblast-like morphology. This consistency in phenotype was maintained up to passage 10 (P10). The cell viability was observed more than 95% and the proliferation was comparable in both OA and RA derived MPCs. The population doubling time of MPCs from OA was 51.33±8.67 hrs, whereas that of RA was 76.51±33.31 hrs. SA β-gal activity was absent in early passage, but present in a few cells in late passages. Colony forming ability analyses showed no noticeable differences between OA and RA samples. ALP activity was observed in the MPCs with or without osteogenic induction. MPCs expressed the mesenchymal markers, but not the hematopoietic markers, and no marked difference in expression was observed between OA and RA samples. Upon induction, MPCs were differentiated along osteogenic, adipocytic and chondrocytic pathways. This was further evidenced by the expression of genes involved in osteogenesis (RUNX2 and osteocalcin), adipogenesis (PPAR-γ2 and lipoprotein lipase) and chondrogenesis (collagen type II, SOX9 and aggrecan). Based on the findings, it is envisaged that the characterized human cartilage derived MPCs could be a suitable choice of cell type for progressing cell-based therapies to repair or regenerate cartilage tissue in OA and RA patients.

Funding Source: This work was supported by Nitte (Deemed to be University), Mangaluru, India.

W-2010

NOTCH SIGNALING ENHANCES STEMNESS BY REGULATING METABOLIC PATHWAYS THROUGH MODIFYING P53, NF-KB, AND HIF-1ALFA

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Human adipose-derived mesenchymal stromal cells (hASCs) are attractive for regenerative medicine, but their limited in vitro life span limits their therapeutic applicability. Recent data demonstrate that hypoxia may benefit the ex vivo culture of stem cells. Such cells exhibit a high level of glycolytic metabolism under hypoxic conditions. However, the physiological role of glycolytic activation and its underlying regulatory mechanism are incompletely understood. We have shown that when activated under conditions of 5% O2, Notch signaling dramatically increases the rate of glycolysis, improves proliferation efficiency, prevents senescence, and maintains the multipotency of hASCs. In the present study, we found that activated Notch1 enhanced nuclear p65 levels, resulting in an increase in glucose metabolism through the upregulation of glycolytic factors, including GLUT3. Notch signaling was also involved in glucose metabolism through p53 inactivation. We also found that NF-κB signaling was regulated by p53. These data suggest that Notch-HES1 signaling enhances the glycolytic pathway through p53 and NF-κB. 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 the coordination of Notch signaling and HIF. Overall, our observations provide new regulatory mechanisms for the glycolysis by Notch signaling to maintain the stemness of hASCs.

MUSCULOSKELETAL TISSUE

W-2012

PERFORMANCE-BASED AUTOMATED SELECTION AND EXPANSION OF CLONAL POPULATIONS DERIVED FROM HUMAN ARTICULAR CARTILAGE TO IMPROVE CELL THERAPY STRATEGIES

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Native tissue sources contain highly heterogeneous populations of progenitors with the capacity to proliferate and differentiate into one or more connective tissues. The clones derived from these founding cells exhibit wide variation in their biological potential (proliferation and differentiation). When unselected cells are placed into culture stochastic variation in the dominance of one clone over the other can result in enormous changes in the biological performance of culture-expanded progeny. We hypothesize that purposeful control over the starting population will provide greater control over the variation in quality and biological potential of the end product. This would provide greater safety and efficacy - and reduce the cost, by limiting investments made in inferior starting materials. The goal of this study is to identify the attributes of cartilage-derived progenitors that are predictive of both high expansion potential and in vitro expression of chondrogenic markers. Articular cartilage (Outerbridge grade 1-2) obtained from six knee arthroplasty patient's were enzymatically digested to isolate cells for 2-D cell culture assay.

Using standardized large field of view image analysis, 24 clonal populations from each patient were identified and "picked" using Cell X Automation Platform. Of these, 12 clonal colonies were expanded to 20 doublings for trilineage differentiation assay and RNA sequencing analysis. Colony founding cells demonstrated wide variation in initial morphology: [circularity (median: 0.665; range:0.15-0.93), area (median: 116.4µm2; range:51.9-204.2µm2)]. Clonal progeny also demonstrated large variation in performance: [doubling time (median:32.9h; range:26.9-41.2h) and colony density (median:6.5%;range:2.3-20.7%)]. Wide variation in differentiation potential and patterns of RNAseq expression are present. Trilineage differentiation in vitro and RNAseq data collection is pending and will be used to define attributes that are most closely associated with the presence or absence of chondrogenic markers. These data will enable prospective assessment of the value of automated selection or removal "weeding" of clonal populations based on quantitative morphological or performance parameters that can be detected non-invasively.

Funding Source: Lisa Dean Moseley Foundation

W-2014

LINEAGE TRACING REVEALS A SUBSET OF MOUSE MUSCLE RESERVE STEM CELLS CAPABLE OF CLONAL EXPANSION UNDER STRESS

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Stem cell heterogeneity is recognized as functionally relevant for tissue homeostasis and repair. The identity, context-dependence, and regulation of skeletal muscle stem cell subsets remain poorly understood. We identify a minor subset of Pax7+ satellite cells (SCs) that is indelibly marked by an inducible Mx1-Cre transgene in vivo, enriched for Pax3 expression and has reduced ROS (Reactive Oxygen Species) levels. Mx1+ SCs possess potent stem cell activity upon transplantation, but minimally contribute to endogenous muscle repair, due to their relative low abundance. In contrast, a dramatic clonal expansion of Mx1+ SCs allows extensive contribution to muscle repair and SC niche repopulation upon selective pressure of radiation stress, consistent with reserve stem cell (RSC) properties. Loss of Pax3 increased ROS content, diminished RSC survival and stress tolerance. Human muscle stem cells also have a subset of SCs capable to tolerate radiation stress. These observations demonstrate that the Pax7+ SC pool contains a discrete population of radio-tolerant reserve stem cells (RSCs) that undergo clonal expansion under severe stress.

W-2016

MODULATION OF SMALL MOLECULES FOR EFFICIENT 3D-CHONDROGENIC DIFFERENTIATION OF TISSUE-SPECIFIC AND INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED HUMAN MESENCHYMAL STEM CELLS (HMSCS)

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Human mesenchymal stem cells (MSCs) are post-natal stem cells considered as excellent cell sources for tissue engineering of cartilage, a tissue without the capacity for regeneration or repair but often injured in activity and with aging. Among them, induced pluripotent stem cell (iPSC)-derived MSCs (iMSCs) are a novel source of MSCs which are strongly proliferative and can be designed to be patient-specific. We therefore studied the use of iMSCs for therapeutic use in cartilage-related diseases. And investigating how 3D culture conditions mechanistically modulate MSC chondrogenesis for discovery of targets on which small molecules can be applied to enhance the efficiency of the process. Our preliminary data demonstrate that one pair of small-molecule agonist/antagonist X and Y, respectively, which could cause opposing effects on MSC chondrogenic differentiation. Furthermore, drug Y was able to induce MSC chondrogenesis in the absence of TGF-β, which upregulated α-SMA expression. We are continuing to understand how these small molecules interact in the 3D culture environment to induce more efficient chondrogenesis in iMSCs.

W-2018

SKELETAL STEM CELLS EXHIBIT ALTERED GENE EXPRESSION IN DISUSE OSTEOPENIA

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Disuse osteopenia (DO) is a complication of prolonged mechanical unloading of the skeleton which results in pronounced bone loss and a concomitant increase in marrow adiposity. This skeletal involution is most severe in astronauts during spaceflight, but also occurs due to prolonged bed rest or limb immobilization. While studies have implicated dysregulated osteoblast and osteoclast activity in the pathophysiology of DO, the cell types do not account for the profound changes in marrow adipose accumulation. Skeletal stem cells (SSCs) are precursors of bone and adipose tissue in adult bone marrow and SSC bifurcation into bone and fat in culture is known to be affected by mechanical forces acting upon cells. Using the hindlimb unloading (HU) model of DO in mice coupled with RNA-Seq analysis of Leptin Receptor positive (LepR+) SSCs sorted directly from bone marrow, we have demonstrated that 6 and 12 weeks of HU results in significant alterations in the transcriptome of SSCs as compared to ambulatory controls. Gene ontology analysis revealed significant changes in genes related to cell division, cytoskeletal organization, RNA catabolism, glycogen synthesis, and long chain fatty acid metabolism. Many of the genes changed are also predicted to be targets of the transcription factor TWIST1, a negative regulator of SSC bifurcation whose transcription was upregulated in SSCs in response to HU. Importantly, TWIST1 has been implicated as a mechanosensor in other systems. Analysis of serum markers indicated that HU did not result in metabolic derangements in mice, while microCT confirmed skeletal involution. Together, these data indicate that SSCs have a cell-autonomous response to mechanical unloading of the skeleton in vivo and their dysfunction may contribute to the DO phenotype.

W-2020

MODULATION OF CARTILAGE RESPONSES AND CHONDROGENIC DIFFERENTIATION IN HUMAN MESENCHYMAL STEM CELLS VIA MECHANOTRANSDUCTIVE L-TYPE CALCIUM CHANNELS

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Articular cartilage due to low self-regenerative capacity is very sensitive to trauma or degenerative diseases such as osteoarthritis, whereas mesenchymal stem cells (MSCs), appear to be promising candidates for cartilage engineering and regeneration. Since hypertension is a common disease in patients with OA, the use of antihypertensive drugs such as nifedipine may affect the course of OA. These molecules regulate intracellular Ca2 + levels and therefore modulate various cellular functions. Aim of this study was to evaluate the effects of nifedipine and L-type calcium channel agonist BayK8644 on chondrogenic differentiation of human bone marrow (BM)-MSCs and mature chondrocytes. For that human BM-MSC and chondrocytes were incubated with nifedipine and BayK8644, and evaluated for chondrogenesis and intracellular calcium levels, as were measured with calcium dye Cal-520 (flow cytometry). Explants of human cartilage tissue were incubated with nifedipine and BayK8644 with / without mechanical load (Flexcell compression system). Chondrogenic differentiation and cartilage responses were assessed by immunohistochemistry (Safranin-O staining and anti-collagen II antibodies) and gene expression assays. We observed reduction of proliferation in BM-MSCs and chondrocytes by nifedipine. Flow cytometry analysis showed different models of intracellular Ca2 + oscillations in both cell types under incubation with L-type calcium channel regulators. More depolarised resting membrane potential in chondrocytes than in BM-MSC was observed by patch clamp. Both nifedipine and mechanical load modulated the expression of collagens I and II, SOX9 and CaV1.2 in cartilage explants. Ca2+ channel blocking reduced mitochondrial respiration and ATP production in MSCs and chondrocytes but increased glycolysis reserve only in chondrocytes (determined by the Seahorse Agilent). Nifedipine and Bay-K8644 stimulated the production of extracellular matrix in both cell types. In summary, nifedipine regulates energy metabolism of human BM-MSCs and chondrocytes and has

beneficial effects on their chondrogenic differentiation, which implies that long-term use of L-type Ca2+ channel inhibitors for cardiovascular disease can modulate cartilage regenerative capacity.

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W-2022

MYF6/MRF4 PREVENTS EXHAUSTION OF THE MOUSE MUSCLE STEM CELL POOL BY A FEED-FORWARD MYOKINE SIGNALING PATHWAY

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The myogenic regulatory factors Myf5, MyoD, Myogenin and Myf6/MRF4 play a crucial role in the execution of a temporally regulated myogenic differentiation program during skeletal myogenesis. We show that Myf6/MRF4 establishes communication between differentiated myotubes and their associated muscle stem cells through myokine signaling. Chromatin immunoprecipitation sequencing (ChIP-seq) in differentiated myotubes revealed that Myf6 not only regulates muscle differentiation genes, but also unexpectedly controls the expression of a vast array of myokines like LIF, EGF, OSM and VEGFA. Myf6-deficient mice are born visibly normal and without any obvious muscle phenotype. However, further immunostaining and genetic analyses revealed that muscle stem cells from these mice exhibit impaired activation of canonical signaling pathways such as EGFR and STAT3 and readily break quiescence, leading to a reduction in MuSC numbers. Together, our data identifies a novel function for Myf6 in sustaining the muscle stem cell pool by a feed-forward myokine signaling loop originating from the myofiber.

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CARDIAC TISSUE AND DISEASE

W-2024

SINGLE CELL TRANSCRIPTOMIC AND FUNCTIONAL ANALYSIS OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED VENTRICULAR CARDIOMYOCYTES FROM PULMONARY ATRESIA WITH INTACT VENTRICULAR SEPTUM PATIENTS

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Pulmonary atresia with intact ventricular septum (PA-IVS), a rare form of congenital heart disease (CHD), is characterized by an atretic right ventricular outflow, hypoplastic right ventricle and various degrees of tricuspid valve and coronary artery anomalies. To date, no genetic mutation has been consistently identified. We report the use of bioengineered cardiac tissues from human induced pluripotent stem cell derived ventricular cardiomyocytes (hiPSC-VCMs) to explore intrinsic functional phenotype of PA-IVS cardiomyocytes and single cell RNA sequencing to decode the functional anomalies at the transcriptomic level. Human ventricular cardiac tissue strips (hvCTS) constructed from PA-IVS hiPSC-VCMs consistently demonstrated reduced contractility when compared with those from healthy controls, caused by downregulation of cardiac contractile apparatus transcripts (ACTA1, MYL2, CSRP3, SORBS2) in PA-IVS hiPSC-VCMs. This is consistent with the clinical observation of impaired right ventricular contraction fraction in PA-IVS patients. Human ventricular cardiac anisotropic sheets (hvCAS) constructed from PA-IVS hiPSC-VCMs did not demonstrate any electrophysiological differences when compared with those from healthy controls, which is consistent with the uncommon clinical occurrence of cardiac arrhythmias in PA-IVS. Single cell sequencing of hvCAS reveals this platform provides a hypertrophic stimulus to hiPSC-VCMs with upregulation of associated genes (NPPB, HOPX, PDLIM3). PA-IVS hvCAS however did not express such genes but instead aberrantly re-expressed immature cardiac contractile isoforms

(MYH6) and cardiac progenitor genes (ISL1, MEF2C, SMYD1) via the canonical Wnt pathway (PSMB9, FRZB, DACT1). We conclude that bioengineered cardiac tissues are capable of capturing the intrinsic functional abnormalities of sporadic CHDs in the absence of secondary remodelling in vivo to the structural alteration.

W-2026

AN EXPERIMENTAL FRAMEWORK FOR IDENTIFYING AND CHARACTERIZING THE PROXIMATE EFFECTS OF FETAL-SPECIFIC CARDIAC REGULATORY VARIANTS ON MOLECULAR PHENOTYPES

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To fully understand the contributions of genetic regulatory variants to human health and disease, it is essential to characterize their proximate effects in both adult and fetal developmental stages. Here, we used iPSCORE (a bank of hundreds of iPSC lines generated from individuals of multiple ethnicities) to characterize molecular phenotypes (RNA-seq, ATAC-seq, ChIP-seq for H3K27ac) in 180 "fetal-like" cardiovascular progenitor cell (iPSC-CVPC) samples derived from 139 individuals. We used single cell RNA-seq to examine eight iPSC-CVPCs and found they were comprised of two distinct cell types, that showed both transcriptional and chromatin states consistent with being cardiomyocytes (CMs) and epicardium derived cells (EPDCs). We established CM-specific and EPDC-specific expression signatures and used them to deconvolute the bulk RNA-seq data for the 180 iPSC-CVPCs, determining the relative ratios of both cell types in all samples. We have leveraged these deconvoluted expression data in combination with whole-genome sequences to perform expression quantitative trait loci (eQTLs), resulting in the discovery of cell-type specific and developmental stage (fetal) specific gene expression regulatory variants that are associated with cardiac disease. Specifically, we normalized the expression of 16,156 expressed genes, adjusted them for multiple covariates (age, sex, ancestry and cell population distribution) and detected eQTLs for 3,163 eGenes, of which 210 were associated with the cell-type. We next investigated if fetal-CM and fetal-EPDC eQTLs provide the same associations between genetic variation and gene expression as adult cardiac tissue eQTLs. We performed a colocalization analysis on all eGenes with eQTL data from GTEx cardiac tissues, and found that 1,570 (49.6%) of the eGenes were not shared with adult tissues. To assess whether fetal-specific eQTLs are associated with complex cardiac traits measured in adult individuals, we colocalized eQTLs with summary statistics from cardiac GWAS, and found 25 fetal-specific eGenes associated with cardiac disease traits. These results show that analysis of the eQTLs in iPSC-CVPCs identifies cardiac disease GWAS regulatory variants that are active in the fetal heart, but not in adult heart tissues, suggesting that they play a role in development.

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W-2030

SARCTRACK: HIGH-THROUGHPUT TRACKING OF FLUORESCENT SARCOMERES ASSESSES FUNCTION, VARIANTS, AND DRUG RESPONSES IN HUMAN IPSC-CARDIOMYOCYTES

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in combination with CRISPR/Cas9 genome editing provide unparalleled opportunities to study cardiac biology and disease. However, sarcomeres, the fundamental units of

myocyte contraction, are immature and nonlinear in hiPSC-CMs, which technically challenges accurate functional interrogation of contractile parameters in beating cells. Furthermore, existing analysis methods are relatively low-throughput, indirectly assess contractility, or only assess well-aligned sarcomeres found in mature cardiac tissues. We aimed to develop an analysis platform that directly, rapidly, and automatically tracks sarcomeres in beating cardiomyocytes. The platform should assess sarcomere content, contraction and relaxation parameters, and beat rate. We developed SarcTrack, a MatLab software that monitors fluorescently-tagged sarcomeres in hiPSC-CMs. The algorithm determines sarcomere content, sarcomere length (SL), and returns rates of sarcomere contraction and relaxation. By rapid measurement of hundreds of sarcomeres in each hiPSC-CM, SarcTrack provides large datasets for robust statistical analyses of multiple contractile parameters. We validated SarcTrack by analyzing drug-treated hiPSC-CMs, confirming the contractility effects of compounds that directly activate (CK-1827452) or inhibit (MYK-461) myosin molecules or indirectly alter contractility (verapamil and propranolol). SarcTrack analysis of hiPSC-CMs carrying a heterozygous truncation variant in the myosin-binding protein C (MYBPC3) gene, which causes hypertrophic cardiomyopathy (HCM), recapitulated seminal disease phenotypes including cardiac hypercontractility and diminished relaxation, abnormalities that normalized with MYK-461 treatment. SarcTrack provides a direct and efficient method to quantitatively assess sarcomere function. By improving existing contractility analysis methods and overcoming technical challenges associated with functional evaluation of hiPSC-CMs, SarcTrack enhances translational prospects for sarcomere-regulating therapeutics and accelerates interrogation of human cardiac genetic variants.

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W-2032

TRANSCRIPTION FACTOR INTERACTOME IN HUMAN IPS-DERIVED CARDIAC PROGENITORS IS ENRICHED FOR PROTEINS ASSOCIATED WITH CONGENITAL HEART DISEASE

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Congenital heart disease (CHD) affects ~1% of live births and remains the leading cause of mortality in infants. While large-scale genetic studies have uncovered genes associated with CHD, distinguishing variants that confer risk from the background noise of inconsequential variants remains a challenge. Causative mutations in transcription factors (TF) essential for cardiovascular development, such as NKX2-5, GATA4 and TBX5, have been identified in familial cases of CHD, however they are rare. To expand our understanding of their molecular function and to test whether their interacting proteins may be enriched for variants associated with CHD. we defined the protein-protein interaction (PPI) network of NKX2-5, GATA4 and TBX5 using unbiased mass spectrometry in human iPSC-derived cardiac progenitors (iPS-CPs). This approach yielded a network of 172 proteins. An interdependent gene-regulatory role has been reported at the DNA-binding level for these 3 TF during cardiac development, and we also found interdependent protein interactomes where loss of one TF affected the interactome of the the others. Interactomes for each were enriched in proteins involved in similar biological processes, such as chromatin remodeling and gene regulation, or previously unrelated processes such as splicing and mRNA transport. Integration of the iPS-CP-PPI network with the CHD-associated damaging variants found in the Pediatric Cardiac Genomics Consortium whole-exome sequencing cohort revealed statistically significant enrichment in the GATA4 interactome for de novo missense variants. In contrast, neither the TBX5 or NKX2-5 PPIs were enriched for either de novo missense or rare damaging variants. Finally, we developed a framework to rank PPIs with reported damaging variants for functional validation studies. Overall, this work identified novel protein interactors of TFs essential for cardiac development, offering new insights regarding their regulatory roles and the mechanisms through which they may cause CHD.

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W-2034

ELASTIN LIKE POLYPEPTIDE ENHANCES MOUSE STEM CELLS DIFFERENTIATION INTO CARDIOMYOCYTE-LIKE CELLS

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Differentiation of stem cells into cardiomyocytes holds great promise for the field of cardiac tissue regeneration. We investigated the effect of elastin like polypeptides (ELPs) on cardiomyocyte differentiation and proliferation of mouse embryonic stem cells (ESC). ELPs possess unique thermoresponsive and elastin-mimicking properties. We hypothesized that these properties might enhance stem cell differentiation. To test this hypothesis, we recombinantly synthesized ELPs. mESC-derived embryoid bodies (EBs) were suspended in culture for 4 days in the presence or absence of ascorbic acid (AA) as the differentiation factor, and the EBs were next transferred to a gelatin or ELP-coated culture dish with a fresh differentiation medium to induce further differentiation for 10 days. ELPs were coated on the culture dish either by simple adsorption (non-crosslinked), or by crosslinking the ELPs using an enzyme (crosslinked). To determine the cardiac differentiation efficiency we measured the beating rate in each group on day 9, and on day 14 the cardiac gene and protein expression was analyzed by qRT-PCR and immunocytochemistry. In the gelatin control group, addition of AA improved cardiomyocyte differentiation, led to larger cardiomyocyte colony clusters that exhibited proliferative spreading as opposed to small beating colonies when AA was not added. When ELPs were used in non-crosslinked form, the percentage of beating colonies were lower, however, the colonies showed greater sarcomere branchings. When ELP was crosslinked, a significant enhancement in cardiomyocyte beating colony was observed, and the beating colonies had a larger area beating in unison as compared to gelatin control. Furthermore, crosslinked ELP group showed higher myocardial related gene expression including Brachury, isl1, GATA4, TBX5, cTNT2 and MLC2a. These findings suggest that crosslinked ELPs could be useful for differentiation of ESCs in to cardiomyocytes for regenerative medicine.

W-2036

MITOCHONDRIA-RICH HUMAN PLURIPOTENT STEM CELL DERIVED-CARDIOMYOCYTES WITH ADVANCED METABOLIC PROPERTIES UNIQUELY RECAPITULATE DISEASE PHENOTYPE AND DRUG RESPONSES

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Differentiation of human pluripotent stem cells (hPSCs) to the cardiac lineage represents a potentially unlimited source of cardiomyocytes (CMs) for research and clinical applications. However, their immature, embryonic-like developmental state, heterogeneity and poorly defined phenotype greatly limits their use. Using a unique strategy for proteomics profiling, we identified proteins on the surface of hPSC-CMs. Our dataset revealed an association between the expression of late protein 1 (LP1), a surface protein involved in metabolism, with a more advanced differentiation stage. CMs positive for this maturation marker are more adult-like in their mitochondrial gene expression pattern, morphology and functions, and are more sensitive to oxidative stress. We next showed that LP1+ CMs can be utilized to study adult disease phenotypes involving mitochondrial dysfunction. LP1+ CMs uniquely recapitulate the effect of cardiotoxic agents and are a significant improvement upon existing hPSC-CMs and animal models. Our work provides important proof-of-principle for the isolation, characterisation and application of mature and defined hPSC-CM subpopulations, and will greatly advance the use of hPSC-derivatives for disease modeling and drug screening.

W-2038

MODELING SUSTAINED VENTRICULAR TACHYCARDIA WITH PATIENT DERIVED IPSC CARRYING A NOVEL SCN5A MISSENSE MUTATION

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Ventricular fibrillations stemming from ventricular tachycardia and abnormal heartbeat may lead to sudden death. In the pediatric or adult cases, fatal and sustained tachycardia are diagnosed based on recording more than 120 beats per minute (BPM) for a defined period. However, it remains elusive whether Ca2+ dynamics and beating capacity of iPSC-derived cardiomyocytes (iPSC-CM) could be developed to recapitulate sustained tachycardia, which could require strong excitability and contractility patterns comparable to adult cardiomyocytes. Therefore, to evaluate the potential to model sustained cardiac tachyarrhythmia, we generated iPSC-CM from two patients from the same family, of whom the son was clinically diagnosed with both junctional ectopic and fascicular ventricular tachycardia, and the mother depicted ventricular extra systoles. Genetic analysis of patients by exome sequencing revealed dominant inheritance of a novel missense mutation at the SCN5A gene. Gene expression and immunostaining analysis did not imply any significant change in mRNA expression or a trafficking defect

of the mutant SCN5A protein. Interestingly, electrophysiological analysis of the patient-specific iPSC-CM by an impedance based hybrid electrode system revealed spontaneous contraction frequency of 150-250 BPM that is sustainable for months, whereas iPSC-CM from healthy subjects exhibited between 45 to 95 BPM. Furthermore, firing rates of patient-specific iPSC-CM could be observed from 180 to a maximum of 355 BPM with shapeless and unrecognizable T-waves for several hours, suggesting that the culture have switched to an intermittent fibrillation-like state reminiscent of the clinical phenotype. Importantly, frequent beating rates and field potential spike amplitude could be significantly and exclusively attenuated by application of Flecainide, a Class 1C sodium channel blocker; in strike contrast to the administration of Mexiletine, the Class 1B antiarrhytmic drug, or a betablocker, atenolol, both of which did not trigger any phenotypic change. To our knowledge, this is the first study specifically recapitulating sustained ventricular tachycardia both singly and accompanied with intermittent fibrillation-like activity in iPSC-CM, as well as mimicking the drug response of patients to the in vitro setting.

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W-2040

IPSC-DERIVED CARDIOMYOCYTES: ESTABLISHING CHARACTERIZATION PARAMATERS IN A 3-DIMENSIONAL CULTURE SYSTEM

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Translating iPSC-derived tissue remains a daunting task, given the lack of definitive process-development standards. Our laboratory is engaged in determining characterization standards for human iPSC-derived cardiomyocyte-lineage cells (iPSC-CL) produced via a 3-dimensional (3D) culture system. We began by selecting assays capable of identifying an iPSC-derived product as “cardiomyocyte-like”. Five iPSC clones were selected based upon data obtained from 2-dimensional (2D) culture: Three clones capable of producing spontaneously beating iPSC-CL, one “marginal” clone, and one clone incapable of cardiac differentiation. DNA fingerprinting confirmed identification; G-banding revealed 4/5 clones exhibited a normal karyotype. MitoSort analysis of mtDNA revealed no detrimental mtDNA heteroplasmy in any of the five lines. Additionally, we treated iPSC-CL with 50 nM of etoposide for 24 hr as a “failsafe” method to eliminate contaminating pluripotent cells. Flow analysis confirmed iPSC-CL post-thaw viability was > 70% for all five products, 3/5 expressed troponin isoform levels > 70% and 4/5 exhibited a proliferation rate < 5%. RT-PCR analyses revealed significant expression of MYH-6 and -7 RNA in 3/5 lines, while pluripotent gene expression was undetectable in all samples, regardless of etoposide treatment. Isoproterenol stimulation resulted in a dose-dependent β-adrenergic response in 3/4 cell lines tested, and all four lines capable of generating 3D iPSC-CL exhibited maximum respiration rates normally observed in healthy cardiomyocytes. Finally, 3/4 beating iPSC-CL displayed an APD90/APD50 ratio < 1.4, typical of a ventricular-like cardiomyocyte subtype. Our results demonstrate that 2D culture is largely predictive of cardiomyocyte differentiation potential in a 3D culture system, and importantly, the differentiation process alone eliminated the presence of pluripotent cells. Moreover, our 3D-iPSC-CL were highly energetic and exhibited sufficient maturity to positively respond to β-adrenergic stimulation. In-process animal studies will provide the ultimate validation of the safety and potency of our 3D iPSC-CL product.

Funding Source: Todd and Karen Wanek Family Program for Hypoplastic Left Heart Syndrome

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

W-2042

RESTORING THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE IN IPSC-DERIVED ENDOTHELIAL CELLS INDUCES SURFACE GLYCOCALYX FORMATION AND ALIGNMENT TO FLOW

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Human induced pluripotent stem cells (iPSCs) are widely used to study organogenesis and disease modelling, and are being developed for regenerative medicine. iPSCs have been differentiated into many cell types, including endothelium; however maturation and stabilization of iPSC-derived endothelial cells (iPSC-ECs) remains challenging. Here, we demonstrate that prolonged exposure to shear stress alone or in combination with pericyte co-culture fails to induce flow alignment and structural maturation of iPSC-EC when compared to microvascular EC. Furthermore, these have a reduced luminal glycocalyx, critical for vasculature homeostasis, shear stress sensing and signalling. We found that iPSC-EC have a dysfunctional mitochondrial permeability transition pore, resulting in reduced mitochondrial function and increased release of reactive oxygen species (ROS). Closure of this mitochondrial membrane transition pore by cyclosporine-A, improves EC mitochondrial function, restores the glycocalyx and subsequent allows for the alignment to flow. These findings indicate that mitochondrial maturation is required for proper iPSC-EC functionality.

W-2044

GENERATION OF A BIPOTENT HEMANGIOGENIC PROGENITOR FROM HUMAN PLURIPOTENT STEM CELLS

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The close temporal-spatial relationship between hematopoietic and endothelial cells during early embryonic development has led to the hypothesis of a common ancestor, the hemangioblast. Here we explored human pluripotent stem cells (hPSCs) endothelial and hematopoietic differentiation via a bipotent progenitor, an in vitro equivalent of the hemangioblast. hPSCs were differentiated into hemangiogenic mesoderm through embryoid body (EB) formation under serum-free culture conditions. After 3,5 days of differentiation, a population positive for CD309 (VEGFR-2), CD144 (VE-Cadherin), CD143 (ACE) and CD34 was isolated based on the expression of CD144 and cultured either under endothelial or hematopoietic conditions. A homogeneous population of functional CD144+CD31+ (PECAM-1) CD34+ and vWF+ endothelial cells was obtained after 4 days of endothelial differentiation. These cells upregulated ICAM upon adding TNF-α, endocytosed acetylated-LDL, expressed eNOS and formed tubular networks when cultured on a matrigel layer. Additionally, passaging and cryopreservation were possible without modifying their phenotypic and functional characteristics. Furthermore, CD144+ EB sorted cells generated blast colonies (BCs) after 4 to 6 days grown in methylcellulose supplied with growth factors. BCs expressed hematopoietic markers such as CD43, CD45 and CD41 and gave rise to in vitro hematopoietic cells under colony forming cell (CFC) assay conditions. Interestingly, we observed an intermediate population at day 4-6 of BC formation expressing both CD144+ and CD45+ suggesting the hemangioblast-like progenitor may undergo an endothelial-hematopoietic transition. These results suggest the existence of an early, isolable and cryopreservable hemangioblast like-progenitor derived from hPSCs. Further experiences aiming to confirm the bipotency of this population in vivo should be carried on in order to assess its potential in future regenerative medicine.

W-2046

TRANSMEMBRANE SCF WITH NANOCARRIERS ENHANCES REVASCULARIZATION IN ISCHEMIA AND INDUCES CD34-/CD133+ ENDOTHELIAL PROGENITOR CELLS

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Diabetes mellitus affects approximately 371 million people worldwide, and one out of every three people with diabetes over the age of 50 have peripheral artery disease. Soluble stem cell factor (SCF) was a promising therapeutic for treating ischemia and stroke but failed clinical trials due to adverse effects including mast cell activation. SCF also exists in a transmembrane form (tmSCF) that has different properties from the soluble form of SCF. We aimed to develop therapeutics based on tmSCF encapsulated in nanocarriers to enhance its activity and maintain solubility in solution. We developed formulations of recombinant tmSCF embedded in the lipid membranes of liposomes and nanodisc (NDs). We validated the creation of these constructs using cryo-EM and dynamic light scattering. We found that both tmSCF proteoliposome (tmSCFPLs) and tmSCF nanodiscs (tmSCFNDs) enhanced revascularization in mice with hind limb ischemia in comparison to control for both WT and ob/ob mice. Histological analysis showed significantly higher number of CD34+VE-cadherin+ cells in the calf and thigh muscle treated with tmSCFNDs after hind limb ischemia surgery. We performed an endothelial progenitor cell (EPC) colony formation assay on bone marrow cells (BMCs) and BM mononuclear cells (BMMNCs) and found a significantly higher number of large EPC colonies in tmSCFPL/ND treated groups. We also incubated BMCs with the tmSCF formulations, quantified the result by flow cytometry, and found a significantly higher numbers of CD34-CD133+CD146+ cells in the tmSCFPLs/NDs treated BMCs. From this result, we hypothesized that tmSCFPLs/NDs act on BMCs and stimulate them to be more like CD34-CD133+EPCs. We further examined the mobilization of CD34-CD133+EPCs to the peripheral blood by subcutaneously injecting our treatments into mice for consecutive 4 days. Flow cytometry analysis revealed that tmSCFND treated mice had significantly higher number of CD34-CD133+EPCs in their blood. An Evan's blue dye assay for vascular leakage in mice demonstrated that the lipid formulations of tmSCF had minimal activation of mast cells in comparison to soluble SCF. In conclusion, our results demonstrate novel therapeutics based on tmSCF that can be used to treat peripheral ischemia without mast cell activation.

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W-2048

NON-CODING GENOMIC VARIANTS INDUCE FUNCTIONAL DEFICITS IN IPSC-DERIVED HUMAN ENDOTHELIAL CELLS

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Genome-wide association studies have correlated single nucleotide polymorphisms (SNPs) in the 9p21 locus with coronary artery disease (CAD). Homozygous carriers, which account for 23% of the US population, have a 60% increased risk of CAD, yet mechanisms are poorly understood as 9p21 variants occur outside of protein-coding sequences. To better understand how non-coding variants affect CAD, we created and differentiated induced pluripotent stem cells (iPSCs) into endothelial cells (ECs) from patients homozygous for the risk (R/R) or non-risk haplotype (N/N); to account for patient variability, the locus itself was deleted to produce isogenic knockouts (KOs). iPSC-VECs cultured in monolayer show ZO1 and VE-cadherin peripheral localization consistent with tight junctions independent of risk haplotype, but despite this, R/R VEC monolayers were 1.25- to 2.5-fold more permeable than their isogenic or non-risk counterparts, respectively. Moreover addition of the inflammatory cytokine TNFalpha increased permeability independent of risk but disproportionately affected R/R VECs. iPSC-VECs cultured in perfusion bioreactors in the presence of acute high stress further confirm functional deficits associated with risk where cells with the risk haplotype disengage from both adjacent cells and basement membrane, leading to enhanced vessel permeability and consistent with CAD phenotypes. Functional deficits are reflected by differences in signaling within the non-coding locus, in particular with changes in the long non-coding RNA, ANRIL. These data further substantiate our previous results indicating that pathologically relevant stress conditions can exacerbate existing differences caused by 9p21 SNPs to lead to disease including CAD.

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HEMATOPOIESIS/IMMUNOLOGY

W-2050

FUNCTIONAL NK CELLS GENERATED FROM HUMAN IPS CELLS WITH 3D-BIOREACTOR FOR IMMUNO-ONCOLOGY

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NK cells are cytotoxic cells critical for innate immune system. Early investigation using CAR-NK cells have shown promising antitumor activities. CAR-T therapy requires either autologous or MHC matching cells to avoid graft-verse-host disease (GVHD), whereas allogeneic NK cells showed substantial anti-tumor activity without GVHD. Therefore, NK population is regarded as the ideal Off-The-Shelf immune cell therapy products. The NK-based immunotherapy, however, has been limited by unsatisfactory sources of NK cells. Human induced pluripotent stem cells (iPSCs) offer unlimited source for manufacturing NK cells. Previously NK cell derivation from hPSCs used feeder cells with multiple scale-up limited processes. In this study we described a novel 3D-bioreactor platform that can continuously generate highly pure NK cells with strong cytotoxic activity. First, undifferentiated human iPS cells were cultured as 3D-spheres with mesoderm inducing conditions, which converted 60-70% iPS cells into hemogenic endothelial progenitor (HEP, CD31+CD144+CD34+) in a week. HEP cells were then cultured under NK cell differentiation condition for up to 40 days. Starting from day 20, NK cells were started to release from these 3D-spheres which can be harvested daily. These iPS-NK cells can be cryopreserved. Human iPS-NK cells display a distinctively homogenous morphology. Over 95% of collected cells express CD56, NKG2D, NKp44 and NKp46 markers; Approximately 30-50% of CD56+ iPS-NK cells express KIR2DL/DS1 and KIR2DS4 receptors; CD56+ PSC-NK cells do not express TCR and CD3, and CD19, which are specific markers for T and B cells, respectively. Surprisingly, we discovered that over 80% CD56+ iPS-NK cells also expressed CD8 as compared to ≈30% of peripheral blood CD56+ NK cells, the increase of which is associated with disease regression in AIDS patients. More importantly, when iPS-NK cells and K562 leukemia cells were mixed together, over 80% of k562 cells were killed within hours as demonstrated both by FACS analysis and time-lapse movie. With the establishment of master iPS-CAR cell lines, our novel technology platform will provide inexhaustible cell sources for the generation of truly off-the-shelf CAR-NK cells suitable for treatment of a large numbers of patients, which can revolutionize the immuno-oncology field.

W-2052

GENOMICS AND PROTEOMICS PROFILING OF PRIMITIVE HSC SUBSETS IN HUMAN BONE MARROW SAMPLES: A MOVE TOWARDS PRECISION MEDICINE

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Development of novel methods in genomics and proteomics will aid in comprehending the biology of primitive hematopoietic stem cells (HSC) and has important implications for precision medicine as well as for basic research. Both CD34+ and CD34- fractions of HSCs exist in murine and human long term repopulating (LTR) in lineage-negative (Lin-CD34low/-). Characterization of the early HSC in BM and identification of an unequivocal marker will facilitate their selection; proliferation and propagation in large scale for clinical usage. In this study Lin–CD34–CD38 Low/– and Lin–CD34+CD38Low/– HSC, were sorted and their molecular and cellular characterization was compared by morphological, gene expression, and proteomics profiling. Around 10 BM discarded units by our blood bank as their recipients were deceased were allocated for this study. Phenotypic study was done by flow cytometry and their ability to differentiate into multi lineages was assessed by colony forming unit (CFU). Gene expression and proteomics technology were applied to compare similarities and differences. Our results showed that CD34+ and CD34- subsets were present in BM samples in variable proportions and their ratios estimated to be 1:2. Furthermore, colonogenic ability as established by CFU assay and measured to be less in CD34- than in CD34+ which gave rise to several hematopoietic cell lineages including CFU-E, BFU-E, CFU-G, CFU-M, CFU-GM, and CFU-GEMM. Additionally, both HPSC subsets expressed pluripotency/ stemness genes i.e. Oct4, Nanong, TERT as quantified by real time PCR. Proteomics analysis revealed that similar spots were aberrant in both HPSCs compartments and more analysis is underway to decipher these spots. In conclusion, distinct biological characteristics of CD34– compared to CD34+ HSC segment and hopefully further analysis will facilitate in the discovery of a universal marker(s) which will facilitate their selection for medical applications.

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W-2054

THE ROLE OF VENTX HOMEOBOX GENE DURING HUMAN HAEMATOPOIETIC DEVELOPMENT

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The Ventx genes are non-clustered homeobox transcription factors that confer a ventral phenotype on mesodermal cells in the developing embryo. Ventx genes are conserved in vertebrates but have been lost in rodents. In the human haematopoietic system, VENTX promotes myeloid differentiation and is expressed in some acute myeloid leukaemias (AML). Using a DOX (Doxycycline) inducible VENTX expression system, we found that VENTX overexpression in hPSC impaired mesoderm formation, but VENTX enforced expression after mesoderm commitment resulted in the emergence of an increased percentage of immature blood cells that co-expressed CD90 and CD34. These cells displayed high clonogenic capacity in methylcellulose, but only after DOX was removed from the media. This suggested that VENTX expression held cells in a non-proliferative state. Transcriptional profiling revealed increased expression of HOXA genes in haematopoietic cells following VENTX induction, consistent with their immature phenotype. Conversely, genes involved in myeloid differentiation were downregulated during VENTX overexpression, as were genes involved in proliferation, such as MYC and MYB. ATAC-sequencing demonstrated that VENTX closes selected chromatin loci, in particular areas targeted by HOXB13 and CDX transcription factors. We hypothesise that VENTX might act as a transcriptional suppressor during haematopoietic differentiation and we are currently investigating the genomic targets of VENTX, combining ATAC-seq and ChIP-seq with the transcriptional profiling. In summary, VENTX overexpression generated immature blood cells in a quiescent state, preventing their proliferation and differentiation into myeloid lineages.

W-2056

DEVELOPMENT OF A NEW SEMI-SOLID MEDIA FOR HUMAN HEMATOPOIETIC PROGENITOR CELL CHARACTERIZATION

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Hematopoietic progenitor cells (HPCs) are a heterogeneous population of progenitor cells that give origin to all the mature blood cell types. The human colony forming cell (CFC) assay is a well-established in vitro function assay used to quantify and characterize the subpopulations of HPCs. Its role in the analysis of differentiation and proliferation patterns of myeloid stem cells has been applied in drug safety and toxicity testing, graft selection, and disease diagnosis. It is considered the gold standard assay for in vitro HPC characterization in the hematological field; therefore, the development of cGMP-manufactured methylcellulose-based CFC media that can be used reliably to characterize HPC colonies under serum-free conditions is essential. Media development at FUJIFILM follows a rational design approach. Our extensive expertise and this approach have led to the development of a serum-free semi-solid solution for HPC characterization, with equal or better performance than other commercially available offers while being cGMP grade. During media development we have identified critical factors that impact the survival and phenotypic characteristics of each HPC-derived lineage. We have found that components present in basal media selection determine the timing of erythrocyte colony pigmentation and growth. The source and grade of albumin play an important role in the maintenance of colonies within the culture, and addition of other serum-replacement components is beneficial to the number and distribution of all HPC colonies. FUJIFILM Irvine Scientific has previously developed a xeno-, serum-free media for HPC expansion (PRIME-XV® Hematopoietic Cell Basal XSFM). The development of a complete serum-free semi-solid media for CFC HPC characterization will provide a complete serum-free cGMP solution for HPC expansion and downstream characterization.

W-2058

HUMAN MESENCHYMAL STEM CELL BASED THERAPY FOR MARROW FAILURES WITH INTRINSIC NICHE DEFECT

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Aplastic anemia (AA), with an estimated incidence of 2-3 per million per year, is a serious and life-threatening disorder. Though immunosuppressive treatments can resolve cytopenia in many patients, 30-40% of these responders' relapse, and 25% show no response. Thrombocytopenia, a major cause of morbidity and mortality in AA is caused by diminished hematopoietic stem cell (HSC) number resulting in decreased megakaryocytes (MK) and impaired platelet generation. Patients with loss-of-function mutations in THPO develop marrow failure and are unresponsive to bone marrow (BM) transplant, suggesting the role of extrinsic factors in the development of this type of AA. Under these circumstances, cell therapies which can generate THPO and facilitate a microenvironment supportive of HSC and platelet generation would be of great clinical benefit. We have recently demonstrated in an animal model, that stromal cells enriched in N-Cadherin+ near the endosteal surface of bone are immature mesenchymal stem cells (MSCs) and can differentiate into different mesenchymal lineages. More importantly, these cells functionally support the most drug-resistant reserve hematopoietic stem cells (HSC) population. To investigate if N-Cadherin+ cells of similar property exist in human bone marrow MSC, we FACS sorted N-Cadherin+ and N-Cadherin- from cultured BM-MSCs, transduced with GFP and tracked their property in vivo by intrafemorally transplanting them into immunodeficient NSG mice. Here, we show that huBM-MSC have a small proportion of N-Cadherin+ MSCs. These cells when intrafemorally xenotransplanted, had were able to engraft and differentiate into osteoblast adipocytes and chondrocytes. The transplanted cells survive long-term, migrate and trans-differentiate into functional hepatocyte-like cells in the liver. Additionally, N-Cadherin+ MSCs had a greater potential to generate huTHPO in liver, bone marrow and blood. More importantly there was a significant increase in hematopoietic stem, progenitor and MK progenitors in mice transplanted with N-Cadherin+ cells. Single cell RNA seq revealed expression of factors that support hematopoiesis and mesenchymal cells differentiation. Altogether our studies demonstrate the clinical relevance of different routes of MSC transplantation for marrow failure.

W-2060

PROSTAGLANDIN E2 INDUCES ENHANCER ACCESSIBILITY AND ACTIVITY THROUGH HISTONE VARIANT H2A.Z ACETYLATION IN HUMAN HEMATOPOIETIC STEM CELLS

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Cell fate decisions are regulated by transcriptional activation in response to external signals such as the inflammatory mediator Prostaglandin E2 (PGE2). The stable derivative 16,16-dimethyl-PGE2 (dmPGE2) enhances hematopoietic stem and progenitor cell (HSPC) engraftment and is currently in its fourth clinical trial to improve HSPC transplantations. To understand the mechanism of action of dmPGE2, we investigated chromatin reorganization and gene expression changes in primary human CD34+ HSPCs after 2 hours of dmPGE2, the time period of treatment in the clinical trials. We mapped genome-wide changes in nucleosome positions and occupancies using micrococcal nuclease sequencing (MNase-seq) and open chromatin regions by ATAC-seq. Surprisingly, we found that dmPGE2 specifically increases accessibility at enhancers yet nucleosomes are retained at these regions upon dmPGE2 treatment. As histone variants improve chromatin accessibility locally and could be present at these dmPGE2-induced enhancers, we compared H2A.Z deposition and binding of downstream transcription factor phospho-CREB (pCREB) at enhancers using ChIP-seq. This revealed that pCREB binds directly to H2A.Z-rich labile nucleosomes which show increased levels of H2A.Z acetylation after dmPGE2 treatment. Direct interaction between pCREB and H2A.Z was validated through co-immunoprecipitation in dmPGE2-responsive U937 cells. We showed that the observed changes in chromatin accessibility at enhancers are not caused by displacement of linker histone H1. This suggests that acetylation of labile nucleosomes provides sufficient DNA access to allow for binding of pCREB at enhancers. By performing RNA-seq, we correlated enhancers to gene expression changes. Enhancer-associated genes that display significant transcriptional changes after dmPGE2 stimulation include known regulators of self-renewal and migration such as NR4A2, EGR1 and CXCR4. Taken together, our data suggests that dmPGE2 induces specific binding of pCREB to and acetylation of H2A.Z-rich accessible nucleosomes at enhancers of genes that promote HSPC fate. The deposition of variant histones at enhancers during development followed by their acetylation and interaction with transcription factors regulates a rapid response to environmental cues that impact cell fate.

W-2062

ANTIBODY CONDITIONING ENABLES MHC-MISMATCHED HEMATOPOIETIC STEM CELL TRANSPLANTS AND ORGAN GRAFT TOLERANCE

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Replacing a patient's diseased blood system by hematopoietic cell transplantation (HCT) can treat or cure genetic disorders of the blood and immune system, including leukemia, autoimmune diseases and immunodeficiencies. In HCT, a patient's blood and immune system are typically ablated using toxic "conditioning regimens" (chemotherapy and/or radiation) and then replaced with donor tissue containing hematopoietic stem cells (HSCs) to regenerate a healthy blood system. While HCT is a foundational treatment, its use and safety are hindered by graft vs. host disease and lethal toxicities caused by the conditioning regimens. Therefore, a decisive goal is to achieve HCT conditioning with more specific and safer agents (e.g., monoclonal antibodies), obviating the need for toxic chemotherapy or radiation, while preventing GvHD by engrafting purified HSCs devoid of T-cells. Here we show that a combination of six monoclonal antibodies can safely and specifically deplete host HSCs, T cells and NK cells of immune-competent mice and permit MHC-mismatched (allogeneic) HSC engraftment. The engrafted donor HSCs were either mismatched at half (haploidentical) or all the MHC genes, and in both cases generated donor blood and immune systems that stably co-existed with host blood cells. These chimeric immune systems were functional: the haploidentical recipients tolerated heart tissue from the same HSC donor strain, while rejecting 3rd party hearts, and the full MHC mismatch recipients were able to develop antibody responses to nominal antigens. These proof-of-concept mouse studies suggest that antibody conditioning can facilitate purified HSC transplantation, and in turn foreign organ transplants and the treatment of diverse, non-malignant blood and immune system disorders.

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W-2064

DECIPHERING HETEROGENEOUS DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS BY MAPPING SINGLE CELL TRANSCRIPTOMES WITH SINGLE CELL FUNCTIONS IN MICE

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Recent studies have suggested that individual hematopoietic stem cells (HSCs) produce different amounts of blood cells and exhibit distinct preferences in producing different types of blood cells. However, little is known about how the heterogeneous differentiation of individual HSCs is regulated. Although population level studies have identified regulatory factors that control overall blood production, intercellular differences between HSCs might be regulated by new classes of mechanisms that are undetectable at the population level. To understand how individual HSCs are differentially regulated, we used a genetic barcode tracking technology to monitor differentiation activities of individual HSCs during serial transplantation in mice. Our data have shown that individual HSCs derived from the same ancestor HSC exhibit similar differentiation characteristics in different mice. This suggests that the specific differentiation program of an individual HSC is not stochastic, but cell autonomous and regulated by HSC intrinsic factors. It also allows us to infer single HSCs' behavior from the genetic barcode based clonal tracking analysis. To identify genes that modulate the functional differences between individual HSCs, we have developed a novel library construction strategy and bioinformatic pipeline to integrate droplet-based single-cell RNA sequencing with our barcode tracking technology. We have successfully mapped hundreds of HSCs across the two datasets, and have identified genes whose expressions are associated with specific self-renewal and differentiation programs of a subset of HSCs. We showed that these genes are significantly enriched for distinct, and in some cases unexpected, biological functions and pathways. Their mutants often cause hematopoietic defects. We have employed CRISPR/Cas9 technology to test the functions of these genes in competitive transplantation. Our new experimental system that integrates single cell gene expression and single cell behavior can be applied to other tissues and organs to identify genes regulating a distinct behavior of a cellular subpopulation without physically isolating these cells.

PANCREAS, LIVER, KIDNEY

W-2066

UTILIZATION OF A PERFUSION BIOREACTOR FOR DECELLULARIZATION OF RAT LIVERS AND RECELLULARIZATION WITH HUMAN MESENCHYMAL STEM CELLS

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Orthotopic liver transplantation is the only definitive treatment for hepatic failure, but there is a great deficit between the total number of donated organs and patients in need of them. Tissue engineering presents itself for producing viable alternatives to eliminate this deficit. Organ decellularization and recellularization is a technique that creates a natural and acellular scaffold maintaining the native characteristics of the utilized organ and making them ideal for the reseeding of cells. Mesenchymal stem cells (MSCs) are a great option for the reseeding of organs as they have the capacity of differentiating into hepatocytes and have a high proliferation rate. In this work, stem cells from human exfoliated deciduous teeth (SHED) were used to recellularize the decellularized extracellular matrix from rat livers using a perfusion bioreactor constructed in the laboratory. The liver decellularization was made with a 0.5% solution of SDS for 24 hours. Sterilization of the scaffold was made with a 0.1% solution of peracetic acid in 4% ethanol for 3 hours. The matrix was washed in PBS with antibiotics. Recellularization was made with 108 SHED and cultivated for 7 days. Functional and structural analyses were made in both matrices. Four decellularized livers and five recellularized livers were used. The decellularized matrices showed an absence of DNA while preserving collagen and glycosaminoglycans quantities, thus confirming the efficiency of the process. The recellularization was carried with 97% retention of the inserted cells. The cells continued to produce albumin and urea at the same levels as the cells cultivated in the culture plates, as shown by the colorimetric enzymatic assay. A rise in LDH levels occurred in the first days of the culture, suggesting that there is cell death immediately after recellularization, though they stabilized on the 7th day. Histological analysis showed conservation of the collagen web and some groups of cells next to the vessels. The construction and use of a bioreactor in the laboratory to decellularize and recellularize the rat livers with SHEDs was successful. The livers were able to survive and be metabolically active during the 7 days in culture. The liver recellularization was carried out with success, creating a new line of research in the laboratory to produce organs in vitro.

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W-2068

MOLECULAR DISSECTION OF HUMAN PODOCYTE DEVELOPMENT REVEALS NOVEL GLOMERULAR ENDOTHELIAL AND MESANGIAL ORGANIZING SIGNALS

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The kidney is essential for metabolic waste excretion, the homeostasis of tissue fluids (water, salt and pH), blood pressure and cell composition, and bone development and metabolism. Filtration is performed within the renal corpuscle by a highly structured cellular device. This comprises a convoluted fenestrated glomerular endothelium supported by mesangial myofibroblasts which releases a plasma filtrate that enters the nephron between slit diaphragms generated by the foot processes of tightly adherent podocytes. Establishment of the glomerular filter is initiated by a stereotypic recruitment of pioneering endothelial cells to the developing podocytes followed secondarily by interstitial cells into the glomerular cleft of and sequential capillary formation. Podocytes support development and maintenance of the glomerular vasculature via VEGFA while endothelial-derived PDGFB signals promote mesangial development prior to RC maturation. Single gene mutations resulting in end stage renal disease cluster in genes showing podocyte-enriched expression, highlighting the central role of podocytes in normal kidney function. To better understand the underlying developmental processes that establish the human RC, we employed single-nucleus droplet-based sequencing to capture thousands of single nuclei from the human fetal kidney cortex. This enabled the identification of distinct cell types of the nephron (podocytes), interstitial (mesangial cells) and vascular (glomerulus) lineages that together generate the renal filter. Computational trajectory analysis identified transient gene expression signatures in the developmental progression of these cell types, predicting early or mature podocytes secrete FBLN2, BMP4 or NTN4, in conjunction with recruitment, differentiation, and modeling of vascular and mesangial cell types into a functional filter. In vitro studies provide evidence these factors exhibit angiogenic or mesangial recruiting and inductive properties consistent with a key organizing role for podocyte precursors in kidney development. Together these studies define a spatiotemporal developmental program for the primary filtration unit of the human kidney and provide novel insights into cell interactions regulating co-assembly of constituent cell types.

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W-2070

APPLYING MOUSE DEVELOPMENTAL PRINCIPLES IN HUMAN PLURIPOTENT STEM CELLS TO PURELY OBTAIN PANCREATIC PROGENITORS

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Type I diabetes (T1D) is caused by a lack of insulin producing beta cells in the islets of Langerhans, which are destroyed by an auto-immune response. Administration of exogenous insulin (i.e. injection) to T1D patients is considered to be a life-saving therapy but does not fully recapitulate the endogenous fine-tuned and stable glycemic control achieved by beta cells. Clinical evidence obtained from whole pancreas and islet transplantations has shown that cellular replacement is an effective solution for T1D patients. Unfortunately, the required widespread use of similar therapies is limited by the shortage and the quality of the available organs. In principle, human pluripotent stem cells (hPSC), including embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) hold the potential to provide an unlimited source of insulin-producing cells, as shown by the capability of hPSC-derived pancreatic progenitors (PP) to give rise to insulin-producing cells in vivo and in vitro. However, the efficiency of PP generation in vitro remains highly variable among labs and cell lines (possibly as a consequence of unexpected divergences between mouse and human development) and this may negatively impact their clinical use. Based on pioneering developmental studies, involving knock-out animals, and available published protocols, we selected and manipulated several signaling pathways in a time-controlled manner during hPSC differentiation. In order to monitor the effect of each signaling or combination of signals, we evaluated the efficiency by which hPSC generate different intermediate stages, essential for the formation and development of the pancreas (using FACS analysis, live-imaging and time-course qRT-PCR). Our work suggests that the timely and fine-tuned control of a combination of signals allows the generation of a nearly pure population of PP, from all hPSC lines, so far tested (using a defined and xenofree extracellular matrix and differentiation medium – GMP compatible protocol). We believe that our protocol will facilitate the production of PP from hPSC for cellular therapies while also shedding light on human endocrine development (endocrine specification and subsequent differentiation into the five endocrine subtypes).

Funding Source: This work is supported by the partners of Regenerative Medicine Crossing Borders (RegMed XB), a public-private partnership that uses regenerative medicine strategies to cure common chronic diseases.

W-2072

AN INDUCED PLURIPOTENT STEM CELL (IPSC) MODEL TO STUDY MECHANISMS OF NON-ALCOHOLIC FATTY LIVER DISEASE ASSOCIATED WITH PNPLA3 POLYMORPHISMS IN HUMAN HEPATOCYTES

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Alcoholic fatty liver disease (NAFLD) is a prominent cause of chronic liver disease and a major indication for liver transplant. Despite a dramatic rise in prevalence, there has been little progress in the pharmacological management of NAFLD. Factors contributing to lack of a specific therapy include current suboptimal disease models and the gap in our knowledge of mechanisms of susceptibility to chronic fatty infiltration of the liver. Our research bridges this gap by using human stem cell and gene editing technology to study the most important genetic susceptibility factor involved in NAFLD progression; the risk variant polymorphism of PNPLA3. The variant of PNPLA3 is highly associated with hepatic fat content, as well as more severe biochemical and histological features of NAFLD. Using TALEN technology, we generated a set of isogenic lines from human induced pluripotent stem cells of known genetic background with the variant and wildtype homozygous alleles of PNPLA3. We have validated this model by showing that variant hepatocytes have abnormal lipolysis as evidenced by accumulation of triglyceride-rich lipid droplets (LDs), and produce pro-inflammatory cytokines involved in NAFLD progression. To further understand the link between fat accumulation and inflammation that determines progression of NAFLD, we are characterizing the morphology, lipid and protein composition of LDs. This aim is motivated by the increasing evidence that LDs are not merely fat reservoirs, but rather metabolically active organelles that can affect cellular function based on their lipid and protein composition. We profile LD number and size by microscopy and flow cytometry, and lipid composition of purified LDs by mass spectrometry to measure amount and subtypes of triglycerides, sterol esters and fatty acids. Our data suggest that

abnormal lipolysis induced by the variant allele of PNPLA3 causes remodeling of LDs, which disrupts bioactive lipid and protein composition. LDs are also involved in compartmentalization and amplification of eicosanoid synthesis, and LD remodeling may induce a pro-inflammatory state via enhanced activity of lipid-derived inflammatory mediators. To test this hypothesis, we are measuring levels of LD- associated bioactive lipids that are known to serve as substrates or mediators of inflammation.

W-2074

GENE-EDITING ENABLES NONINVASIVE IN VIVO TRACKING OF HIPSC-DERIVED LIVERBUDS

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Human induced pluripotent stem cell (hiPSC)-derived hepatocytes (HLCs) have clinical transplantation potential. Preclinically, variations in transplantation site, cell format and stage of differentiation are reported with no standard protocol for HLC transplantation. Whole-body in vivo imaging would enable transplanted cell survival and/or expansion to be monitored non-invasively over time, allowing robust comparisons between technologies and transplant modalities. Radionuclide imaging is ideal for this purpose, offering high sensitivity compared to other imaging modalities, quantification of deep tissue signals and direct applicability to the clinic. The human sodium iodide symporter (hNIS) can be exploited as a radionuclide reporter gene for positron emission tomography (PET) or single photon emission computed tomography (SPECT)-afforded whole body in vivo cell tracking. We used transcription activator-like effector nuclease gene-editing to incorporate a hNIS-GFP fusion gene within the AAVS1 safe harbour locus of a cGMP compliant hiPSC line, enabling constitutive hNIS-GFP expression in hiPSCs and their differentiated progeny. hNIS-hiPSCs retained comparable pluripotency and HLC-differentiation capacity to parental cells (shown by qPCR and immunostaining). Subsequently, they were used to produce multilineage liver buds (LBs) by combining three differentiated liver progenitor populations (immature HLCs, endothelial cells and septum transverse mesenchyme). LBs were transplanted into kidney capsules of NOD SCID and TK NOG mice and PET imaged in vivo at various timepoints post-transplant. Cells retained their differentiated function as demonstrated by human albumin presence in mouse sera and immunostaining of human: CD31, CD144, CK18 and albumin. This study is the first to show hNIS-expressing hiPSC progeny retain their differentiated function and can be tracked noninvasively in vivo by PET. LBs were chosen for tracking as scalability to clinically relevant capacity has been previously demonstrated, their use with a clinically applicable radiotracer and imaging modality highlights the potential for this strategy to answer wider questions in the HLC field.

Funding Source: Japan Society for the Promotion of Science; Guy's and St Thomas' Charity

W-2076

ROLE OF PRMT1 IN HUMAN ESCS DURING DIFFERENTIATION INTO PANCREATIC ENDOCRINE CELLS IN VITRO

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Neurogenin 3 (NGN3) is an essential transcription factor that determines the fate of endocrine cells in developing pancreas. It has been reported that protein arginine methyl transferase 1 (PRMT1) knockout (KO) mouse embryos show abnormal expression of NGN3 and pancreas hypoplasia from E14.5 days to postnatal period. PRMT1 is a major asymmetric arginine methyl transferase for cellular arginine methylation in mammalian cells. However, the exact mechanism of how PRMT1 regulates NGN3 still remains elusive. To understand the relationship between NGN3 and PRMT1, human embryonic stem cells (hESCs) were differentiated into pancreatic endocrine cells by using a stepwise protocol. The hESCs were first induced to definitive endoderm (DE), and then subsequently differentiated pancreatic endoderm (PE), endocrine progenitor (EP), and endocrine cells (EC). NGN3 is transiently expressed in EP and disappears in the process of development to the ECs. The protein level of PRMT1 was highest in DE and gradually decreased during subsequent differentiation to ECs. To explore the role of PRMT1 on the NGN3 expression, the vector that contains CRISPR-Cas9 components using tet-ON system was constructed to knockout PRMT1 by treatment with doxycycline. It was confirmed that infection of PRMT1-KO lentivirus decreased the PRMT1 protein level in 293T cells. When the PRMT1-KO lentivirus was infected into hESC-derived EPs, the PRMT1 protein level was reduced and the NGN3 expression was upregulated. Our results represent that PRMT1 may regulate the expression of NGN3 in hESCs during pancreatic development.

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EPITHELIAL TISSUES

W-2078

HUMAN STEM CELL-DERIVED ALPHA 2-HS GLYCOPROTEIN (FETUIN) AND ITS EFFECT ON SKIN AGING

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Daily expose to environmental stressor, including ultraviolet (UV) light and pollution, increases oxidative stress and leads to decline in restorative properties of the skin. The idea to use stem cell–derived growth factors was based on the premise that the extrinsic aging process of skin is like that of a wound healing and it can be mitigated using topical growth factors and proteins. The studies presented here examine the effect of stem cell-derived skin lineage precursor secretions on human cells in culture and on aged skin in the clinical setting, using a simple topical formulation. Human stem cells cultivated in balanced conditions were differentiated into skin lineage precursors and shown to secrete large amounts of fetuin as well as multiple growth factors beneficial for human skin development and maintenance. The effects of these cell secretions were investigated in an IRB-approved 12-week human trial that included 25 subjects in each group. Subjects were examined at 2, 4, 8, and 12 weeks by a dermatologist to evaluate safety, trans- epidermal water loss, wrinkles, firmness, radiance, texture, softness, and overall appearance. A sub-group of subjects from each group consented for biopsies for histological analyses. Clinical investigation demonstrated significant amelioration of the clinical signs of intrinsic and extrinsic skin aging, findings that were confirmed by significant changes in skin morphology, filaggrin, aquaporin 3, and collagen I content. This data strongly supports the hypothesis that topical application of stem cell-derived skin lineage precursor secretions containing fetuin and growth factors is beneficial for human skin development and maintenance, due to clinical and histological evidence of amelioration of the intrinsic and extrinsic signs of skin aging.

W-2080

REGULATION OF LUNG PROGENITOR CELLS DIFFERENTIATION BY THE LIN28A/LET-7 PATHWAY

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Lin28 is a RNA binding protein that regulates gene expression via inhibition of the Let-7 microRNA maturation or by Let-7 independent mechanism. In vertebrates, Lin28A and its paralog Lin28B are highly expressed in stem and progenitor cells of the early embryo, and play important roles in the balance between self-renewal, proliferation, and differentiation. Lin28 is also one of the “reprogramming factors” and overexpression of Lin28 has been detected in many malignancies. We have shown previously that global Lin28A overexpression during mouse embryogenesis results in perinatal lethality. However, the reason for this early lethality has not been elucidated. Here we show that Lin28A overexpression and the resulting Let-7 downregulation prevent normal lung development, thus causing the perinatal lethality. Notably, we found that the Lin28A/Let-7 pathway delays the transition between one developmental stage of the lung epithelial progenitor cells to another but does not completely abrogate their differentiation capacity. Using the Cre-Lox system for tissue specific overexpression we show that Lin28A expression in the embryonic lung mesenchymal cells is sufficient to recapitulate the epithelial lung phenotype derived by global Lin28A overexpression while its expression in the epithelial progenitor cells results in a much milder developmental phenotype. Finally, we defined the specific time window wherein Lin28A expression exerts its effect on the development of the lung and showed the relevance of our findings also for human lung development. To conclude, our findings define the Lin28/Let-7 pathway as a heterochronic regulator of the lung progenitor cells’ differentiation. While the precise molecular mechanism of this heterochronic regulation is yet to be determined, we identified several pathways that might play a critical role in this process.

W-2082

CLAUDIN-18 REGULATES AIRWAY PROGENITOR CELL HOMEOSTASIS AND DIFFERENTIATION FOLLOWING INJURY

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The epithelium lining the internal surface of the lung varies in cellular composition along its proximal-distal axis. The proximal airway epithelium is comprised of three putative progenitor populations, basal cells (BC), pulmonary neuroendocrine cells (PNEC) and club cells, and two non-progenitor cell populations namely goblet and ciliated cells. The alveolar epithelium is comprised of alveolar epithelial type 2 (AT2) cells which serve as progenitors and more terminally differentiated type I (AT1) cells. Claudin-18 (Cldn18) is a tight junction protein that maintains epithelial barrier properties. The lung-specific isoform, Cldn18.1, is highly expressed in alveolar epithelial cells. We recently reported that Cldn18 deletion in knockout (KO) mice leads to dramatic AT2 progenitor cell expansion and proliferation. In the current study, we investigated effects of Cldn18 deletion on airway progenitor cell homeostasis at baseline and in response to injury. Surprisingly, despite only sparse expression of Cldn18 in ciliated cells and not progenitor cells in airways of wild type (WT) mice, Cldn18 KO mice demonstrated expansion and ectopic localization of all airway progenitor cell populations. Following naphthalene (NAP, 225 mg/kg intraperitoneally) injury, WT mice showed the expected depletion of club cells by day 3 with spreading of ciliated cells to cover the epithelial surface. Repopulation of the airways by variant club cells (not susceptible to NAP) was completed by day 21, with the epithelium being reconstituted with club and ciliated cells. In contrast, Cldn18 KO mice developed marked goblet cell hyperplasia with airway cells expressing markers for both club (CC-10) and goblet (MUC5AC) cells. Given that Cldn18 is only sparsely expressed at baseline in airway epithelium and predominantly in ciliated cells, these findings suggest that the observed changes in club cell differentiation are mediated through cell-cell communication. These results demonstrate a novel role for Cldn18 in regulation of airway progenitor cell homeostasis and specification. Given that Cldn18 is downregulated in asthma, a disease characterized by goblet cell hyperplasia, modulation of Cldn18 and its downstream pathways may be beneficial in the treatment of airway diseases.

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W-2084

EZH2 AND INTESTINAL STEM CELLS ARE INVOLVED IN THE PATHOGENESIS OF NECROTIZING ENTEROCOLITIS

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Necrotizing enterocolitis (NEC) is characterized by a reduction in actively-proliferating Lgr5+ intestinal epithelial stem cells (ISCs), which is linked to impaired intestinal regeneration. Enhancer of zeste homologue 2 (Ezh2) regulates ISCs by supressing gene expression through histone methylation, promoting stemness and limiting their differentiation. We have previously demonstrated that Ezh2 is impaired in experimental and human NEC. The aim of this study is to determine whether Ezh2 ablation in Lgr5+ ISCs leads to NEC-like injury. In vivo study, to conditionally knockout Ezh2 in Lgr5+ ISCs, experiments were performed on Lgr5-GFP-IRES-CreERT2;Ezh2f/f mice (Lgr5ΔEzh2) and Ezh2+/+ controls. All mice were administered tamoxifen once/day via oral gavage on postnatal days 3-5 (P3-5). Mice were sacrificed on P9 and morphology of ileal sections were analyzed blindly using a specific scoring system. Lgr5ΔEzh2 mice showed higher injury scores compared to controls, indicating greater intestinal injury. They also had decreased Lgr5 expression and

Ki67+ cells/crypt. Ex vivo study, Intestinal epithelial crypts were isolated from Lgr5ΔEzh2 and control terminal ileal tissue and were grown into intestinal organoids in Matrigel domes. Growth and differentiation were compared by analyzing organoid size and budding after 7 days. Similar to NEC organoids, intestinal organoids grown from Lgr5ΔEzh2 crypts were smaller in size, had more buds, and reductions in Ezh2, Lgr5, and Ki67 expression. We demonstrated that eliminating Ezh2 from intestinal stem cells induces NEC-like intestinal injury. These data suggest that Ezh2 and intestinal stem cells are important in the pathogenesis of neonatal intestinal NEC, indicated a potential target to offset the NEC injury.

W-2086

DEFINING CELL FATE SPECIFICATION OF MOUSE MAMMARY STEM CELLS IN 4D

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The remarkable capacity of the mammary gland for rapid growth and regeneration has been attributed to the presence of mammary stem cells (MaSCs). Our recent results showed that, while multipotent MaSCs exist in the early stages of mammogenesis, all embryonic mammary cells become lineage restricted at embryonic day E15.5, coinciding with remarkable epithelial remodelling during the first morphogenetic events that establish the mammary ductal network. We also found that constitutive Notch1 activation imposes a premature differentiation to multipotent embryonic MaSCs and, remarkably, ectopic Notch expression in BCs can also lineage convert committed unipotent adult cells, implying that similar mechanisms control embryonic cell fate determination and adult cell plasticity. However, these signalling circuits are poorly defined and their coordination with cell migration and organisation during branching morphogenesis is unknown. To address this, and to further characterise epithelial cell dynamics and lineage specification during mammary gland development, we combine multicolor lineage tracing approaches with 4D imaging of embryonic mammary explant cultures. Using a method to grow embryonic mammary placodes in culture, the fate of individual labelled embryonic MaSCs during branching morphogenesis can be followed in real time by time-lapse confocal microscopy. These experiments allow us to delineate the relationship between the initial sprouting events during mammary morphogenesis and the precise timing of stem cell commitment. In addition, we aim to apply this powerful tool to investigate the impact of aberrant activation of oncogenic signaling on cell migratory dynamics, morphological changes, proliferation, cell death and rearrangements during branching morphogenesis. Conditional mutant transgenic models will be used to study the role of Wnt and Notch signaling, key pathways in mammary development and fate allocation. Through real time imaging of mutant embryonic mammary placodes in culture, the lineage potential of targeted mutant cells can be correlated with their migratory dynamics and behaviour during branching morphogenesis. This approach will provide fundamental insights into the mechanisms governing mammary cell fate decisions during both normal and pathological development.

W-2088

INDUCED PLURIPOTENT STEM CELL DERIVED BASAL CELLS PROVIDE A NOVEL SOURCE OF MULTI-LINEAGE AIRWAY EPITHELIAL CELLS

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Engraftment of gene-corrected autologous cells is a potential therapy for genetic respiratory disorders such as cystic fibrosis and primary ciliary dyskinesia. Basal cells are critical stems for repair and maintenance of the respiratory epithelium. Differentiation of isolated basal cells at an air-liquid interface (ALI) is the gold-standard model for human respiratory epithelium, yet use of these cells for study of genetic disease is limited due to inherent difficulties in procurement, expansion, and genetic manipulation. iPSC derived basal cells (iBasal) offer a potentially unlimited source of readily manipulated patient specific cells for both in vitro disease modeling and autologous cell therapy. Building on our airway differentiation protocol, we have derived a strategy for generation, isolation and expansion of iBasal, improving the efficiency of generating respiratory epithelium from iPSC. Our modified cell culture protocol directs differentiation of iPSC to Nkx2.1 expressing lung progenitors, purified based on a CD47hi/CD26lo expression profile. Isolated lung progenitors are subsequently cultured in the presence of SMAD inhibitors and evaluated over 5 passages for basal cell marker expression, clonogenicity, and differentiation capacity in both ALI and spheroid cultures. iBasal transcriptomes were compared to those of primary basal cells by single cell RNAseq. iBasal form lung tracheospheres as well as single cell colonies on NIH3T3-J2 feeders. Flow cytometry and immunofluorescence characterization indicates that single cell derived colonies express epithelial calmodulin (EpCAM) and basal cell markers cytokeratin 5 (krt5), p63, integrin alpha 6 (IGTA6), and nerve

growth factor receptor (NGFR) when passaged. Furthermore, iBasal have the capacity for differentiation into a functional, tight junction forming epithelium containing both mucus secreting and motile multiciliated cells at the ALI. We have generated iBasal from four independent iPSC lines, with marker expression and differentiation capacity akin to that of primary basal cells. iBasal, therefore, provide the opportunity to generate sufficient functional and gene-corrected autologous basal cells to develop patient specific, high throughput screening platforms overcoming the current limitations.

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EYE AND RETINA

W-2090

DIABETIC VASCULAR PROGENITORS DIFFERENTIATED FROM HUMAN NAÏVE IPSC DISPLAY IMPROVED GENOMIC INTEGRITY AND AUGMENTED ENGRAFTMENT AND MIGRATION INTO ISCHEMIA-DAMAGED RETINA

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Human induced pluripotent stem cells (hiPSC) could provide unlimited amounts of embryonic vascular progenitors (eVP) to repair ischemic diabetic tissue. We have demonstrated that highly prolific CD31+CD146+CXCR4+ eVP differentiated from patient-specific hiPSC can repair damaged adult retinal blood vessels in a humanized NOD-SCID ischemia/reperfusion (I/R) model. We also established naïve hiPSC (N-hiPSC) stably reverted to a preimplantation epiblast-like pluripotent state following culture with a novel cocktail of LIF-2i supplemented with the tankyrase inhibitor XAV939 (LIF-3i). LIF-3i-reverted N-hiPSC possessed enhanced multi-lineage differentiation potential compared to isogenic conventional hiPSC and eliminated interline variability. To test the functionality of N-hiPSC-derived eVP, we reprogrammed skin fibroblasts of type-I diabetic patients using an episomal system. These diabetic hiPSC (D-hiPSC) were reverted to a naïve state using LIF-3i. Isogenic primed D-hiPSC and N-D-hiPSC were differentiated in parallel to the vascular lineage to compare endothelial function and in vivo regenerative capacity in the I/R model. N-D-hiPSC acquired markers of naïve pluripotency (e.g., p-STAT3, DNMT3L, TFCP2L1), global reduction of DNA CpG methylation, increased 5-hydroxymethylation, and decreased H3K27me3 at bivalent promoters. N-D-hiPSC differentiated without re-priming into CD31+CD146+ eVP more efficiently than isogenic primed D-hiPSC, and possessed higher proliferation, more efficient acetylated-Dil-LDL uptake, and improved tube formation in Matrigel. N-D-hiPSC eVP expanded with reduced senescence and lower sensitivity to DNA damage. Injection of N-D-hiPSC eVP into I/R-damaged mouse retinas resulted in dramatically higher frequencies of engraftment of human-specific (HNA+) cells than primed controls up to 4 weeks following intra-vitreal injections. One week after injection, CD34+ human N-D-hiPSC eVP were detected throughout the ganglion cell layer and up to the inner nuclear layer of the retina. In contrast, isogenic D-hiPSC eVP displayed poor migration, and remained in the superficial retinal layers. These studies demonstrate that N-hPSC-derived eVP with improved genomic integrity possessed improved functionality compared to eVP generated from conventional hiPSC.

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W-2092

IDENTIFICATION OF SPECIFIC CELL SURFACE MARKERS FOR RETINAL PIGMENTED EPITHELIAL CELLS AND ESTABLISHMENT OF A XENOFREE AND DEFINED MONOLAYER HPSC-RPE DIFFERENTIATION METHODOLOGY

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Recent clinical trials have suggested that transplantation of human pluripotent stem cell (hPSC) 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 hPSC-RPE cells over other unsought cell types that can emerge along the way, which impairs the large-scale production of these cells and also complicate their clinical implementation. Aiming to overcome such limitations, we sought to identify unique cell surface markers for the hPSC-RPE cells. For that purpose, we screened hPSCs and hPSC-RPE cells against an antibody library recognizing 240 different cell surface markers. From this screening and subsequent validation, we identified a set of unique cell surface markers that were able to discriminate hPSC-RPE cells from the bulk of cells that emerged after hPSC differentiation, as well as markers that could be used to evaluate the degree of maturation of the hPSC-RPE in culture. Subsequently, our identified cell surface proteins were used to quantitatively asses the performance of alternative hPSC-RPE differentiation protocols, proving to be useful in identifying hPSC lines with poor differentiation outcomes. Finally, through scRNA-seq analysis, we demonstrate that a higher degree of purity of the final hPSC-RPE cell product could be achieved through cell enrichment using our selected cell surface markers. Altogether, we foresee that the implementation of our novel xeno-free 2D hPSC-RPE differentiation protocol combined with the use of the set of surface markers identified will have important implications for the development of a safe, robust and scalable cell replacement therapy for macular degeneration.

W-2094

NANOCONJUGATE-BASED GENE THERAPY NORMALIZES HUMAN CULTURED STEM CELL-ENRICHED DIABETIC LIMBAL EPITHELIAL CELLS AND ORGAN-CULTURED CORNEAS

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Diabetic corneas have persistent epithelial defects and impaired wound healing, which may be caused by the dysfunction of limbal epithelial stem cells (LESC). We developed gene therapy for delivery of antisense oligonucleotides (AON) targeting diabetes-associated genes, using novel polymalic acid (PMLA)-based nanoconjugates carrying AONs to cathepsin F (CF) and to miR-409 that would activate c-met expression. Nanoconjugates contained cell targeting antibody to transferrin receptor, and morpholino AONs (at 5 μM each) to CF and to miR-409-3p that targets c-met proto-oncogene, or a scrambled AON (control). Healing of scratch wounds in limbal epithelial cell (LEC) cultures and of heptanol-induced corneal epithelial wounds was monitored. Apoptosis was assessed with AnnexinV staining. Previously, we showed that organ-cultured corneas were safely transduced with adenoviral (AV) constructs carrying c-met or CF shRNA. However, AV transduction of cultured LEC even at low 80 pfu/cell caused significant cell rounding and apoptosis. Conversely, nanoconjugate with AON miR+CF at 5-30 μM AON showed no increase in cultured LEC of AnnexinV-positive apoptotic cells (<5%) from untreated or control nanoconjugate-treated LEC. As expected, this nanoconjugate caused an increase of its target c-met and a decrease of CF in diabetic LEC and organ-cultured diabetic corneas. This treatment also upregulated stem cell markers ABCG2 and keratin 15 in diabetic LEC. Similarly, stem cell (ABCG2, keratin 17 and ΔNp63) and diabetic (integrin α3β1 and nidogen-1) markers were upregulated, and wound healing was significantly accelerated after nanoconjugate treatment of organ-cultured diabetic corneas. Treatment also normalized the expression of activated/phosphorylated signaling intermediates, EGFR-Akt and p38 in organ-cultured diabetic corneas. Overall, non-toxic nanoconjugates provide a new alternative to viral gene therapy in normalizing diabetic limbal epithelial stem cells and organ-cultured corneas.

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W-2096

PURIFICATION AND MOLECULAR CHARACTERIZATION OF MOUSE RETINAL STEM CELLS

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Retinal stem cells (RSCs) are a rare, multipotent population of quiescent cells residing in the pigmented layer of the adult ciliary epithelium at the retinal periphery. While scarce, RSCs preserve regenerative potential within the eye: RSCs give rise to all cell types within the neural retina, as well as retinal pigmented epithelial cells. Unfortunately, RSCs can be identified only retrospectively through in vitro colony-forming assays as no RSC-specific markers have been discovered. To find such markers, we sought to purify the RSC population. This was

accomplished via a microfluidic chip and magnetic nanoparticle antibody selection process. Various cell surface antigens known to identify other stem cell populations or to influence RSC behaviour in vitro were screened. This approach established that a combination of Frizzled 1, ABCG2, and Notch 1 (FAN) significantly enriches RSCs to approximately 1 in 2 cells from primary retinal dissection. Single cell RNA Sequencing was performed on the FAN-enriched RSC population, leading to the discovery of novel cell surface markers: Cnr1, Grm7, Il15ra and Nptxr. Most important, these new markers purify RSCs comparable to the FAN enrichment. These antigens were validated by in vivo expression patterns: only a small percentage of cells within the ciliary epithelium were positive for each marker as assessed by antibody staining. In addition to highlighting these markers, unsupervised clustering identified 5 cell populations with significantly different gene expression patterns. Only two of the clusters (2 and 4) contained the clonal sphere forming cells, that is the RSCs, while the other three clusters contained markers that indicated that we had isolated cells from the cornea, endothelium and trabecular meshwork in the primary tissue dissection. The differential expression of transcription factors of each cluster suggested that Cluster 2 may contain quiescent RSCs while the other may contain RSCs more poised to proliferate. Indeed, cell surface sorting of cluster 2 versus 4 revealed 3 fold more sphere forming cells from cluster 4 cells. The molecular signature of retinal stem cells may be useful in understanding the mechanisms by which stem cells retain quiescence.

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W-2098

IPS-DERIVED MESENCHYMAL STEM CELLS TRANSFER MITOCHONDRIA TO CORNEAL ENDOTHELIAL CELLS

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Since corneal endothelial cells (CECs) cannot regenerate itself under normal physiological conditions, loss of CECs lead to devastating consequences in the patients, including severe vision loss. Currently, corneal transplantation is the only recognized therapy for such disease conditions. In this study, we sought to investigate whether mitochondrial uptake of CECs from healthy neighboring cells preserves their function from degeneration in vitro. CECs with mitochondrial complex I inhibitor, rotenone, induced stress were co-cultivated with human induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs). Extracellular oxygen consumption rate (OCR) was performed to test the mitochondrial function of CECs. Immunofluorescence (IF), transmission electron microscope (TEM) analysis, confocal microscopy imaging, transwell assay, and RNA-Seq were conducted to investigate mitochondrial transfer from healthy cells to stressed CECs. The results revealed CECs could uptake mitochondria from adjacent cells via tunneling nanotubes (TNT) -like structures. Furthermore, the donated mitochondria effectively protected against CECs death and largely preserved cell function with rotenone stress. Importantly, the effects of mitochondrial donation from iPSC-MSCs to damaged cell were associated with F-actin expression under using of Y-27632/ROCK inhibitor. iPSC-MSCs can effectively donate functional mitochondria to CECs and protect against cell loss in stressed condition. Our study uncovered a critical role of promoting of mitochondrial donation in protection of CEC from early degeneration, and highlight a viable therapeutic strategy by mitochondrial donation for the treatment of CEC degeneration.

W-2100

LEVELS OF WNT6 DIFFERENTIALLY REGULATE HUMAN LIMBAL STEM/PROGENITOR CELLS

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Visual acuity is reliant on limbal stem/progenitor cell (LSC) mediated corneal regeneration. Continuous regeneration is critical to corneal transparency, and a lack or loss of LSCs results in corneal hazing and deteriorating vision. Previously, we have found that activation of the Wnt signaling pathway with lithium chloride increased LSC proliferation in vitro in addition to maintaining LSC characteristics. Of the 19 known Wnt ligands, 4 were found to be highly expressed in the limbus with one being Wnt6. Expansion of freshly isolated limbal epithelial cells (LECs) on Wnt6 overexpressing 3T3 feeder cells (Wnt6-3T3) maintained stem/progenitor characteristics and increased proliferation of LSCs under high levels. Short term exposure to Wnt6, under 1 hour, revealed that both canonical (β-catenin) and non-canonical (RhoA, CamKII) pathways were activated. Signaling, quantified by protein levels, was detected within minutes, but the level of activation within specific pathways varied with Wnt6 concentration and colony density. Characterization of long term Wnt6-LSC cultures showed that although colony forming efficiencies (CFEs) remained similar between all groups, high levels of Wnt6 increased proliferation of LSCs by 17.1% ($p<0.05$). Medium and high levels of Wnt6 also increased the percentage of small cells by 10.8% and 20.4%, respectively. In parallel, the percentage of cells expressing K14 was between 91.4% and 92.1%, however, the expression of K12 was reduced by 40.6% and 54.6%, in medium and high Wnt6 cultures. Wnt6 control of LSCs appears to demonstrate dynamic regulation of both the canonical and non-canonical pathways as the concentration of Wnt6 appears to have varying effects on human LECs in vitro.

High levels of Wnt6 support increased proliferation and better maintains the stem/progenitor phenotype while low levels tend to differentiate LECs. Wnt6 regulation appears to require tight control of both canonical and non-canonical pathways to regulate LSC expansion and phenotype.

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W-2102

DISTINCT CONE MATURATION FEATURES IN FETAL AND HESC-DERIVED RETINAE

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The human retina resembles that of other vertebrates in that it develops centrally to peripherally and generates retinal cell types in a biased order. However, animal models do not recapitulate some human retina features such as foveal structures, expression of a cone precursor proliferation-related program, and the cone precursors' proliferative response to RB loss. Human stem cell-derived retinal organoids may be used to interrogate human development and disease, yet their accurate portrayal of human-specific features has not been evaluated. Here, we assessed the verisimilitude of retinal organoid cone photoreceptor development by comparing the cone precursors' response to RB loss, proliferation-related protein expression, and single cell transcriptomic states in human fetal retina and in retinal organoids produced using three methods. In contrast to maturing cones in the fetal retina, maturing cones in retinal organoids did not proliferate in response to RB1 depletion. They also showed divergent timing, intensity and localization of proliferation-related proteins when compared to fetal retinal tissue. Single cell RNA-sequencing performed on photoreceptor precursors enriched from retinae (gestational weeks 15 – 21) and from retinal organoids (culture days 55-140) by CD133+/CD44,49blow FACS yielded transcriptomes of rod, cone, and retinal progenitor cell populations from both tissues. However, clustering and trajectory analyses indicate that organoid cone and rod photoreceptors traverse early maturation states that do not exist in fetal retina samples, prior to forming more mature photoreceptors that cluster together with their fetal counterparts. These data suggest that photoreceptor maturation in current organoid systems differs from that of fetal tissue, yet the photoreceptors that are ultimately produced are comparatively similar to fetal photoreceptors and thus might be valuable for modeling retinal development and diseases that manifest after photoreceptor differentiation.

STEM CELL NICHES

W-2104

INTRA-ARTICULAR DELIVERY OF MESENCHYMAL STEM CELLS REPAIRS THE DAMAGED CARTILAGE IN AN EXPERIMENTAL MONKEY OSTEOARTHRITIS MODEL

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Osteoarthritis (OA) is the most common type of degenerative arthritis affecting humans worldwide, caused by the inflammation, breakdown, and eventual loss of cartilage in the joints. However, there is no cure for OA and no medication can reverse the damaged cartilage. There is unmet needs to develop new therapeutics which can restore the damaged

cartilage of joints. Stem cell therapy is the rapidly growing and most promising strategy for curing OA. In this work, human mesenchymal stem cells (MSC) were intra-articular delivered into the knees of experimental rhesus macaques OA model to investigate the efficacy of MSC treatment for OA.Experimental OA was induced surgically in the knees of 12 rhesus macaques by bilateral anterior cruciate ligament transection (ACLT) and a complete radial transection of the medial meniscus. A single dose of 2.5×107 MSC were intra-articular delivered in the OA joint and the cell medium was used as the control 8 weeks from the surgery. Eight weeks after MSC injection, knee MRI was performed to evaluate the volume of knee cartilage. Cartilage and synovial histological sections were stained with HE and Safranin O/fast green to assess the severity of the pathology. The expression of extracellular matrix molecules, such as collagen I and II, inflammatory markers, such as tumor necrosis factor-α (TNF- α) and IL-1, were detected by immunohistochemistry. The volume of the knee cartilage analyzed by MRI was significantly increased after the treatment in animals received MSC injection (215.8 ± 9.0 vs 229.4 ±13.3, $p < 0.01$) while there was no significant difference after MSC medium treatment (209.6 ±14.3 vs 205.1 ±15.5). Pathology analysis showed that intra-articular MSC administration reversed the damage of the cartilage. Immunohistochemistry results indicated increased collagen II expression and decreased collagen I staining. MSC also attenuated the inflammation evidenced by the decreased expression of TNF- α and IL-1 in the animals treated with MSC. MSC promoted cartilage repair and attenuated inflammatory events in OA. intra-articular MSC administration may be a promising therapy for inhibiting the progression of OA.

W-2106

CELL DELIVERY VEHICLE FOR HUMAN EMBRYONIC STEM-CELL REGENERATION THERAPY IN THE INNER EAR: UTILIZATION OF HYDROGEL-ENCAPUSULATED OTIC NEURONAL PROGENITOR SPHEROIDS

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The effectiveness of neuronal differentiation from human pluripotent stem cells (hPSCs) and poor post-transplantation survival of single suspended cells has long remained an obstacle for clinically applicable stem cell replacement therapies in the inner ear. Here, we proposed two novel vehicles for cell delivery: otic neural progenitor (ONP) spheroids and hydrogel microspheres. Both methods share the common goal of increasing cell-to-cell interactions and signaling to mimic in vivo conditions. Human ESC-derived ONPs were generated based on a protocol developed in our laboratory using StemFit (Nacalai USA). Microspheres were created with a laminar flow patterning device. A low adhesion EZSPHERE plate (Nacalai USA) was also used for the growth of isolated spheroids in 3-D culture. Growdex (UPM Biomedical) and PODS (Cell Guidance Systems) were also used to support a stem cell niche. Both spheroids and microspheres were characterized using immunocytochemistry. NeuroFluor NeuO (Stemcell Technologies) live neuronal assay was also used for neuronal characterization. The laminar flow patterning device was able to successfully create uniform microspheres. The NeuroFluor NeuO dye showed that our hydrogel microspheres were capable of differentiating mid-stage ONPs to a neuronal state. Immunocytochemistry shows that our late-stage ONP spheroids are positive for the ONP markers (Nestin, Beta-III Tubulin, and PAX8). Hydrogel encapsulated microspheres and spheroids derived from hPSCs present a viable option for transplanting single cell suspensions with the possibility of increasing cell survival due to protection from shear forces and increased paracrine signaling. In addition to microspheres, the production of hPSC derived ONP spheroids is a feasible method for cell transplantation. The spheroids are small enough to be transplanted into mouse cochlea, yet durable enough to be manipulated using a negative pressure microinjector. Further testing is needed to determine the ability of hESC-derived ONPs to differentiate into auditory neurons within the mouse cochlea.

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W-2108

HUMAN UMBILICAL CORD PROVIDES A RELIABLE SOURCE FOR IMMUNE-INHIBITORY MESENCHYMAL STROMAL CELLS OVER SEVERAL MONTHS

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Mesenchymal stromal cells (MSCs) are used in hundreds of clinical trials for tissues replacement, trophic support, anti-tumor therapy and immune inhibition. Bone marrow is the most frequently used source for MSC, followed by umbilical cord (UC). Generally, UC-MSCs are harvested by the cultivation of tissue pieces, which leads to the continuous outgrowth of MSCs over a period of several months. For clinical use, only the first outgrown MSC culture is expanded and applied in patients. Here, we characterized consecutively generated MSC cultures, taken from different time points within several months after UC preparation. First, we analyzed the clonal composition of consecutive MSC-EMs, since long-term culture and subsequent clonal dominance raised concerns regarding transformation. With an improved preparation technique, we efficiently transduced whole UC pieces by lentiviral vectors harboring a genetic barcode and observed sustained polyclonality after several months of induction. We furthermore analyzed the transcriptome of early (first MSC culture) and late (two months post UC preparation) induced cultures, and observed only minor changes. In contrast, upon activation with IFN-γ and TNF-α, the transcriptome drastically, but similarly changed for early and late cultures. Analysis of the secretome with a cytokine 27-plex assay showed higher production of cytokines for later induced MSC cultures. To test whether the different cytokine levels were in a therapeutically relevant range, we used conditioned medium in an in vivo killing assay. We observed a higher immune-inhibitory capacity of the conditioned medium (CM) from late compared to early induced MSC cultures. Thus, our results indicate that cultured UC maintains a microenvironment for the generation of polyclonal MSC initiating cells. Since transplantation of cell-free CM was sufficient to mediate an immunomodulatory effect, there was no risk associated with the long-term-culture of UC-MSCs. Most importantly, the cultures induced 2 months after the UC preparation were at least as capable to inhibit the immune system as the first induced MSC culture. Since hundreds of clinical trials rely on the immune inhibitory function of MSC, our suggested technique could drastically increase the amount of therapeutic MSC for a substantial amount of patients.

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W-2110

A DYNAMIC WNT-SECRETING NICHE REGULATES MOUSE AND HUMAN PROXIMAL AIRWAY REGENERATION

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The proximal airways play a vital role in host defense via a specialized mucociliary epithelium that arises from airway basal stem cells (ABSCs). Dysregulated repair can lead to ABSC hyperplasia and squamous lung cancer (SLC), warranting understanding of airway homeostatic mechanisms. The subepithelial intercartilaginous zone (ICZ) of the airway harbors diverse cell types that comprise an intricate ABSC niche. However, interactions between ABSCs and the ICZ regulating homeostasis are poorly understood. Using transgenic mouse and pharmacologic studies, we found that β-catenin signaling within ABSCs was essential for repair post-injury in vivo, mediated by β-catenin phosphorylated at tyrosine 489. ABSCs and PDGFRα+ fibroblasts in the ICZ niche are induced to transiently secrete Wnt ligand post-injury concomitant with ABSC proliferation. To directly examine the role of different cellular compartments on Wnt-driven repair, we employed transgenic mouse models to selectively prevent Wnt secretion from either ABSCs or ICZ fibroblasts. We demonstrated that ABSC autocrine Wnt signaling was dispensable while the mesenchymal Wnt-secreting niche was sufficient to drive airway regeneration, underscoring the importance of this dynamic niche. The ICZ niche is dynamic during repair, but also displays striking changes during aging. Subepithelial Wnt-secreting cells persist in the uninjured aged ICZ, indicating a primed, actively signaling niche at baseline. Constitutively active β-catenin in young mice was sufficient to promote formation of age-related glandular structures in the ICZ that contribute to airway epithelial repair post-injury. Further, there is an emergence of a mesenchymal Wnt-producing niche in human patients with SLC, implicating its role in disease pathogenesis. These studies collectively elucidate a novel proximal airway niche that exhibits tremendous dynamism, appreciable during normal injury repair, temporally across the aging spectrum and with disease progression.

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W-2112

THE MOUSE FETAL LIVER STROMA: SIGNALS FOR HSC EXPANSION

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During development, hematopoietic stem cells (HSCs) encounter different microenvironments known to be critical for cell fate decisions. Whereas the adult bone marrow (BM) niche has been associated with maintenance of semi-quiescent HSCs, the fetal liver (FL) microenvironment is assumed to support proliferation being the only organ that accommodates HSC expansion. In the BM the factors essential for expansion and differentiation of hematopoietic cells are largely produced by mesenchymal and endothelial cells, however very limited data is available on the nature of the FL niche. Aiming at analyzing the stromal compartment of the FL and its function in regulating hematopoiesis we performed 16-color cytometric analysis of FL cells from E10 to newborn, which allowed the identification of endothelial, mesenchymal, hepatic and hematopoietic populations. The majority of Ter119-CD45- cells are Myb-dependent erythroid progenitors of yolk-sac origin that persist up to E14. Our analysis of the FL further reveals that only around 5% of the cells are non-hematopoietic. From the latter, NG2+ pericytes are the main source of the Cxcl12 chemokine and hepatoblasts the major producers of Scf, Tpo and differentiation signals, emphasizing the crosstalk between the progenitors of two different systems, the hepatic and the hematopoietic. Concurrently we have been analyzing these populations in situ by 15-color imaging mass cytometry, which enables identification of different hematopoietic populations and stromal FL cells within the same tissue section. Ultimately, the use of animal models in which specific hematopoietic cytokines are deleted in hepatoblasts will validate the role of these cells in HSC expansion. The identification of the cues driving HSCs self-renewal would bring new insights for ex vivo HSC expansion and reshape the future of the most well established stem cell-based therapy.

W-2114

MICRORNAS AS PROGNOSTIC MARKERS FOR CHONDROGENIC POTENCY OF MESENCHYMAL STROMAL CELLS DERIVED FROM EQUINE CORD BLOOD

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Multipotent Mesenchymal Stromal Cells (MSCs) are a heterogeneous population of cells with varying chondrogenic potency. Biomarkers predicting the chondrogenic potential of MSCs would allow for more time- and cost-effective identification of MSC cultures suitable for cartilage repair strategies compared to current post-chondrogenic induction determination of potency. MicroRNAs (miRNAs) are involved in the regulation of many cell functions and are often secreted by the cells. The miRNAs may be useful as biomarker to determine the chondrogenic potential of undifferentiated MSCs. We hypothesized that equine Cord Blood derived MSC (eCB-MSC) cultures exhibiting variable chondrogenic potency are associated with differential expression of miRNAs. The objective of this study is to assess the prediction value of miRNAs for chondrogenic potential of eCB-MSC cultures. In this study, ten eCB-MSC donors were initially evaluated for their ability to produce neocartilage using standard chondrogenic differentiation assay consisting of 3D pellet cultures in the presence of TGF-beta-3. The chondrogenic differentiation potential was scored based on histological matrix formation, quantitative glycosaminoglycan deposition and mRNA expression levels of chondrogenic marker genes. Subsequently, total RNAs were isolated for determination and differential expression of a panel of microRNAs (miR-34a, miR-140, miR-148a, miR-199a, miR-410) and their target genes between eCB-MSC cultures with high and low chondrogenic potential. Three eCB-MSC cultures out of 10 exhibited low chondrogenic potential, whereas 3 showed high and 4 moderate chondrogenic potential. Expression analysis of candidate microRNAs and their target genes, previously implemented in chondrogenesis, did not show a consistent pattern between highly and lowly chondrogenic eCB-MSC cultures. In conclusion, miRNA profiling of eCB-MSC cultures showed differential miRNA expression among eCB-MSC cultures although no miRNA consistently distinguished between cultures with high or low chondrogenic potential. An unbiased approach to microRNA profiling, using next generation sequencing (NGS), is ongoing and may reveal novel transcripts with predictive value.

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CANCERS

W-2116

IPSC-BASED SCREEN REVEALED AN IMPACT OF DISREGULATED NFKB ACTIVITY IN AML1-ETO RELATED LEUKEMIA

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Onset of acute myeloid leukemia (AML) has been accounted for by cooperation between multiple genetic alterations inducing abnormal control of cellular pathways. However, the detailed mechanisms of how they work in the early stages of leukemogenesis and what unknown "cooperative" cues function in those periods remain unclear. From this viewpoint, in order to identify novel cellular molecules involved in the acquisition of leukemic phenotypes, we have conducted the gene-trap strategy-based phenomic screen in the use of pluripotent stem cell (PSC)-derived hematopoietic culture. Through the gene-trap strategy-based phenomic screen in the use of PSC-based hematopoietic culture, we found that the knockdown of NSFL1c, a gene negatively regulates NFkB pathways by reducing IKK activities, enhanced the leukemic properties of hematopoietic progenitor cells harboring AML1-ETO (AE) fusion gene: Cells differentiated from AE-PSCs which have additionally the poly A trapping sequence inserted in NSFL1c locus showed doubled efficiency in engraftment into immunodeficient NOG mice than cells without trapping (3.1% v.s. 1.3%, 16 weeks after intra bone marrow transplantation), and also showed the significantly higher colony replating efficacy in methylcellulose colony forming assay. Interestingly, those activities were cancelled in the absence of AE expression. In AML, elevated NF-κB pathways have been detected in more than 30% of patients. Although NF-κB signaling networks have proved induced by inflammatory and immune signals, and previous studies showed their abnormal activities make leukemia cells escape from cell death and go into abnormal proliferation, the detailed mechanisms, in particular at the very early stages of the leukemogenesis, are well not defined. Our data indicate the novel mechanisms behind the deviation of progenitor cell fate from normal to abnormal pathway leading to the emergence of leukemic initiating cells, and suggested the myeloid-biased leukemogenic potentials.

W-2118

THE EPIGENETIC CONTROL OF STEMNESS IN GLIOBLASTOMA STEM LIKE CELL FATE COMMITMENT

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The heterogeneity of glioblastoma (GBM) causes more people to die than any other brain tumors despite existing alkylating chemotherapy. GBM stem-like cells (GSCs) contributes to the complexity of GBM and their chemoresistance. Yet, it remains challenging to identify adequate GSCs or factors controlling their activity. Here, we identified a specific GSC subset and described that their activity is regulated by methyl CpG binding domain 3 (MBD3). CK1α and β-TrCP E3 ubiquitin ligase binds to MBD3, triggering MBD3 degradation through its phosphorylation and ubiquitinylation. Mbd3 loss or degradation induced by CK1α activator, pyrvinium pamoate (Pyrpam), leads to GSC differentiation and inhibition of tumor growth in xenograft model of GBM resistant to TMZ chemotherapy. Pyrpam blocks recruitment of the repressive MBD3/NuRD complex at the neural differentiation-associated gene loci, leading to increased acetyl histone H3 activity and GSC differentiation. Our work demonstrates that targeting CK1α/β-TrCP/MBD3/NuRD axis inhibits GSC activity and hence chemoresistant GBM.

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W-2120

INHIBITION OF DYNAMIN TARGETS LEUKEMIA STEM CELLS AND OVERCOMES CHEMORESISTANCE

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The hierarchical model posits that acute leukemias arise from leukemia stem cells (LSCs), which display stem cell-like properties that include long-term self-renewal and differentiation to generate the heterogeneity observed in the tumor at diagnosis. Elimination of LSCs and their ancestral clones, pre-leukemic stem cells (pre-LSCs), is critical for long-term cure as they are the source of relapse following chemotherapy. How these cells survive high-dose chemotherapy remains poorly defined, but may include quiescence and pro-survival signals provided by the microenvironment. Strategies to interfere with specific growth factor-induced signals from the niche have shown promising results, but may be subject to compensatory mechanisms due to the inherent plasticity of relapse-inducing cells. The signalling network downstream of many of these growth factors is controlled by receptor-mediated endocytosis, a generic process dependent on the Dynamin family of large GTPases. Given the important role of the microenvironment for relapse-inducing cells, we postulated that inhibition of Dynamin might impair their stem cell-like properties and sensitize them to chemotherapy. Here, we show that the Dynamin small molecule inhibitor Dynole 34-2 simultaneously blocks multiple signalling pathways in pre-LSCs and LSCs by preventing receptor-mediated endocytosis. Using the Lmo2-transgenic mouse model of T-cell acute lymphoblastic leukemia (T-ALL), we found that Dynole 34-2 impairs self-renewal of pre-LSCs and sensitizes relapse-inducing cells to chemotherapy. We also found that inhibition of receptor-mediated endocytosis by Dynole 34-2 delayed leukemia onset in mice treated with high-dose therapy. Treatment of different patient-derived xenografts of human T-ALL revealed that that inhibition of Dynamin activity with Dynole 34-2 represents an effective therapeutic strategy for different subtypes of human T-ALL. In summary, our finding that Dynamin activity is essential for the maintenance and therapeutic resistance of relapse-inducing cells in acute leukemia paves the way for novel therapeutic strategies.

W-2122

NEURAL STEM CELL DELIVERED ONCOLYTIC VIROTHERAPY PRIMES OVARIAN TUMORS TO CHECKPOINT INHIBITOR IMMUNOTHERAPY

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Ovarian cancer will be responsible for an estimated 13,980 women deaths in the US alone in 2019. Current chemotherapy regimens for late stage ovarian cancer often have limited benefits and are not well-tolerated by the majority of patients. Several promising studies have demonstrated increased efficacy with combination oncoviral and immunotherapies. Treatment with replication-competent oncolytic viruses followed by checkpoint inhibitors (e.g., PD-1/PD-L1) may result in improved clinical efficacy. This is attributed to the 'unmasking' of tumor antigens exposed following oncolytic tumor cell lysis. Here, we used an HLA Class II negative, tumor-tropic neural stem cell (NSC) line to deliver a chimeric orthopoxvirus (CF33) to ovarian tumors. Using the NSCs as a virus delivery vehicle also provides protection from neutralizing Abs. CF33 is deficient for thymidine kinase, a protein necessary for viral replication that is overexpressed by cancer cells. As a result, CF33 selectively replicates in cancer cells while also inducing an anti-tumoral immune response that may be potentiated by checkpoint inhibitors. In vitro studies with CF33-producing NSCs (CF33-NSCs) and murine or human ovarian cancer cell lines at a ratio of 1:1000 demonstrated increased tumor cell expression of PD-L1 after 1 day. We thus hypothesized that CF33-NSCs would infect tumor cells in vivo, causing increasing expression of PD-L1 and cell lysis. We postulated that blocking T cell PD-1 receptors would lead to further anti-tumor activity in CF33-NSC treated mice. In vivo experiments in immunocompetent murine models of intraperitoneal (IP) ovarian metastases consisted of 3 weekly IP injections of CF33-NSCs, free CF33 or PBS starting 1 week post-tumor implantation. A single IP administration of PD-1 Ab was given 2 days before, after, or concurrently with the first CF33-NSCs, free CF33 or PBS injection. Weekly bioluminescence imaging (BLI) was conducted to monitor tumor growth/regression, as mice were followed for long-term survival. Mice receiving concurrent CF33-NSCs and PD-1 Ab combination therapy exhibited the lowest BLI tumor signal and highest long-term survival rate vs. groups receiving PD-1 Ab before or after. These data warrant further investigation for potential clinical translation and improved outcomes for ovarian cancer patients.

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W-2124

STORE OPERATED CALCIUM CHANNELS (SOC) REGULATE CANCER STEM CELLS SELF-RENEWAL IN HUMAN GLIOBLASTOMA

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Gliomas are primary brain tumors whose most aggressive and lethal form is glioblastoma. Despite multimodal treatment, glioblastoma tumors recur in more than 90 % of patients and the average life expectancy does not exceed 15 months. Within the tumor, a cell subpopulation called cancer stem cells (CSC) is more resistant to radiation and chemotherapy and therefore may be responsible for tumor relapse. A growing body of experimental and clinical data supports that glioblastoma CSC may arise, at least partly, from neural stem cells with whom they share several features. Calcium channels produce spatiotemporal fluctuations of intracellular concentrations of calcium ions. Expressed by both excitable and non-excitable cells, the store-operated calcium channels (SOC) transduce extracellular signals in an intracellular calcium response in numerous cell types. SOC control various cellular functions including cell proliferation, differentiation and migration. In a previous work, we found that SOC regulate neural stem cells activation and self-renewal in mice. Although some previous studies suggest that alterations in SOC may represent a proximal cause associated with cancer, their potential role in CSC has not been investigated yet. Accordingly, we assessed the expression of SOC in eight patient-derived cell cultures grown in a cell culture medium used for CSC and evaluated SOC functions in CSC by using three different pharmacological SOC inhibitors. We studied the involvement of SOC in glioblastoma cell proliferation, and in CSC ability to self-renew. We found that glioblastoma cells derived from patient expressed core components of SOC supporting store-dependent calcium entries. Pharmacological inhibition of SOC reduced proliferation of glioblastoma cells. Moreover, we found that CSC in these cultures expressed SOC, and that SOC inhibition reduced CSC ability to self-renew. Our data showing the presence of SOC in CSC and their requirement for CSC self-renewal pave the way for a strategy to target the cells that convey resistance to cancer treatment.

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W-2126

NEURAL STEM CELL DELIVERED CONDITIONALLY REPLICATION-COMPETENT ONCOLYTIC ADENOVIRUS (CRAD-SPARC-PK7) FOR THE TREATMENT OF GLIOBLASTOMA

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Oncolytic virotherapy is a promising treatment approach for refractory glioblastoma (GBM) given that oncolytic viruses can lyse even quiescent tumor cells and, secondarily, expose tumor antigens that can stimulate an anti-tumor immune response. To address current challenges of oncolytic virus distribution to distant tumor foci, our lab has demonstrated that a clinically relevant tumor-tropic neural stem cell (NSC) line (HB1.F3.CD21) can deliver virotherapy agents to multiple tumor sites, improving efficacy. A first-in-human newly diagnosed GMB patient trial assessing the safety of intracranial NSC-delivered CRAd-Survivin-pk7 (NSC.CRAd-S-pk7) in combination with radiation and chemotherapy is ongoing. Replication of the CRAd-S-pk7 virus is driven by survivin, which is upregulated in glioma cells in response to radiation. While the use of NSCs overcomes key barriers of virotherapy distribution to tumor sites, it has been recognized that the tumor-associated stroma and microenvironment represent another barrier to amplification and spread of oncolytic viruses. To address this, Dr. Curiel's lab has defined a novel promoter element, SPARC, which is overexpressed in both tumor cells and stroma, and contains enhancer elements which facilitate viral replication in hypoxic and inflammatory tumor microenvironments. The objective of

this work is to perform comparative efficacy studies of NSC-mediated delivery of one of two viral payloads: CRAd-SPARC-pk7 vs. CRAd-S-pk7. To compare the relative CRAd potencies, a fiber knob modification was performed on CRAd-SPARC-pk3/5 to create CRAd-SPARC-pk7. The viral uptake and lysis kinetics of NSC-producing CRAd-SPARC-pk7 (NSC.CRAd-SPARC-pk7) were then optimized in vitro. CRAd-SPARC-pk7 tumor cytolysis was confirmed using multiple murine and human glioma cells. In vivo efficacy studies comparing NSC.CRAd-S-pk7 to NSC.CRAd-SPARC-pk7 are in progress. Thus far, we have observed increased NSC.CRAd-SPARC-pk7 distribution and spread in tumors, tumor-associated stroma and tumor microenvironments. Given the prominent stroma component within GBMs, ongoing experiments are expected to demonstrate improved anti-tumor efficacy of CRAd-SPARC-pk7, resulting in extended long-term survival.

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W-2128

UNFOLDED PROTEIN RESPONSE PROMOTES GLIOBLASTOMA STEM CELL SURVIVAL AND PROLIFERATION

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Glioblastoma is the most common primary malignant brain tumor with a median survival of only 12-15 months. Glioblastoma stem cells (GSCs) drive chemoresistance and radioresistance and are capable of thriving in harsh environments characterized by nutrient deprivation, DNA damage and high reactive oxygen species. GSC resistance to environmental stress, such as that induced by therapeutic challenge, is in part driven by upregulation of heat shock proteins (HSPs) and the unfolded protein response (UPR). Inhibition of these pathways impairs GSC survival and induces chemoresistance. To identify novel potential drivers of glioblastoma maintenance, we performed an in silico analysis utilizing The Cancer Genome Atlas (TCGA). Genes were prioritized that were overexpressed in glioblastoma relative to normal brain tissue and informed poor patient survival. Next, further selection of molecular targets that might specifically regulate the GSCs focused on genes overexpressed in GSCs relative to neural stem cells. The top in silico hits underwent functional validation using an in vitro cell viability assay. Mediators of UPR emerged as the most critical mediator of GSC survival, with knockdown inducing rapid and widespread cell death. The UPR was activated through HSP binding with UPR mediators marked by a GSC-specific histone 3 lysine 27 acetylation peak at its promoter absent in differentiated glioblastoma cells, suggesting a stem cell-specific regulatory mechanism. Ongoing studies are defining the upstream regulatory mechanisms through which the UPR is specifically regulated in GSCs with functional validation in inherent stem cell phenotypes. Thus, targeting the UPR may be a novel sensitizing mechanism to prevent GSC escape of existing chemo- or radio-therapeutic modalities.

NEURAL DEVELOPMENT AND REGENERATION

W-3002

HUMAN OLIGODENDROCYTE PROGENITOR CELL-BASED ASSAYS FOR DRUG DISCOVERY: EFFECTS OF BMP INHIBITORS ON DIFFERENTIATION AND MYELINATION

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Mechanistic studies of human oligodendrocyte differentiation and functional myelination have been hindered by the lack of in vitro human specific oligodendrocyteculture system. Here we describe the development of a robust in vitro protocol for the derivation of human oligodendrocytes from pluripotent stem cells (hPSC). Under this protocol, hPSCs are differentiated toward glial restricted cell (hGRC) population, which are then sorted for enrichment in oligodendrocyte precursor cells (hOPC) using O4 antibody. The hOPCs are further expanded and kept frozen as cell banks. Upon thawing, hOPCs efficiently differentiate into mature and functional oligodendrocytes. We set up a high-throughput and high content screening platform using hOPCs to analyze the activity of potential compounds on hOPC proliferation, differentiation and myelination. We tested escalating doses of Noggin (an antagonist of bone morphogenetic protein (BMP) -family cytokines) on hOPC culture. We found that Noggin significantly increased the number of Olig2 and O4 positive cells, number of processes per cell, total length of the processes and branching of processes in a dose-dependent manner. The 50% effective dose (EC50) of Noggin was found to be 1.57 nM. The assay's robustness was confirmed by a significant Z' factor (Z'>0.3). Based on these results we screened several small molecules with a known inhibitory activity on BMP family cytokines. Among these molecules we found that LDN-193189 (inhibitor of Alk2, 3, and 6) promoted oligodendrocyte differentiation and myelination (IC50 30nM) in a co-culture system of hOPCs with rodent dorsal root ganglia (DRGs) neurons. The effect of LDN-193189 was further

evaluated in vivo, in an EAE-MOG animal model. We found that the combination of LDN-193189 and Methylprednisolone ameliorated diseases symptoms, as compared to control groups. This new human OPC cell-based assay platform creates new opportunities to discover compounds with a therapeutic potential for the treatment of demyelinating diseases and disorders.

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W-3004

EFFECTS OF ROS LEVELS IN HUMAN OPCS GROWTH AND MALIGNANCY

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Human oligodendrocyte progenitor cells (OPCs) can give rise to different glial cell types such as astrocytes and oligodendrocytes. OPCs can be used to create a humanized glial microenvironment in mice to model brain tumors and investigate the interaction between human glia with neurological diseases. Recently, we have successfully generated human OPCs from induced pluripotent stem cell (iPSC) and embryonic stem cell (ESC) cultures. However, one disadvantage of using human OPCs to model brain tumors is their slow rate of differentiation and proliferation. It has been shown that environmental factors and intracellular signaling pathways may play key roles in neural stem cell (NSC) proliferation and self-renewal. Recent studies have suggested that hypoxia can increase the endogenous reactive oxygen species (ROS) levels by activating the NOX pathway in NSCs. Additionally, ROS can play roles as second messengers and activate cellular processes such as the PI3K/Akt/mTOR pathway via reversible inactivation of the PTEN protein. In this study, we sought to determine whether altering exogenous or endogenous ROS levels in OPCs affects cell proliferation and survival rate. Thus, we tested this hypothesis by targeting the NOX pathway via culturing the OPCs in hypoxia and by administering apocynin as a NOX inhibitor. Our preliminary in vitro findings suggest that hypoxic condition induces proliferation in OPCs by regulating processes other than the NOX pathway. Hydrogen peroxide treatment of the human OPCs caused no significant change in cell count. We speculate that this is because of the inactivation of the hydrogen peroxide prior to the experiments.

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W-3006

DISSECTING THE ROLES OF STORE-OPERATED CALCIUM ENTRY DURING DEVELOPMENT OF THE MAMMALIAN CEREBRAL CORTEX

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Calcium signaling has been reproducibly implicated in a variety of developmental processes in the embryonic brain, including neural induction, neural progenitor cell (NPC) proliferation, neuroblast migration and differentiation. In the embryonic rodent cortex, agonist-induced calcium waves, mediated by the release of intracellular calcium stores, propagate through the germinal zones to modulate aspects of NPC proliferation. It is unclear, however, how internal calcium stores are regulated in cortical neural progenitors and how they are linked to the regulation of progenitor cell function. Store operated calcium entry (SOCE), a mode of calcium influx tied to depletion of intracellular ER calcium stores, has been shown to regulate proliferation of NPCs in vitro, but its role in the embryonic cortex remains unknown. Here, using a combination of pharmacology and in utero gain- and loss-of-function approaches, we interrogate specific roles for SOCE in NPCs of the embryonic cortex. We have found that functionally distinct splice isoforms of the STIM family of endoplasmic reticulum (ER) calcium sensors are dynamically regulated during neuronal differentiation, such that an inhibitory isoform of Stim2 that suppresses SOCE is upregulated in young neurons. This observation is in line with previous work demonstrating that robust SOCE responses in NPCs in vitro are abolished upon differentiation into neuroblasts. We have also found that manipulating the levels of Stim2 splice variants using in utero electroporation at embryonic day 13 (E13) bidirectionally regulates cell cycle exit in dividing cortical NPCs. We are further exploring this finding using live imaging approaches, single cell RNA sequencing, and a variety of molecular and biochemical

assays. Collectively, our data suggests that dynamic regulation of SOCE mediators and downstream calcium signaling plays essential roles in the regulation of proliferation and differentiation in the developing cortex.

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W-3008

FOXP1 PROMOTES NEURAL STEM CELL SELF RENEWAL IN THE DEVELOPING MOUSE CORTEX.

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The laminar architecture of the mammalian neocortex depends on the orderly generation of distinct neuronal subtypes by apical radial glia (aRG) during embryogenesis. Here we identify critical roles for Foxp1 in promoting self renewal thus maintaining aRG identity and gating the temporal competency for early neurogenesis. High levels of Foxp1 are associated with early aRG and are required to promote proliferation, influence cell division symmetry and promote self renewal, favoring aRG expansion and production of early born neurons. The potent pro-progenitor functions of Foxp1 are further revealed by our demonstrating its ability to preserve a population of aRG cells throughout development that have the potential to generate early born neurons. Furthermore, Foxp1 promotes the formation of cells resembling basal radial glia, a progenitor group implicated in the increased size and complexity of the human cortex. Consistent with this role, we show that FOXP1 is associated with the initial formation and expansion of bRG during human corticogenesis.

W-3010

NESTED OSCILLATORY DYNAMICS IN CORTICAL ORGANOIDS MODEL EARLY HUMAN BRAIN NETWORK DEVELOPMENT

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Structural and transcriptional changes during early brain maturation follow fixed developmental programs defined by genetics. However, whether this is true for functional network activity remains unknown, primarily due to experimental inaccessibility of the initial stages of the living human brain. Here, we developed cortical organoids that spontaneously display periodic and regular oscillatory network events that are dependent on glutamatergic and GABAergic signaling. These nested oscillations exhibit cross-frequency coupling, proposed to coordinate neuronal computation and communication. As evidence of potential network maturation, oscillatory activity subsequently transitioned to more spatiotemporally irregular patterns, capturing features observed in preterm human electroencephalography. These results show that the development of structured network activity in the human neocortex may follow stable genetic programming, even in the absence of external or subcortical inputs. Our approach provides novel opportunities for investigating and manipulating the role of network activity in the developing human cortex.

W-3012

GPNMB NEGATIVELY REGULATES NEURAL STEM CELL DERIVED OLIGODENDROGENESIS

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Neural stem cells residing in the subventricular zone (SVZ) of adult brains are a source of remyelinating oligodendrocytes. In particular, a subset of these cells which express the transcription factor Gli1 in the ventral SVZ, have been shown to migrate to demyelinating lesions and differentiate into oligodendrocytes in the corpus callosum. However in the healthy brain, these cells do not generate oligodendrocytes, instead they differentiate into neurons in the olfactory bulb. Using a transcriptomic analysis of neural stem cells, we identified a type-1 trans-membrane protein, glycoprotein nonmetastatic melanoma protein B (GPNMB) as one of the genes responsible for the inhibition of oligodendrocyte generation in the adult brain. Our data shows that GPNMB is expressed in neural stem cells and not in the oligodendrocyte progenitor cells in the adult brain. Further, in vitro overexpression of GPNMB in the adult neural stem cells inhibits the generation of oligodendrocytes. These results suggest that GPNMB inhibits the differentiation of oligodendrocytes from adult neural stem cells and may help guide efforts to enhance remyelination.

Funding Source: National Multiple Sclerosis Society, New York STEM, Wisconsin Alumni Research Foundation

W-3014

XENO-FREE DERIVATION OF NEURAL CREST STEM CELLS FROM HUMAN PLURIPOTENT STEM CELLS USING A SIMPLE AND DEFINED MEDIUM

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Neural crest stem cells (NCSCs) retain unique characteristics including multipotency to become derivatives of neuroectodermal and mesodermal lineages. Many protocols to derivate NCSCs from human pluripotent stem cells (hPSCs) so far have been using blockers of BMP, Activin/Nodal, and WNT signaling pathways. In this study, we established a novel protocol to generate NCSCs from hPSCs using a simple, defined, and xeno-free medium. The resulting NCSCs were shown to retain multipotency and became peripheral neural cells as well as mesodermal cells in response to proper culture conditions. In conclusion, our study provides a platform to generate xeno-free NCSCs and will be used to facilitate clinical applications of NCSCs to treat many incurable diseases.

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W-3016

IN VIVO REPROGRAMMING FACTOR OCT4 EXPRESSION ALLEVIATES MYELINOPATHY IN A MOUSE MODEL OF HUNTINGTON DISEASE

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Introduction: Huntington's disease (HD) is an incurable neurodegenerative disorder. Recent studies reported that white matter atrophy is an early symptom of HD. Therefore, this study investigated the effects of overexpressing the octamer-binding transcription factor 4 (OCT4) reprogramming factor in vivo as a treatment for dysmyelination in HD. Methods: Adeno-associated virus serotype 9 (AAV9) was used as a vector for OCT4 overexpression in mice. Each group of R6/2 mice was injected with AAV9-OCT4, phosphate-buffered saline (PBS), or AAV9-Null on both sides of the lateral ventricles. The groups were compared by performing behavioral tests such as rotarod test and grip strength test, and histochemistry analyses. Results: The behavioral tests such as rotarod test and grip strength test showed that the AAV9-OCT4 group displayed significantly improved performance compared to the control groups (PBS and AAV9-Null). The subventricular zone in the AAV9-OCT4 group had significantly higher numbers of BrdU+Nestin+, BrdU+NG2+ and BrdU+Olig2+ cells than the control groups. The AAV9-OCT4 group had significantly higher expression levels of PDGFRa, WNT3, myelin regulatory factor (MYRF), and glial-derived neuroprotective factor (GDNF) in the striatum and the frontal cortex. The myelin basic protein (MBP) level was significantly higher in the frontal cortex of the AAV9-OCT4 group than in the control groups. In addition, striatal DARPP32+ GABAergic neurons significantly increased in AAV9-OCT4 group compared to the control groups. Conclusions: These results suggest that oligodendrogenesis was induced by in vivo overexpression of the reprogramming factor OCT4 in the subventricular zone, thereby attenuating dysmyelination. GDNF released by oligodendrocyte progenitor cells might exert neuroprotective effects on striatal GABAergic neurons, which explains the behavioral improvement in R6/2 mice treated with AAV9-OCT4. Taken together, in vivo reprogramming factor OCT4 expression could be a therapeutic strategy for alleviating disease progression in HD.

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NEURAL DISEASE AND DEGENERATION

W-3018

IDENTIFICATION OF POTENTIAL THERAPEUTIC AGENTS FOR IMPAIRED MITOPHAGY IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disease caused by selective loss of midbrain dopaminergic (DA) neurons. Although more than 90% of PD cases are sporadic without any identified causative genes, iPSC-based models of familial PDs with specific cellular defects are useful for disease modeling and drug screening. We have established and reported disease-specific iPSCs from two types of the familial PDs, PARK2, caused by PRKN mutation, and PARK6, caused by PINK1 mutation, with impaired mitochondria clearance. Then, several PD-related phenotypes, including impaired mitophagy, ROS accumulation, and increased apoptosis in PARK2 and PARK6 dopaminergic neurons were quantified automatically by using IN Cell Analyzer. We performed library screening (320 compounds) and identified 4 compounds that improve multiple phenotypes observed in PARK2/PARK6 DA neurons. These candidates showed enhancing mitochondrial clearance and anti-apoptotic effect against CCCP-induced mitochondrial damage in dose-dependent manner. We also found that 3 of 4 compounds rescued PARK2/6 DA neurons from apoptosis in the regular culture conditions, without artificial mitochondrial damage. We then confirmed that these candidate drugs could recover PD-phenotypes of PARK6 fly models and apoptotic phenotypes in iPSC-derived neurons derived from sporadic PD patients. This high-throughput phenotype detection system is an effective tool for drug screening to explore disease-modifying drugs in PD.

W-3020

MODELING VARIANTS OF LATE-ONSET ALZHEIMER'S DISEASE BY GENERATING ALLELIC SERIES OF GENETIC RISK FACTORS IN HUMAN PLURIPOTENT STEM CELLS

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Alzheimer's disease (AD) is the leading cause of dementia worldwide, and there is no effective treatment which reverses or halts disease pathology. The societal and economic burdens resulting from AD make the development of novel therapeutic strategies of paramount importance. AD has traditionally been divided into two categories: rare early-onset (EOAD, often familial) and more common late-onset (LOAD, defined age 65 and above, > 95% of cases) subtypes. The majority of animal and cellular models have focused on EOAD, in particular by using autosomal dominant mutant forms of the amyloid precursor protein (APP) or presenilins (PSEN1/2) that cleave APP to form Abeta and other toxic proteolytic fragments. Therapeutic strategies attempting to either block Abeta generation or promote its clearance via monoclonal antibodies have failed thus far in the clinic. While some of these approaches may prove to be more efficacious if given prophylactically before the appearance of AD symptoms, another possibility is that LOAD reflects a broader spectrum of disease states. In this model, particular sub-variants of LOAD may respond better to therapeutics targeting pathways dysregulated by specific LOAD genetic risk factors. In order to begin addressing this possibility, we have generated allelic series of LOAD risk factors in the same genetic background by using CRISPR/Cas9 to knockin disease-relevant variants into a control iPSC backbone. This includes the SORL1 E270K and ABCA7 rs142076058 (44 bp deletion) mutations, which were identified in Caribbean Hispanic and African American populations respectively. We have also generated patient-specific iPSCs for each of these mutations, and are in the process of gene-correcting them. LOAD mutants and isogenic controls are being differentiated into neurons, microglia, and multicellular cortical organoids. Unbiased transcriptomic and other omics profiling will be conducted to identify key pathways dysregulated in mutant cells, in addition to functional assays including, but not limited to, APP processing in neurons and phagocytosis/cytokine assays in microglia. Preliminary results will be presented.

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W-3022

ADULT HIPPOCAMPAL NEUROGENESIS IN HUMAN MESIAL TEMPORAL LOBE EPILEPSY

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Adult neurogenesis is a dramatic form of brain plasticity in which new born neurons and astrocytes modify existing neural circuitry. Alterations in adult neurogenesis can initiate disease, as evidenced in epilepsy models, or slow disease progression, as shown in Alzheimer's disease models. While adult neurogenesis is generally conserved across mammals, its existence in humans is currently controversial. We used histology, multielectrode array (MEA), single cell RNA-sequencing (scRNA-Seq) and cell culture to comprehensively analyse adult neurogenesis in hippocampal resections from Mesial Temporal Lobe Epilepsy (MTLE) patients. Convergence from these approaches identify new born granule neurons in the human dentate gyrus (DG) of MTLE patients. Yet, the number of newly generated granule neurons drastically decreases to undetectable levels during epilepsy progression. Meanwhile, new born astroglia continue to be generated in the DG and increase in number with disease progression. These cells remarkably display both neuronal and astroglial characteristics. Newborn astroglia are responsive to the excitation levels of local circuits, which influence migration to the hilus and molecular layer. This study provide more definitive evidence of rare adult new born neurons in human tissue and indicates the human adult neurogenesis process is greatly impacted by epilepsy progression.

W-3024

DEVELOPMENTAL ASPECTS OF AMYOTROPHIC LATERAL SCLEROSIS: FINDINGS IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MOTOR NEURONS WITH A4V AND G93A SUPEROXIDE DISMUTASE 1 MUTATIONS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the gradual degeneration and elimination of motor neurons (MNs) and skeletal muscles leading to paralysis, respiratory insufficiency, and death. While ALS presents clinically in adults, the pathogenic onset and true disease duration are unknown. Specifically, it is not known when ALS begins mechanistically, functionally, and pathologically and how the central nervous system might compensate. Some familial ALS is linked to mutations in copper, zinc superoxide dismutase 1 (SOD1) and its mutant forms are believed to acquire an adverse property. However, underlying therapeutically relevant mechanisms on how mutant SOD1s cause neurodegeneration are also unknown. Here, using human induced pluripotent stem cells (iPSCs) with heterozygous A4V or G93A mutations, we studied their directed differentiation into spinal motor neurons and identified neurodevelopmental defects when compared to the wild-type. These abnormalities include smaller colony size, less number of colonies, and decreased total cell numbers in pluripotent stem cell (PSC) and neuroepithelial progenitor (NEP) stages. Our study suggests a new concept of understanding the disease course, particularly possible non-clinical latent phases. This idea could lead to the identification of novel mechanisms of ALS pathogenesis masked by long periods of biological compensation, and could be therapeutically relevant for effective mechanism-based therapies.

W-3026

NEURAL CREST-DERIVED HUMAN CRANIAL PERICYTES MODEL PRIMARY FOREBRAIN PERICYTES AND PREDICT DISEASE-SPECIFIC CRANIAL VASCULATURE DEFECTS

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Forebrain pericytes are critical players in the blood-brain barrier (BBB). Defects in or loss of functional forebrain pericytes leads to compromised microvessel function and ultimately breakdown of the integrity of the BBB, causing leakage of toxins and pathogens into the brain and aggrevating neuroinflammation.

Defective blood vessels and leakiness of the BBB has recently been found to play a part in numerous neurodegenerative diseases, most notably Alzheimer's disease (AD), and tortuous vessels have been detected prior to onset of dementia in AD patients and carriers of AD risk alleles. Despite their importance, little is known about forebrain pericytes and what makes this population of pericytes both able to maintain the BBB and become prone to damage with aging and disease. Utilizing a method to generate in vitro-derived cranial pericytes, I have been able to identify a set of defects inherent in pericytes in AD. My project focuses on taking steps toward understanding the molecular mechanisms underlying the defects in AD pericytes. I plan to incorporate epigenomic profiling with in vivo AD rat studies to further characterize the AD pericytes and identify what regulates their dysfunction with aging. These approaches will help the field of pericyte biology to gain a better understanding of forebrain pericytes, as well as open the door for potential therapeutic avenues to delay or stem the onset of AD.

W-3028

MESENCHYMAL STEM CELL TRANSPLANTATION PROMOTES FUNCTIONAL RECOVERY THROUGH MODULATING ASTROGLIOSIS AFTER SPINAL CORD INJURY

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Treatment with mesenchymal stem cells (MSC) in spinal cord injury (SCI) has been highlighted as therapeutic candidate for SCI. Although astrogliosis is a major phenomenon after SCI, the role of astrogliosis is still controversial. In this study, we determined whether acute transplantation of MSC improves the outcome of SCI through modulating astrogliosis. Bone marrow derived rat MSCs were transplanted one week after the induction of acute SCI rat model. Spinal cords were harvested, and matrix metalloproteinase (MMP) and neuro-inflammatory pathway was analyzed for acute astrogliosis at 1, 3 and 7 d after SCI. Functional outcome was analyzed serially at postoperative one day and weekly for 4 weeks. Histopathologic analysis was undertaken at 7 and 28 d following injury. Transplantation of MSCs decreased IL-1α, CXCL-2, CXCL-10, TNF-α and TGF-β in a rat model of contusive SCI. Protein level of NF-κB p65 was slightly decreased while level of STAT-3 was increased. In immunohistochemistry, MSC transplantation increased astrogliosis whereas attenuated scar formation with sparing white matter of spinal cord lesions. In RT-PCR analysis, mRNA levels of MMP2 and MMP9 were significantly increased in MSC transplanted rats. In BBB locomotor scale, the rats of MSC treated group exhibited improvement of functional recovery. In conclusion, transplantation of MSC reduces the inflammatory reaction and modulates astrogliosis via MMP2/STAT3 pathway leading to improve functional recovery after SCI in rats.

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W-3030

JC VIRUS PROPAGATION IS POTENTIATED BY GLIAL REPLICATION AND IS ACCELERATED BY DEMYELINATION-ASSOCIATED GLIAL PROLIFERATION

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Progressive multifocal leukoencephalopathy (PML) is a demyelinating infection of the brain of immunosuppressed individuals, mediated by the gliotropic polyomavirus JCV. JCV replicates in mitotically-competent human glial progenitor cells and astrocytes, which are triggered to divide in the setting of viral T antigen-mediated cell cycle entry, allowing viral replication; the death of mitotically-incompetent oligodendrocytes occurs secondarily, largely through T antigen-mediated apoptosis. This observation suggested that JCV infection might be potentiated by astrocytic replication, and hence accelerated in the setting of mitotic gliogenesis. To test this hypothesis, we tagged dividing human glia in vitro with bromodeoxyuridine (BrdU), then infected them with JCV MAD1, and confirmed that proliferating human astrocytes are more supportive of JCV propagation than mitotically quiescent cells. In vitro, scratch assays confirmed that viral propagation was greatly enhanced in peri-scratch regions of dividing glia. Mice were neonatally-implanted with human pluripotent stem cell-derived glial progenitor cells,

which then colonized their host brains so as to yield human glial chimeras. JCV infection in human glial chimeras established that infection was greatly accentuated by cuprizone-mediated demyelination, which was associated with increased glial progenitor cell proliferation. JCV infection in vivo was associated with caspase3-defined death of uninfected as well as infected oligodendrocytes, suggesting the contribution of bystander death to JCV-associated demyelination. These results suggest that JCV propagation in PML may be potentiated by glial cell division, and that the accentuated glial cell division and hence DNA replication attending acute demyelination might provide an especially favorable environment for JCV propagation and PML progression. These data thus argue for the aggressive prevention of new demyelinating events in patients at risk for PML, while providing a humanized model by which therapeutics targeting human-specific infectious diseases of the brain may be evaluated in vivo.

Funding Source: PML Consortium

W-3032

DEVELOPING A NOVEL HUMANIZED MOUSE MODEL WITH TRANSPLANTED HIPSC-GLIAL ENRICHED PROGENITOR CELLS/HESC-OLIGODENDROCYTE PROGENITOR CELLS

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White matter stroke (WMS) accounts for 30% of all stroke events and is caused by the development of ischemic lesions in the connecting regions of the brain, designated the white matter tracts. After a WMS occurs there is very limited motor and cognitive recovery. A main aspect of WMS is the damage to glial cells such as astrocytes and oligodendrocytes, resulting in the loss of myelin. These neural glial cells are vital in maintaining the central nervous system (CNS) and without them deficits occur. To develop new treatments for this disease, it is important to determine how these human glial cells react to a WMS. We first developed a mouse model with human astrocytes and oligodendrocytes. This was done by deriving glial enriched progenitor cells (GEPs) from human induced pluripotent stem cells (hiPSC) and oligodendrocyte progenitor cells (OPCs) from human embryonic stem cells (hES). After differentiating the stem cells into GEPs and OPCs they were transplanted into 2 day old mice. Data indicate that transplanted GEPs and OPCs populate the mouse brain with human cells and create a humanized environment. This can serve as to better study human glial cells in a humanized mouse model and in the future be used as a cell based therapy for WMS.

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W-3034

TRANSCRIPTOMIC AND PROTEOMIC ANALYSES OF IPSC-DERIVED IBMPFD/ALS DISEASED CELLS

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IBMPFD/ALS (inclusion body myopathy, Paget's disease of bone, frontotemporal dementia/amyotrophic lateral sclerosis) is an untreatable and fatal neurodegenerative disease. Pathogenic p97/VCP (valosin-containing protein) mutants are clearly causative proteins of IBMPFD/ALS diseases in humans, thus making them to be potential therapeutic targets. Among 45 known p97 mutations, R155H is the most common mutation and accounts for 50% of the clinical prevalence. To design rational therapies, we must first define the pathogenic mechanisms by which R155H-p97 causes the disease. To perform our experiments in disease relevant cells, we reprogrammed fibroblasts from 10 patients with heterozygous p97 mutation-R155H and 3 unaffected family members with WT p97 into iPS cells. Next we used CRISPR to edit iPS cells to generate isogenic controls. After that, all iPS cells are differentiated into motor neurons. Quantitative RT-PCR and immunofluorescence are used to monitor expression of specific markers for each stage of differentiation. From our preliminary RNA-seq data, 43 interesting genes were identified by comparing the derived motor neurons of four patients to their isogenic controls. These differentially expressed genes will be further analyzed and confirmed by RT-qPCR to explain the altered cellular functions. Meanwhile, we will use mass spectrometry to identify the total proteome in diseased and normal cells and compare the protein-protein interaction profiles between R155H and WT p97 proteins, identifying any alterations. Overall, we hope to reveal the role of p97 mutation in neurodegenerative disease and overcome the critical barrier to find treatment for IBMPFD/ALS patients who have p97 mutations.

W-3036

CHARACTERIZATION OF HUMAN IPSC-DERIVED MICROGLIA-LIKE CELLS FOR THE STUDY OF AMYOTROPHIC LATERAL SCLEROSIS

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Microglia, the resident macrophages of the brain, play a pivotal role in several mechanisms underlying development, homeostasis and disorders of the Central Nervous System (CNS). In this study, we present an adapted method to robustly derive human microglia-like cells (hMg) from embryonic and induced pluripotent stem cells (hESC/hiPSC) for the study of neurodegenerative diseases (Abud et al., 2017). hiPSC-Mg present similar morphology to primary microglia cultured in vitro and express markers of CNS-resident myeloid cells. hiPSC-Mg are highly motile and are able to phagocytose fluorescently-labelled beads in vitro. Co-culture of these cells with mixed hiPSC-derived neuro-astroglial populations increases ramication of hiPSC-Mg and conrms their tendency to be highly motile and form numerous cell-to-cell contacts with the neuro-astroglial cultures, similar to microglial behaviour in the CNS. Moreover, single cell RNAseq analysis demonstrated how hiPSC-Mg present gene expression proles that resemble those of in vitro cultured human primary microglia and allowed their stratication into different hiPSC-Mg sub-populations in vitro. We hope to utilise this platform to widen our knowledge in human microglial functions, responsiveness as well as their ability to support neuronal health and further investigate their misregulation in neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS).

W-3038

CHARACTERIZING CELLULAR DISTRIBUTION OF AMYLOID PRECURSOR PROTEIN IN HUMAN IPSC-DERIVED NEURONS PROVIDES INSIGHTS INTO APP TRAFFICKING AND PROTEOLYTIC PROCESSING

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Alzheimer's disease (AD) is a devastating, fatal neurodegenerative disorder pathologically characterized by the presence of extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) in the brain. Aβ peptide, the primary component of these Aβ plaques and potential contributor to development of NFTs, is produced by the sequential proteolytic cleavage of the transmembrane amyloid precursor protein (APP). There is extensive research in the field examining the modulatory effects of APP, its AD-associated mutations, and enzymatic cleavage variations on the generation of Aβ peptides, however, there is no existing research establishing endogenous cellular levels and distribution of APP and its amyloidogenic fragments in naive human neurons. Creating a steady-state profile of APP in a normal-expression system is essential to providing a basis for determining differential expression, processing and trafficking of APP. Therefore, in this study, we utilized a non-artificial system expressing APP endogenously to analyze full-length APP and its amyloidogenic fragments' cellular distribution and trafficking using control Craig Venter (CVB) human iPSC-derived neurons. First, through a combination of immunocytochemistry and classic biochemical techniques we systematically assessed and quantified APP levels and localization in different cellular and sub-cellular compartments; secondly, we utilized a time-course dependent uptake assay using fluorescently-tagged endogenous APP to characterize its endocytosis, trafficking and degradation trajectory. Our analysis yielded a dynamic distribution of APP, varying from the biosynthetic to endosomal compartments. This investigation allows us to determine the endogenous localization and distribution of APP in a physiologically relevant model of AD, which will hopefully provide a basis for further analysis of the molecular mechanisms underlying the generation of Aβ in AD.

ORGANOIDS

W-3042

FUNCTIONAL AND MECHANISTIC NEUROTOXIC PROFILING USING HUMAN IPSC-DERIVED NEURAL SPHEROID 3D CULTURES

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Environmental toxins, air pollution, and medications have all been implicated in the development of neurologic disorders and diseases. While the mechanisms of action and outcomes are known for some of these chemicals, many remain a mystery. To speed up the development of more effective and safer drugs, there is an increasing need for more complex, biologically relevant, and predictive cell-based assays for drug discovery

and toxicology screening. We have used 3D neural culture assay platform containing human iPSC-derived functionally active cortical glutamatergic and GABAergic neurons. The impact of various compounds on the patterns of neural spontaneous activity was monitored by changes in intracellular Ca2+ oscillations measured by fast kinetic fluorescence with calcium-sensitive dyes. Advanced image analysis methods were implemented to provide multi-parametric characterization of the Ca2+ oscillation patterns. In addition, we used high content imaging methods to characterize compound effects on morphology, viability, and mitochondria potential of neural cells. This phenotypic assay allows for the characterization of parameters such as oscillation frequency, amplitude, peak width, rise and decay times, as well as cell viability and morphological characteristics. Here, we report on application of the 3D neurospheroid assays for neurotoxicity profiling of a library of compounds that contained drugs, pesticides, flame retardants, polycyclic aromatic hydrocarbons (PAH)s, and industrial chemicals. Spheroids were exposed to each of chemicals and calcium oscillations and cellular and mitochondrial toxicity were quantified. Our results showed that 56% of the compounds significantly impacted calcium handling in the spheroids, compared to only 21 and 26% of the chemicals exhibiting effects in the cytotoxicity assays. After accounting for mechanism of actions, the sensitivity of assays using calcium handling as a neurotoxicity screening biomarker increased to 61%, with pesticides (91%), flame retardants (84%) and drugs (53%) showed the greatest sensitivity in this model. Our results show that microBrain 3D is a promising, biologically-relevant tool for assessing the neurotoxic potential of drugs and environmental toxins.

W-3044

SINGLE CELL ANALYSIS OF HUMAN FETAL RETINA WITH STEM CELL-DERIVED RETINAL ORGANOIDS REVEAL CONSERVATION OF DEVELOPMENTAL TIMING, BUT NOT CELLULAR COMPOSITION

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Human stem-cell derived retinal organoids represent a highly accessible and amenable system for studies of retinal development and disease. They can accurately mirror early stages of human retinogenesis in a stepwise, temporal sequence, and the organization and expression of major classes of retinal neurons is recapitulated. Organoids have been especially successful in facilitating the differentiation and maturation of photoreceptors, which acquire the initial stages of outer segment morphology and phototransduction protein-expression in long-term 3D cultures. However, development and maturation of inner layer retinal neurons such as bipolar and ganglion cells are limited in organoids. Additionally, it is not clear if organoids can reproduce the cellular composition, diversity and genesis of the human fetal retina, as direct comparisons of organoids with the fetal retina have been limited. This is particularly relevant, since the human retina develops along a large spatial-temporal gradient, where the central retina is accelerated by several weeks compared to the periphery, and it is not known if retinogenesis in organoids recapitulates the developmental axis of the retina. Therefore, we used single cell RNAseq (10x Genomics) analysis, Immunostaining and RNA seq analysis to compare organoids to analogous stages of the fetal retina. Our results demonstrate for the first time that organoids follow similar pseudotime retinal lineages as the fetal retina, but differ in their cellular composition and maintenance of inner retinal organization at later stages. To test if this lack of organization in organoids is due to culture conditions, we compared the morphology and single cell RNAseq data of organoids with fetal retina tissue maintained in vitro. Our analyses show that inner retinal cell types, such as bipolar cells, amacrine and horizontal cells develop well in cultured fetal retina but not in organoids, indicating that culture conditions alone do not account for the deficiencies seen in retinal organoids. Overall, these experiments represent the first direct single cell analysis comparisons of organoids to fetal tissue, and will help to identify strategies to better facilitate organoids for translational studies of the retina.

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W-3046

GENERATION OF 3D CORTICAL MODELS BY BIOPRINTING HUMAN IPSCS-DERIVED NEURONS

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Conventional 2D cell cultures fail to represent the complexity of the brain and novel 3D systems are emerging as more realistic and representative models. 3D bioprinting is a biofabrication method that uses as an ink a combination of biocompatible non-living materials and cells (bioink). This technique provides the possibility to combine cells in a controlled way, to build structures that closely mimic natural tissues. A number of possible biomedical applications have been proposed for 3D bioprinting, ranging from regenerative medicine to disease modeling. However, only recently 3D bioprinting has been applied to build 3D models using human induced Pluripotent Stem Cells (iPSCs) as the building block. Here we report the generation of 3D cortical models generated from iPSC-derived neural cells by bioprinting. We have used a custom 3D extrusion bioprinter developed in-house. This instrument incorporates a microfluidic printing head that allows the deposition of multimaterial and/or multicellular bioink within a single scaffold, thus providing control on the relative position of different cell types within the 3D construct at the micrometer scale with increased reproducibility. Post-printing, we observed high survival rate of cortical neurons in the 3D construct, a reticulum, which allowed optimal perfusion of culture medium without the need of a bioreactor. Neuronal cells projected their axons and dendrites both within and across the fibers. Marker and functional analysis (sodium and potassium currents; calcium transients) suggested that, compared to conventional 2D cultures, acceleration of neuronal in vitro maturation may occur in the 3D model. Collectively, these preliminary data provide the basis for a novel field of application of bioprinting: creation of a variety of 3D models of the human nervous system, starting from neural (and possibly non-neural) cells derived from iPSCs.

W-3048

FUSION BRAIN ORGANOIDS DEMONSTRATE COMPLEX NEURAL NETWORK AND OSCILLATORY ACTIVITIES

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Human brain organoids are a 3D culture system where brain-like structures are created from human embryonic or induced pluripotent stem cells (hESCs or hiPSCs). This compelling new platform recapitulates unique aspects of human brain development and cytoarchitecture and has already provided novel insights into human neurological disease. However, much of the current literature on brain organoids has focused on utilizing the anatomical and cytoarchitectural characteristics of the organoid to model brain diseases that impact neurogenic development and has not focused on the physiological activity or network architecture of these remarkable structures. Here, we leveraged recent advances in organoid culture that have permitted the formation of cerebral cortex-ganglionic eminence "fusion" organoids in which excitatory and inhibitory neuron populations integrate to generate organoids with complex oscillatory and network events. Using two photon based calcium imaging and unbiased post imaging algorithmic analyses, we show that excitatory-inhibitory fusion organoids have unique patterns of calcium network activation not seen in control organoids. We next used traditional extracellular recording of local field potentials and showed that the fusion organoids have sustained low frequency oscillations that are not seen in controls. Together, these data suggest that excitatory-inhibitory fusion organoids have unique and complex neural circuit activities. Further understanding of the underlying mechanism of these physiological activities may allow fusion organoids to provide novel insights into human brain disease.

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W-3050

ESTABLISHMENT OF EXPERIMENTAL PARADIGM FOR GENERATION OF FUNCTIONING ENTERIC NEURONS FROM HUMAN PLURIPOTENT STEM CELLS USING SINGLE CELL TRANSCRIPTOMICS AND HUMAN INNERVATED COLONIC ORGANOIDS

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Generation of fully-functioning cells from human pluripotent stem cells (hPSCs) remains challenging. In this study, we performed single-cell RNA sequencing (scRNA-seq) to systematically analyze the lineage commitment process after the hPSCs exist

their pluripotent state and the timing of various differentiation cues underlying the generation of neural crest (NC) and their subsequent neuronal lineage differentiation and established a hPSC-derived innervated colonic organoid (HCOs) model for appraising the function of hPSC-derived enteric neurons. scRNA-seq analysis revealed five main clusters of cells from the pool of hPSC-derived NC, each cluster of cells exhibited unique expression pattern resembling NCs at different developmental stages in vivo. In particular, we found that HEDGEHOG (HH) pathway is activated in the post-migratory NC-like cells. Using chemical- and gene-targeting-mediated modulation of HH signaling, we further defined the critical treatment window for HH to increase the NC-yield from hPSC. By profiling the single cell transcriptomes of hiPSC-derived NC cells and their neuronal progenies, we further delineated how HH alters the topology of the neuronal differentiation path of NC and primes NC toward the neurogenic lineage. Subsequent in vitro differentiation assay further indicated that activation of HH signaling during the hPSC-to-NC transition can greatly improve the subsequent neuronal lineage differentiation of NC. More importantly, we established a differentiation protocol for the generation of a human innervated HCOs model and used them for assessing the functional competency of hPSC-derived enteric neurons. Our innervated HCOs model contained defined crypts, colonic epithelium, various types of colon cell (e.g. Goblet- and endocrine-like cells) as well as functional enteric nervous system. With our innervated HCOs model, we further demonstrated that activation of HH during NC induction can greatly improve the functional competency and the neuromuscular coupling of hPSC-derived enteric neurons with high reproducibility across hPSC lines. In summary, we established an experimental paradigm to systematically optimize the differentiation protocol for the generation of functioning NC in a systematic way.

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W-3052

INVESTIGATING SOX2 AND SOX9 FUNCTION IN HUMAN FETAL LUNG PROGENITOR CELLS

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The human lung is a complex organ whose primary function is gas exchange. This is fulfilled by a tree-like epithelium terminating at numerous gas exchange units known as alveoli. Our current understanding of human lung development comes mostly from intensive mouse research. We have previously shown that during human lung development, the fetal epithelial tip progenitors are analogous to those of the developing mouse lung and both self-renew and differentiate into all lineages of pulmonary epithelial cells. However, SRY-related HMG-box (SOX) family proteins-SOX2 and SOX9, which are essential for mouse lung development, have a different expression pattern in the developing human lung epithelium compared with the mouse. Here, we investigate the role of SOX2 and SOX9 specifically in human lung development using our human fetal lung tip organoid culture system. We have developed efficient methods for genetic manipulation in our lung organoid platform, including for gene-targeting. We systematically optimised the efficiency of CRISPR-Cas9 mediated gene-targeting in this system by targeting the highly expressed endogenous ACTB locus to generate an ACTB-GFP fusion protein. Delivery of Cas9 ribonucleoprotein (RNP) complex by nucleofection was identified as the most efficient method for organoid genomic engineering. Thereafter, the SOX2 endogenous locus was correctly targeted with the auxin inducible degron (AID) system to down-regulate SOX2 protein. By contrast, inducible SOX2 and SOX9 overexpression were achieved using the PiggyBac transposon system and lentivirus. Preliminary results suggest that SOX2 overexpression leads to self-renewal breakdown, whereas SOX9 overexpression does not interfere with progenitor self-renewal.

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W-3054

INVESTIGATION OF THE ROLE OF CHD8 IN HUMAN BRAIN DEVELOPMENT AT SINGLE-CELL RESOLUTION

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The ATP-dependent chromatin-remodeling factor CHD8 is one of the most commonly mutated genes in sporadic autism spectrum disorder (ASD), and is associated with a high prevalence of macrocephaly. Limited information is available on

the cell-type specific cellular and molecular defects induced by CHD8 mutation in human brain cells. To address this question, we used pluripotent stem cell (PSC) lines heterozygous for a CHD8 loss of function mutation to generate different 3D brain organoids. Whole-brain organoids gave rise to many cell types of the endogenous human forebrain. Interestingly, these CHD8+/- organoids recapitulate the macrocephaly phenotype observed in several of the ASD patients carrying this mutation. In order to gain information on specific cell types affected by the CHD8 mutation, we initially performed single-cell analysis on cells taken from 4 month old CHD8 mutant and control whole-brain organoids using the 10X Genomics Chromium system. Clustering and differential gene expression analysis revealed that some clusters are well represented by both genotypes, and that the differentially expressed genes from these reproducible clusters showed overlap with known risk associated genes for ASD and schizophrenia. However, certain clusters of ventral origin had only control cells. To investigate whether we could rescue these cells, and generate inhibitory neurons, we ventralized the whole-brain protocol and successfully made significant numbers of ventral cell-types. However, both whole-brain protocols are still subject to some heterogeneity in the cell-types produced. To increase the reproducibility, we used a dorsal patterned protocol and found, at the single cell level, more consistent replicate contribution to cell-type clusters. Next, we patterned for ventral forebrain organoids, but while significantly more reproducible than whole-brain organoids, the patterned ventral protocol still gave some heterogeneity across cell-type clusters. However, we find that both patterned protocols allow us to reliably make certain cell types of the dorsal and ventral forebrain. Further analysis of these data should provide the first insights into cell-type specific cellular and molecular abnormalities associated with CHD8 mutation during human brain development.

W-3056

HUMAN RETINOBLASTOMA IN RETINAL ORGANOIDS DERIVED FROM EMBRYONIC STEM CELLS WITH TARGETED RB1 MUTATIONS

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Retinoblastoma (Rb) is a primary intraocular cancer in children caused by biallelic inactivation of the retinoblastoma 1 (RB1) gene. Nowadays the 'cell origin', tumorigenesis of Rb remain elusive due to the unavailability of human Rb models. Human embryonic stem cells (hESCs)-derived retinal organoids provide an extraordinary platform for retinal disease modeling, allowing us to develop an ideal in-dish Rb model called human Rb organoids (hRORs). RB1-mutant (RB1Mut/Mut) and -null (RB1-/-) hESCs were generated by CRISPR/Cas9 mediated genome-editing, its genetic integrity, pluripotency were evaluated to confirm their differentiation capacity into retinal organoids. Stepwise differentiation of RB1Mut/Mut and RB1-/- hESCs into hRORs in vitro were carried out. The molecular, cellular, histopathologic, and morphometric characteristics of hRORs were identified by RNA sequencing (RNA-seq), single-cell RNA-seq, whole-genome bisulfite sequencing (WGBS), assay for transposase-accessible chromatin with high throughput sequencing (ATAC-seq), transmission electron microscopy (TEM), immunostaining, and subretinal engrafting in SCID mice. We generated the RB1Mut/Mut (c.958C>T; p.R320*) and RB1-/- hESCs remained in an undifferentiated state, which were successfully differentiated into hRORs. Developing hRORs progressed through molecular, cellular, histopathologic, and morphometric stages that were nearly identical to the tumorigenesis and development of primary Rb. Subretinal engrafting of hRORs further validated its proliferative capacity similar to the Rb tumor cell line (Y79). We observed Rb initiation from cone precursors which are more sensitive to RB1 inactivation, and ARR3+ cells were primarily present in cultured hRORs. The PI3K/AKT pathway was found to be aberrantly regulated in hRORs, indicating a therapeutic target. We also demonstrated that hRORs are suitable for evaluation of drug effects in the treatment of Rb. We successfully developed a novel hROR in-dish model, and reported the development of hRORs for the study of human Rb tumor initiation, progression, and response to perturbation. Our developed hRORs will provide a valuable complement to the current basic and preclinical models for mechanism study and drug screening.

W-3058

HUMAN CEREBRAL ORGANOIDS ESTABLISHED USING URINARY EPITHELIAL CELLS ISOLATED FROM URINE

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The potential of organoids developed from human pluripotent stem cells (hPSCs) for basic science research and clinical applications have been increasingly noticed. We reason that cerebral organoids can be derived from cells sourced from human urine samples and present with cellular plasticity that could be leveraged to form either cerebral or non-cerebral neural tissue. The development of such organoids began with the collection of urinary epithelial cells (UECs) from urine samples of individuals with distinct ethnicity and ages. We have obtained multiple lines of human UEC-derived induced pluripotent stem cells (hUEC-iPSCs) by cell reprogramming. Cerebral organoids (COs) were generated from hUEC-iPSCs using a protocol optimized by our group. To comprehensively characterize the cellular and molecular features of our COs, we collected samples at different developmental time points for analysis. The hUEC-iPSC-developed COs exhibit normal development with neurogenesis and maturation of neuronal cells forming brain layers. Notably, these COs produce neurotropic and anti-inflammatory factors that are presumably critical for neurogenesis and repair of injured neural tissues. Several metalloproteases that may facilitate cell migration and microenvironment rearrangement are also present. After transplantation into the mouse cerebrum, vascularization develops quickly around and in the implanted COs, suggesting their viability and ability to interact with the environment. To gauge cellular plasticity of hUEC-iPSC-developed COs along their development, we subjected the COs that were developed using three different protocols to culture media containing FGF2 and FGF19 and harvested them at distinct time points. Multiple markers of the developing hindbrain were highly upregulated in COs initially committed to telencephalic development, indicating that they retain plasticity and could be reprogrammed into non-cerebral neural tissue upon optimized stimuli. Overall, our work begins to reveal the promise of generating personalized COs from cells that are isolated from urine samples. These COs present with cellular plasticity that permits the possibility to generate neural tissue of various brain regions by converting telencephalic organoids into a mesencephalic or rhombencephalic fate.

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TISSUE ENGINEERING

W-3060

HOW TO BUILD A SYNTHETIC TISSUE: ACTIVATION OF TRANSGENES FROM A MATERIAL SURFACE

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Since the earliest days of medical science scientists have dreamt of building replacement organs for patient specific tissue replacement. Current approaches to generate multicellular structures in vitro are not able to control spatial organization at the cellular scale. Synthetic biology has generated in recent years novel receptor-transgene mechanisms to turn on/off genes based on communication signals from engineered cell types. The advent of technologies, such as synthetic notch (synNotch), that tie specific cell inputs to user-defined cellular responses are providing novel modular molecular mechanisms for engineering and testing induction pathways. We expanded this technology by using synNotch to build a scaffold-cell communication platform. Now for the first time, we have developed a series of methods for spatially controlled activation of engineered transgenes using material-bound ligands. Here we demonstrate a novel ligand-driven communication network which bridges the gap between extracellular matrix materials and engineered cells. In our experiments, we have been able to demonstrate activation of both a reporter gene and a transdifferentiation-inducing master transcription factor (myoD) from ligands presented on a spatially patterned material surfaces, in both 2D and 3D models. Our technology allows for the activation of transgene cassettes in a subpopulation of cells within a greater population. This yields two distinct spatially defined populations of cell within a larger population. Starting with embryonic fibroblasts, we can generate patterned differentiation into multinucleated, alpha-actinin positive myotubes. We anticipate that when this technology will be used within more mature in vitro differentiation technologies like organoids and tissue engineering, it will contribute to generate tissue-constructs with enhanced cellular precision, paving the way for a new class of synthetic multicellular tissue to be used as developmental models, drug discovery tools, and patient-specific engineered simple tissue replacement.

W-3062

ENGINEERING OF MULTICELLULAR ORGANIZATION AND MORPHOGENESIS OF HUMAN PLURIPOTENT STEM CELLS USING AUTOMATED PREDICTION

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Embryonic morphogenesis is a critical determinant of tissue generation, yet many regulatory mechanisms remain elusive due to the complex nature of multicellular interactions, and the limited tools to manipulate these systems at single cell resolution. Similarly, morphogenesis of pluripotent stem cell derived organoids proceeds largely through self-organized pattern formation that crudely mimics organogenesis. Controlling specific morphogenic processes would greatly enhance our ability to create bona fide human tissue; however, robustly directing morphogenesis requires novel control approaches. The objective of this work was to develop an in vitro system to interrogate multicellular organization within human induced pluripotent stem cell (hiPSC) colonies. To achieve this, we combined CRISPR technologies with computational modeling, machine learning, and pattern optimization to control hiPSC self-organization. Since many morphogenic processes require changes in adhesion properties, we engineered hiPSCs expressing an inducible CRISPR interference system to silence regulators of cellular mechanics: Rho-associated coiled-coil containing kinase-1 (ROCK1) and E-cadherin (CDH1). Knockdown of ROCK1 or CDH1 in a sub-population of hiPSCs induced symmetry breaking (a pre-requisite of morphogenesis), resulting in cell sorting and multicellular organization in 2D and 3D. Combining a Cellular Potts-based computational model and a pattern recognition framework, we created an in silico system to predict specific experimental parameters to generate desired patterns, such as a Bullseye, where one centrally located cell population is surrounded by a second cell population. Executing the in silico derived experimental setup in vitro resulted in multicellular organization that remarkably reflected the in silico predictions in both frequency and extent of pattern formation. Furthermore, differentiation of patterned hiPSC colonies resulted in divergent cell fate commitment, indicating that directed multicellular organization impacts lineage co-emergence. These results demonstrate that we can predict morphogenic dynamics in silico to accurately manipulate hiPSCs in vitro producing desired morphogenetic events, a critical first step towards engineering human organoids and tissues.

W-3064

GENE AND STEM CELL THERAPY FOR LUNG INJURY: IN VIVO REPROGRAMMING OF ALVEOLAR EPITHELIAL CELLS

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Lung is a vital organ with highly complex architectural structure and contains a variety of cell populations. Lung diseases, such as acute respiratory distress syndrome (ARDS) or chronic obstructive pulmonary disease (COPD), are both major public health problems but currently without any effective pharmacologic approach for the treatment. Stem cell therapy based on transplantation of in vitro propagated stem/progenitor cells has been proposed as a potential solution to restore lung functions. However, due to the complexity of cell source and lung microenvironment, whether transplanted cells have differentiated for reconstitution of airway/alveolar epithelium were questioned. Furthermore, safety issues have raised concerning the use of stem cells in vivo. Our lab has focused on in vivo gene delivery of stemness genes in somatic lung epithelial cells using PEI nanoparticles for lung injury treatment. In mouse model of elastase-induced emphysema or bleomycin-induced fibrosis, we found that the transient gene delivery of a gene (indicated as COP-X1 here) in alveolar epithelial cells post-injury induced efficient regeneration of alveolar epithelium and improved pulmonary function. The regenerated regions showed normal alveolar epithelial phenotype and extracellular matrix components, without the symptoms of neoplasia. The COP-X1 target cells, including alveolar epithelial cells type 1 and 2, were sorted and cultured in vitro, which formed alveolar-like spheroids more efficiently than control cells. These cells were also analyzed for transcriptome and epigenetic profiles using NGS to verify the potential in vivo reprogramming mediated by the stemness gene delivery. In summary, our study suggests that in vivo delivery of stemness genes in somatic cells in pathologic loci could be a feasible approach for tissue regeneration. The target cells could transiently acquire the stemness property, which proliferate and differentiate for tissue regeneration more efficiently due to the native identity and microenvironment. In vivo gene delivery thus holds promise for the future treatment of lung diseases such as ARDS or COPD in clinic.

W-3066

3D BIOPRINTING TOOLS FOR ENGINEERING COMPLEX HUMAN NEURAL TISSUES FROM STEM CELLS

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Neurological drugs entering clinical trials fail over 90% of the time due to lack of efficacy or unforeseen toxicity. Better pre-clinical tools for predicting the effectiveness and toxicity of potential drug targets would significantly lower the chance of drug failure during clinical trials, reducing the cost of drug development and decreasing healthcare costs. Microfluidic 3D bioprinting technology from Aspect Biosystems provides a way to generate novel 3D multicellular neural tissue models from human induced pluripotent stem cells (hiPSCs). These tissue models can recapitulate the features of neurodegenerative diseases, serving as a convenient drug screening tool with increased physiological relevance. Here we demonstrate that the low-shear conditions of this unique microfluidic printing technology enable hiPSC-derived neural progenitors to be bioprinted into functional 3D neural tissues without triggering hiPSC death (~81% viability post printing) when used in combination with a novel, fibrin-based bioink developed by our team. Bioprinting also did not induce premature differentiation as evidenced by maintained expression of the pluripotency marker SSEA-4 in iPSCs post-printing. After printing, we treated these tissues with a set of small molecules to induce mature neuronal differentiation. We then validated that these tissues mimic the properties of native human brain tissue using flow cytometry and immunohistochemistry. These bioprinted neural tissues expressed the neuronal marker Beta-tubulin-III (45 ± 20.9%) after 15 days of culture and markers associated with spinal cord (SC) motor neurons (MNs), such as Olig2 (68.8 ± 6.9%), and HB9 (99.6 ± 0.4%) as indicated by flow cytometry. The bioprinted neural tissues also expressed the mature MN marker, ChaT, after 30 days of culture as indicated by immunocytochemistry. We have also printed 3D tissues with hiPSC-derived neural aggregates, which were bioprinted for the first time by our team, and human glioblastoma cells while maintaining high levels of viability (>90% post printing) not previously observed.

W-3068

THE THERAPEUTIC EFFECTS OF SPHERICAL HUMAN ADIPOSE-DERIVED STEM CELLS ON SKELETAL MUSCLE OF MDX MOUSE

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The Duchenne muscular dystrophy (DMD) is a recessive X-linked form of muscular dystrophy caused by a mutation in the dystrophin gene in male infants. For the treatment of DMD, stem cell therapy is one of the essential strategies for repairing degenerative muscles. Here, we prepared and transplanted human adipose-derived stem cell (hADSC) spheroid or single cells in hind limb muscle of mdx mice. The transplantation of hADSC spheroid in mdx mice was showed to recover the expression of dystrophin gene through the paracrine secretions at 4 weeks. After 4 weeks, we confirmed that hADSC spheroid group was significantly reduced the percentage of central nuclei and enhanced diameter of myofiber compared to transplantation of hADSC single cells. Also, hADSC spheroid group showed higher neovessel formation via capillary and arterioles staining. Furthermore, an expression of dystrophin, hADSC spheroid group was showed a significantly higher number of dystrophin-positive fibers compared to single cell treatment. We confirmed that human nucleus antigen was appeared to be merged with dystrophin-positive fibers. These findings suggest that hADSC spheroid promote the regeneration and survival of host muscle cells by paracrine secretion, thereby alleviating muscle degeneration in mdx mice.

Funding Source: This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. 2018M3A9E2023259)

W-3070

NEURAL STEM/PROGENITOR CELLS ON COLLAGEN WITH ANCHORED BASIC FIBROBLAST GROWTH FACTOR AS POTENTIAL NATURAL NERVE CONDUITS FOR FACIAL NERVE REGENERATION

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Introducing neural stem/progenitor cells (NS/PCs) for repairing facial nerve injuries could be an alternative strategy for nerve gap reconstruction. However, the lack of success associated with current methods of applying NS/PCs to neurological disease is due to poor engraftment following transplantation into the host tissue. In this work, we developed rat-tail collagen-based nerve conduits to repair lengthy facial nerve defects, promoting NS/PC

proliferation in the natural nerve conduits with anchored bFGF to improve the therapeutic effects of cell transplantation. In vitro studies showed that heparinized collagen prevented leakage of bFGF and NS/PCs expended in the rat-tail collagen gel with the anchored bFGF. The natural nerve conduits were implanted to connect 8-mm facial nerve defects in rats. The repair outcomes including vibrissae movements, electrophysiological tests, immunohistochemistry and remyelination analysis of regenerated nerve were evaluated. At 12 weeks after implantation, only natural nerve conduits treated group showed Hoechst labeled NS/PCs. Besides, the natural nerve conduit significantly promoted functional recovery and nerve growth, which was similar to those of the gold standard, an autograft. The animal experiment results suggesting that the natural nerve conduits were valuable for facial nerve reconstruction.

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W-3072

OLDER PERIODONTITIS MODEL MICE SHOWED SEVERER BONE DEFECTS AND SPARSER MESENCHYMAL STEM CELLS DISTRIBUTION WITH MORE DIFFUSE PAN T CELLS INFILTRATION

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Periodontal disease is one of the common causes of tooth loss, characterized by both inflammation and alveolar bone loss. The prevalence and its related tissue destruction increase along with aging. On the other hand, Mesenchymal stem cells (MSCs) play a role in the repair and the regeneration of damaged tissues and the control of inflammation. Aging-induced deterioration of MSCs functions might contribute to the pathophysiology of the various aging-associated disorders. Here, we examined the correlation in the number of the tissue specific MSCs and T lymphocytes with severity of bone destruction of periodontitis in young verses old mice. A ligature- induced periodontitis model was developed at mandibular 1st molar of young (5-week) and old (50-week) in C57BL/6J mice (N=3 each). Mice were sacrificed at 0, 3 and 10 day after ligation. In the micro CT image, bone defect area in the furcation area was measured by Image-J software. To check the distribution of MSCs and T lymphocytes, the PDGFRa and CD3 positive cells were counted respectively in the immunohistochemical staining image of the furcation area of the 1st molar. The bone defect area was significantly higher in the old mice compared to young mice ($p<0.01$, one-way ANOVA/ Turkey) at 10 day after ligation. In addition, the bone destruction could be observed even in 3 day after ligation in the old mice while the young mice showed no bone resorption. In the histological analysis, the old mice showed lower number of PDGFRa positive cells (112±21.13, 169±17) compared to the young mice (178±16.8, 257±80) at 3 day and 10 day after ligation respectively. The number of CD3 positive cells in the old mice (232.67±42, 466±54.4) was also increased when compared to the young mice (63±9.2, 122.67±23) on 3 day and 10 day after induction of periodontitis. In this study, we found that severe bone defect and fewer number of MSCs in the old age group. More importantly, greater number of T cells was infiltrated around furcation area in the old age periodontitis group compared to the young age group, indicating less number of MSCs might fail to inhibit T cell proliferation at the site of inflammation. These data suggested that there might be some correlation between tissue deterioration in periodontal disease and the decreased number of tissue specific MSCs associated with aging.

Funding Source: JSPS, Grant-in-Aid for Scientific Research (B)

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

W-3074

CELL-BASED MEAT: HOW A NEW GENERATION OF STEM CELL SCIENTISTS CAN SHAPE A CHANGING PUBLIC PERCEPTION OF STEM CELLS

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The utilization of animal stem cells to grow muscle and fat tissues in vitro for consumption, dubbed “cell-based meat,” offers an unprecedented opportunity to transform animal agriculture and produce meat in a humane and sustainable way. This technology now puts the public face-to-face with stem cells in new ways, casting society’s ingrained perceptions of stem cell ethics and legal frameworks in a new light together with external considerations such as food systems and climate change. Here, we discuss how recent decisions suggest that many of the legal frameworks used in the production of biologics and cellular therapies will be used by the FDA to regulate the cell-based meat industry and how we can best approach public education and outreach as it pertains to the use of stem cells and the technologies used to grow these cells. Lastly, we discuss how a new generation of stem cell scientists can have an impact in this fast-growing nascent industry.

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W-3076

HUMAN-ANIMAL NEUROLOGICAL CHIMERAS: "HUMANIZED" ANIMALS?

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In 2015, the National Institutes of Health (NIH) issued a notice regarding a moratorium on funding research projects involving injection of human stem cells into pre-implantation animal embryos, a method central to blastocyst complementation which can help mitigate the need for human tissues and organs in regenerative medicine. A major concern by the NIH was the contribution of human cells to the brain of animals thus creating "humanized" animals. We attempted to address this concern through an in-depth review of the neural transplantation literature to determine how the integration of human cells into the non-human neural circuitry has altered the behavior of the host. We analyzed the outcomes of 150 transplantation studies in 112 peer-reviewed publications where human cells were targeted to the mammalian CNS. From each study, we ascertained whether the transplanted human cells enhanced the cognitive/behavioral function of the host to levels above wild-type animals. Nearly all studies showed survival of the implanted human cells, and differentiation into various neuronal cell phenotypes. Some provided evidence of transplant-to-host nerve fiber innervation. Some studies demonstrated restoration of lost functions in animal models of neurological disorders. However, none of the 150 transplantation studies provided evidence to suggest humanization of the animal host. In summary, we found that concerns over humanization should not prevent research on blastocyst complementation to continue. We suggest proceeding in a controlled and transparent manner, however, and include recommendations for future research with careful consideration for how human cells may contribute to the animal host nervous system.

W-3078

SCOPE OF TRAINING OF PHYSICIANS OFFERING UNAPPROVED STEM CELL TREATMENTS

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In the US, over 700 clinics advertise unlicensed stem cell-based interventions (SCBIs) for a wide range of conditions. Various physical harms, high costs and misleading marketing practices have been reported. While many studies examine marketing claims of SCBI businesses, little is known about the providers at these clinics. To address this gap, we investigated the professional backgrounds of SCBI providers in 3 states with the greatest number of clinics. Using clinics previously reported, we included 91% (N=166) of the businesses and identified the backgrounds of 608 providers. Most clinics employed only 1 (40%) or 2-3 providers (27%). While the majority of providers listed were physicians (66%), others included PAs (9.0%), nurses (6%), podiatrists (5%), physical therapists (3%), chiropractors (3%), MS/PhD scientists (2%), and dentists (1%). Among physicians, most were men (92%) with a mean age of 55 years; the majority completed medical training in the US (81%). Most trained in orthopedic (31%), anesthesiology (16%), PM&R (11%), or family medicine (10%) residencies. The most frequent fellowships included orthopedics (28%), sports medicine (24%), and pain medicine (22%). We also examined whether businesses employed at least one physician with residency or fellowship training relevant to the clinic's marketed SCBIs. We found that half of the clinics (52%) had at least one physician practicing within his or her scope of training. Orthopedics-focused clinics were more likely to have at least one physician offering SCBIs within their scope of training compared to non-orthopedic focused clinics (77% vs. 19%, $p<0.0001$). Similarly, businesses with at least one fellowship-trained physician were more likely to offer treatments within their scope of training compared to those having none (63% vs. 32%, $p=0.0002$). When investigating possible regulatory non-compliance, state medical boards may wish to prioritize businesses advertising SCBI for non-orthopedic conditions given the higher prevalence of out-of-scope practice. However, practicing within one's scope of training is no guarantee of adherence to state and federal regulations. Regulators therefore need to consider scope of training as one of numerous factors when making decisions to allocate resources and initiate investigations.

Funding Source: Federation of State Medical Boards Foundation Grant

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

W-3082

FACTORS DETERMINING TARGET-SPECIFIC PROJECTIONS AND APPROPRIATE SYNAPTIC INPUTS OF HUMAN ESC-DERIVED DOPAMINERGIC GRAFTS IN A RAT MODEL OF PARKINSON´S DISEASE

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Human embryonic stem cell (hESC)-derived ventral midbrain-patterned progenitors grafted into the dopamine-depleted adult rat brain survive long-term, mature into dopamine neurons, integrate synaptically with host neurons, and extend dopaminergic axons to fill functionally-appropriate target structures and reverse motor deficits. In the clinical setting, midbrain-patterned cells are grafted heterotopically into the striatum, rather than homotopically into the substantia nigra. To determine the factors dictating the appropriateness of graft integration into the host basal ganglia, we have compared the axonal outgrowth from and synaptic inputs to midbrain- and forebrain-patterned cells placed either in the striatum or the substantia nigra of dopamine-depleted host rats. We found that graft-derived axonal outgrowth to dopamine target regions depended on midbrain patterning of the transplanted cells, whereas the anatomical location of host cells making monosynaptic contact with graft neurons depended on the location of the transplant. Moreover, there was a significant anatomical and phenotypic overlap with regions known to regulate the function of intact midbrain dopamine neurons. These results suggested that grafts placed in the clinical location – heterotopically in the striatum – may nevertheless receive input from the brain regions in the host that project to the endogenous midbrain dopamine neurons in the substantia nigra. Conventional retrograde tracing performed concurrent to graft-initiated rabies tracing confirmed that grafts placed in the striatum in fact received synaptic input from individual neurons that maintained simultaneous collateral projections to the substantia nigra. In summary, our data shows that functionally-appropriate subtypes of host neurons provide monosynaptic input to grafts, regardless of clinically-analogous heterotopic graft placement into the striatum.

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W-3084

HUMAN AMNIOTIC EPITHELIAL CELLS TRANSPLANTATION FOR PATIENTS OF PREMATURE OVARIAN INSUFFICIENCY

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Premature ovarian insufficiency (POI)/Premature ovarian failure (POF) affects about 1% of women below the age of 40. As for the vital role of healthy ovarian function for women, the consequences of POI consist of infertility, worse sexual performance, cardiovascular diseases, cognitive dysfunction and so on. So far, there is still no cure for POI. Herein, we presented the study of human amniotic epithelial cells(hAECs) transplantation therapy for POI patients which was approved by the ethics review committee of International Peace Maternity and Childhealth Hospital (IPMCH), which has registered at clinicaltrials.gov. (NCT02912104). Patients provided written informed consents. The clinical-grade hAECs was prepared and the phenotype of hAECs were characterized by flow cytometry. Then the patients underwent right femoral artery puncture with Seldinger technique under local anesthesia and a 4F sheath (Cook Medical) was inserted. 3×107 of hAECs in 10 ml NS (Normal Saline, NS) were separately and slowly injected along the microcatheter (Cook Medical) to the ovarian artery under fluoroscopy. The two cases follow-up hitherto did not exhibit any infectious complications or intolerance to cellular treatment. Then patients were followed up 14 months after treatment. We observed that the serum estrogen level elevated, FSH level deceased, however, LH level changed little, especially. The ultrasonographic examination revealed that the endometrial thickness increased, from 4-5mm to 10-15mm and follicle developing to 1.8-2 cm was found by ultrasonography at 5 and 11 months after hAECs transplantation. The women's subjective improvement was determined by using the menopausal KI. Total KI scores before and after treatment ranged differently in these two cases. Two month after transplantation, KI scores was significantly reduced by 60-70%. Further significant reductions in KI scores occurred over the next 12 months. The relief symptoms including hot flushes, insomnia, fatigue and vaginal dryness showed the most significant reduction. This present study showed hAECs transplantation via bilateral ovarian artery is safe and feasible. The primary efficacy of ameliorating perimenopausal syndrome suggesting hAECs is a promising cell source for stem cell therapy for POI patients.

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W-3086

MACROPHAGES-PRIMED ADIPOSE-DERIVED STROMAL CELLS DECREASE INFLAMMATION AND MULTIPLE ORGAN LESIONS TO IMPROVE SURVIVAL AFTER SEPSIS IN MICE

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Sepsis is a life-threatening illness and a public health issue due to its high frequency, its high annual cost and the increase of bacterial resistance to antibiotics. Sepsis results from an overwhelming inflammatory host response to infection that can lead to organ failure. Attenuation of the inflammation could be a new therapeutic approach. In the last few years, mesenchymal stromal cells (MSCs) and in particular adipose-derived mesenchymal stromal cells (ASCs) have been considered as a new therapeutic for sepsis. Indeed, ASCs modulate immune response and therefore could decrease general inflammation. Our recent study demonstrated that contact with pro- (M1-Mφ) or anti-inflammatory (M2-Mφ) macrophages modulate the immunosuppressive capacities of MSCs in vitro (Espagnolle et al, 2017). Therefore, the objective of this study was to determine the effect of different Mφ-primed ASCs in a model of sepsis induced by cecal ligation and puncture (CLP) in C57BL/6 mice.The intraperitoneal injection of Mφ-primed ASCs and especially M1-primed ASCs significantly increases the survival rate compared to unprimed-ASCs. Indeed, 70% of the M1-ASCs-treated mice survived 5 days post-CLP whereas 30% survived in the PBS-treated group. Moreover, injection of Mφ-primed ASCs decrease organ damage that is associated with the modification of recruited immune cells in the organs. Mφ-primed ASCs also modified the immune response in the peritoneal cavity, maintaining macrophage population in the cavity. Finally, M1-primed ASCs restore normal plasma level of lactate and decrease general inflammation. Altogether, our results suggest that Mφ-primed ASCs modulated modifies the immune response after sepsis and are more protective than the injection of unprimed ASCs. Therefore, Mφ-primed ASCs, associated to antibiotics, could improve the clinical outcomes in patients with sepsis.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

W-3088

MODELLING EARLY EMBRYOGENESIS WITH STEM CELLS

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At implantation, the mammalian embryo consists of the extra-embryonic trophectoderm (TE) and primitive endoderm (PE), progenitors of placenta and yolk sac; and pluripotent epiblast which generates the new organism. These extra-embryonic tissues are not only important to nourish the growing embryo, but they also provide cross-talk to pattern the embryonic epiblast, regulating early morphogenetic events to define the precise body plan. Establishing exactly how these cells interact to shape the embryo in vivo had been difficult, as the embryo becomes inaccessible when it implants into the uterus. For this reason, developing methods to recapitulate the principles of early development in vitro is gaining momentum, and recent advances using stem cells were able to faithfully model fragments of natural embryogenesis. We have generated a novel platform to mimic specific lineage organisation and subsequent morphogenesis of the pre-implantation embryo by utilising their in vitro stem cell analogues. We demonstrate that the generation of these structures occurs through self-assembly and self-organisation of 'naïve' state of embryonic (ESCs) and trophoblast (TSCs) stem cells from an initially homogenous population. We show that these embryo-like structures recapitulate cell fate decisions and early axis patterning events associated with early mouse embryo development. Further, these undertake similar morphogenesis events to give rise to post-implantation embryo-like structures akin to mouse egg-cylinder, which has 'primed' state of the embryonic and extra-embryonic tissues. This system can be used in developmental studies to investigate the dynamic interactions between embryonic and extra-embryonic tissues during early mammalian development, providing a complementary and relatively simple platform on which to dissect the physical and molecular processes that shape the embryo.

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W-3090

RECONSTRUCTION OF MAMMALIAN EMBRYOGENESIS: FROM SINGLE CELL SORTING THROUGH TO EARLY PATTERN FORMATION USING STEM CELLS IN VITRO

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Successful embryogenesis requires cross-talk between embryonic and extraembryonic tissues in order to regulate morphogenesis and establish the body plan. Dynamic patterning events between embryonic/extraembryonic tissues leading to postimplantation embryo formation in vivo are largely unresolved, as during implantation into uterus the embryo becomes inaccessible for direct observation. This difficulty has prompted efforts to model embryogenesis in vitro, using cell lines, which are more amenable to genetic manipulation. We developed a method to reproduce and study the complex biophysiology of the cells/tissues of early mammalian embryo by using various types of stem cells. In our 3D-model system, we combine single-cell suspensions of embryonic (ESC), trophoblast (TSC) and extraembryonic endoderm (XEN) stem cells to build embryo-like structures, having three discrete compartments resembling mouse postimplantation embryos, without provision of any external signal. Spatial transcriptomic analyses demonstrate that the establishment of signalling interactions between the embryonic/extraembryonic stem cells enables the robust specification of the molecular identity of anteroposterior axis. XEN cells regionalise first to develop specific cellular morphology and gene expression resembling visceral endoderm (VE) compartmentalization; then to initiate anterior VE formation which directs anterior development by repressing Nodal and Wnt3 signalling, as in the natural embryo. Further, BMP4 production from TSCs induces the establishment of a posterior identity. ESC-derived embryonic compartment transforms from being a single layer to multi-layered tissue by recapitulating postimplantation morphogenetic events including epithelial-mesenchymal-transition, mesoderm segmentation and definitive endoderm specification, processes defining early gastrulation. These results reveal the ability of three stem cell types to self-assemble and self-organise in vitro into structures similar to mouse postimplantation embryos in morphogenesis and spatial patterning of germ layers. This 3D-stem cell based model system offers a tremendous potential in advancing our knowledge of the dynamics of early embryogenesis that can provide new roadmaps for exploring human development and disease.

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W-3092

A PAX5-OCT4-PRDM1 DEVELOPMENTAL SWITCH SPECIFIES HUMAN PRIMORDIAL GERM CELLS FROM PLURIPOTENT STEM CELLS

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Human germ cells are unique and responsible for passing the DNA of one generation to the next. Dysregulation of genetic pathways during human germ cell development is a common cause of human infertility that afflicts 10-15% of couples. However, at least in part due to a lack of models of human germ cell development, most of the underlying cellular and molecular correlates of infertility remain unknown. Here, we analyzed bona fide human primordial germ cells (hPGCs) to probe the developmental genetics of human germ cell specification and differentiation. We examined the distribution of OCT4 occupancy in hPGCs relative to human embryonic stem cells (hESCs). We demonstrate that development, from pluripotent stem cells to germ cells, is driven by switching partners with OCT4 from SOX2 to PAX5 and PRDM1. Gain- and loss-of-function studies revealed that PAX5 encodes a critical regulator of hPGC development. Moreover, analysis of epistasis indicates that PAX5 acts upstream of OCT4 and PRDM1. The PAX5-OCT4-PRDM1 proteins form a core transcriptional network that activates germline and represses somatic programs during human germ cell differentiation. These findings illustrate the power of combined genome editing, cell differentiation and engraftment for probing human developmental genetics that has historically been difficult to study. Our work not only enables us to directly use genetic tools to identify and characterize the fundamental basis of human germ cell development, but also provides the foundation for establishing a robust human genetic system in vitro to model human germ cell development and develop novel therapies for human infertility.

W-3094

ANTAGONISTIC SIGNALS BETWEEN BMP4 AND FGFS SPATIO-TEMPORALLY DETERMINE MOUSE GERM CELL FATES

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Germ cells are the carrier of hereditary information and genetic variation in metazoans. The specialization of primordial germ cells(PGCs) is a crucial first step for germline development. Germ cell can be specified either by maternally inherited determinants (preformation) or by inductive signals (epigenesis). In mice, BMP signals produced in the extra-embryonic ectoderm are essential for Blimp1 positive PGC induction in the posterior-proximal epiblast at E6.25. However, the mechanisms how BMP4 saptio-temporally determine germ cell fate are unknown. Here, we performed temporal transcriptome analysis by RNA-Seq during in vitro PGCLC specialization. Our findings reveal that BMP4 and Wnt signals temporally repress FGFs and FGF receptors expression. Conditional knockdown FGFs or knockout FGFR1 increase Blimp1 expression and accelerate PGCLC induction. Furthermore, we combined conditional over-expression and CRISPR/Cas9 knockout methods to find key transcriptional factors directly regulate Blimp1 expression. This approach

identified a new transcriptional factor which is critical for PGC specialization both in vitro and in vivo. These results help to reveal the molecular mechanism how signaling niche ensure PGC specialization in the right space at the right time.

CHROMATIN AND EPIGENETICS

W-3096

GABRA2 GENETIC VARIANTS AND CHROMATIN CONFORMATION IN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL CELLS

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Approximately 8.5% of American adults are afflicted by either moderate or severe alcohol use disorder (AUD), a substance use disorder that places significant psychological, physiological, emotional, and financial burdens on patients and their families. Globally, AUD contributes 15 million disability-adjusted life years to the global estimate. Heritability of increased genetic susceptibility to developing AUD is estimated to be between 50-60%. The Collaborative Study on the Genetics of Alcoholism used linkage analysis to suggest, in European Americans, a significant association between alcohol dependence and a 140kb haplotype block in GABRA2. Synonymous SNP rs279858 tags this AUD-associated haplotype block, and has a minor allele frequency of 0.45. Neuro-endophenotypes including increased activation of the insular cortex and nucleus accumbens in reward anticipation and differential activation of the ventral tegmental area and medial frontal cortex in response to alcohol cues are associated with this haplotype block. The chr4p12 locus codes for γ1, α2, α4, and β1 GABA receptor subunits; patient-generated iPSC-derived neural cell lines carrying the minor allele at rs279858 have significantly reduced expression not only of GABRA2, but also GABRB1, approximately 800kb away from rs279858 in linear distance. The observation of this correlation between the genotype of the tag-SNP marking the AUD-associated haplotype block in GABRA2 with expression of GABRB1 in iPSC-derived neural cells suggests a putative long-range interaction between these two genes. Virtual circular chromatin conformation capture (4C-seq) data supports this hypothesis of cis regulation of GABA gene expression. Additionally, the assay for transposase-accessible chromatin (ATAC-seq) is being used to identify allele-dependent regions of open chromatin. Identification and functional characterization of genetic variants in GABRA2 associated with increased susceptibility to developing AUD may aid in: 1) counseling young adult heavy drinkers with respect to their risk of progression to development of a full-fledged AUD; and 2) creating tailored intervention strategies for those who have already developed an AUD.

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W-3098

PHASE SEPARATION IS REQUIRED FOR XIST LOCALIZATION AND GENE SILENCING IN X-INACTIVATION

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X-chromosome inactivation (XCI) is an essential silencing process in female placental mammals. A prevalent view is that XCI is mediated by Xist RNA and occurs through the ability of Xist to act as a modular scaffold for a diverse set of proteins, to spread over X-linked genes, and to induce gene silencing. Current models do not account for how ~1000 genes on the X-chromosome can be silenced by many fewer (~10-fold less) Xist molecules. Here we identify a new mechanism underlying XCI - the Xist-dependent formation of a multivalent higher-order protein-protein assembly essential for XCI. We show that a repetitive 1.4kb region of Xist, known as the E-repeat, co-operatively binds many molecules of PTBP1, a well-known regulator of alternative splicing, in vivo and in vitro and that the E-repeat RNA strongly promotes liquid-liquid demixing of PTBP1 in vitro. In differentiating female mouse embryonic stem cells (ESCs), we find that Xist transcripts lacking the E-repeat initially coat the future Xi but over time, disperse across the nucleus. This time-dependent localization defect is accompanied by the loss of gene silencing, chromatin compaction and H3K27me3 enrichment. The recruitment of multiple PTBP1 molecules to Xist lacking the E-repeat averts these phenotypes and enables

faithful XCI. We find that PTBP1 interacts with MATR3, TDP-43 and CELF1, which also bind the E-repeat directly. Together, these proteins engage in protein-protein and protein-RNA interactions that are required for XCI and for the rescue of the E-repeat deletion phenotype. Conversely, mutations within MATR3 and TDP-43 known to interfere with their assembly into phase separated compartments do not promote rescue. We conclude that Xist, through its highly repetitive E-repeat sequence, seeds a multivalent protein-protein network to maintain both Xist localization and gene silencing. We propose that this higher-order network, likely formed via phase separation, super-stoichiometrically enriches all Xist interactors, including silencing proteins, and explains how a limited number of Xist molecules can silence hundreds of genes along the X-chromosome. Our results suggest a critical role for phase separation in lncRNA-mediated regulation of gene expression and an alternative role for RNA processing factors in mediating heterochromatin formation.

W-3100

GENOME-WIDE DNA METHYLATION IN MOUSE EMBRYONIC STEM CELLS USING MRR-LIKE ENZYME FSPE1

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Mrr-like enzymes are modification-dependent restriction endonucleases like MspJ1, FspE1, LpnPI, R1aI, AspBHI,SgrTI etc. We used FspE1 for our studies. FspE1 recognizes 5-methylcytosine (5-mC) and 5-hydroxymethycytosine (5-hmC) in CmC and mCDS sites (in presence of activator; D=A or G or T; S=C or G). It introduces double-stranded nicks at fixed distances (N12/N16 from mC) at the 3' end of methylated cytosine creating four base 5' overhangs. For symmetrically methylated target sites, FspE1 can cut in a bidirectional manner generating 32 bp fragments with mC in the center. This enzymatic trait allows for screening of approximately all restriction sites in the genome in parallel without the drawbacks of sequenceable fragment size (generally less than 500 bp). In this study, we assessed genome-wide DNA methylation in mouse embryonic stem cells (mESCS) that have been treated with Lsd1 inhibitor, pargyline or tranylcypromine (TCP). Lsd1 is a histone demethylase which demethylates lysine 4 on the histone H3 protein (H3K4) in pluripotency gene (PpG) enhancers and promoters promoting targeting of DNA methyltransferase Dnmt3a especially to the PpG enhancers thereby silencing them during differentiation. Using Mrr-like enzymes, through methylation dependent PCR studies we have shown reduced DNA methylation at PpG enhancers after mESC differentiation indicating the significant role of Lsd1 in regulation of DNA methylation and consequently gene repression in ESCs. We combined the specific restriction by FspEI with high throughput genome sequencing to study the effects of Lsd1 inhibitor on DNA methylation genome wide during ESC differentiation. Our data show that blocking of Lsd1 activity inhibits gain of DNA methylation by 80% at Lsd1 bound enhancers. These studies become important since maintenance of proper DNA methylation patterns is essential for cellular identity, failure of which can lead to aberrant gene activation which can lead to tumorigenesis and developmental anomalies.

W-3102

REVEALING THE PATHOPHYSIOLOSY OF NEUROCRISTOPATHIES USING PATIENTS DERIVED IPSC

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Some multiple congenital anomaly disorders are related to the abnormality of neural crest development. ATRX is known to have characteristic face and is suggested to be related to the defects of neural crest development. The causative gene is ATRX. This gene also related to regulate DNA methylation. It remains unclear whether neural crest cells are actually dysfunctional in ATRX syndrome patients. To better delineate neural crest defects in ATRX, we generated induced pluripotent stem cells (iPSCs) from ATRX patients with truncating mutations in ATRX and typical syndrome manifestations, and characterized neural crest cells differentiated in vitro from these iPSCs (iPSCNCCs). Using patient-iPSCs derived NCCs, we found defective migration in neural crest from ATRX patients. These results support the historical inference that ATRX syndrome patients exhibit defects in neural crest .

PLURIPOTENCY

W-3104

ROUTINE MONITORING OF COMMON GENETIC ABNORMALITIES IN HUMAN PLURIPOTENT STEM CELLS USING THE HPSC GENETIC ANALYSIS KIT

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Cell culture acquired chromosomal aberrations have been widely reported in human pluripotent stem cells (hPSCs). These cytogenetic changes comprising numerical aneuploidies, chromosomal rearrangements and sub-microscopic changes can affect hPSC growth rates, cell survival and differentiation potential. Karyotypic abnormalities that arise in vivo during embryonic development account for the majority of non-viable embryos, an observation that highlights the importance of understanding genetic stability and its impact on hPSC culture quality and applications. With a renewed focus on hPSC quality, and concerns over data reproducibility due to poor monitoring practices for cell quality, detection methods for genetic variants that arise in hPSC cultures are urgently required. The hPSC Genetic Analysis Kit is a qPCR-based method designed to rapidly detect the most common genetic abnormalities observed in hPSC cultures. Specifically, primer-probe assays were optimized to amplify minimal critical regions on chromosomes 1q, 8q, 10p, 12p, 17q 18q, 20q and Xp, as well as a control region on chromosome 4p. These regions represent approximately 70% of all reported abnormalities in hPSC cultures. Amplification efficiencies for all primer-probe sets were measured at ≥ 90% (n = 2). Abnormalities were detected in 4 different hPSC lines each containing a 1q duplication, 10p deletion, 12 trisomy or 20q duplication ($p < 0.001$), with no other genetic abnormalities detected in other regions ($p > 0.1$). Duplication of 20q11.21 is a submicroscopic abnormality often missed when using G-band karyotyping. The hPSC Genetic Analysis Kit was able to detect the 20q11.21 duplication in three hPSC cultures reported as karyotypically normal using conventional G-band karyotyping. This duplication was confirmed by fluorescence in situ hybridization. To determine assay sensitivity, fluorescently-labelled hPSCs known to be abnormal for 10p, 12p and 20q were mixed with unlabelled diploid hPSC at varying ratios. Results indicate that our qPCR-based approach was able to detect genetically abnormal hPSCs when present at a minimal frequency of 30% (n = 3; $p < 0.05$). In summary, the hPSC Genetic Analysis Kit offers researchers a reliable, fast and cost-effective tool to routinely monitor and screen hPSCs for recurrent genetic abnormalities.

W-3106

TRANSCRIPTIONAL NETWORK VARIATIONS DURING CELL CYCLE PROGRESSION IN HUMAN EMBRYONIC STEM CELLS

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Chromatin organisation and transcriptional networks are tightly controlled during cell cycle progression in stem cell in order to maintain their identity. The cell cycle machinery has been shown to directly control maintenance of pluripotency and initiation of differentiation. More precisely, the activity of Activin/Nodal signalling in human Embryonic Stem Cells (hESCs) is cell cycle regulated and it affects differentiation propensity via the dynamic binding of its main effector SMAD2/3. However, the impact of these mechanisms on the broader transcriptional networks characterising hESCs remain to be fully uncovered. Here, we utilise the FUCCI reporter system to show that chromatin accessibility, gene expression and binding of key transcription factors change during cell cycle progression in hESCs. We show that the most dynamic phase of the cell cycle is the Early G1, and this correlates with an increase in CTCF binding. In the Late G1 phase, CTCF binding decreases and core pluripotency factors (OCT4, SOX2, NANOG) being to occupy a large number of genomic regions. This corresponds to transcriptional leakiness for key developmental regulators while the full pluripotency network is re-established only in the S/G2/M phase. Importantly, this binding pattern is required to induce differentiation in G1 and to preserve pluripotency in the S/G2/M phase. Our results demonstrate for the first time that key transcription factors change genomic location during cell cycle progression and that this dynamic binding pattern is required for induction of differentiation. Ultimately, this highlights the importance of studying transcriptional and epigenetic regulation in the context of the cell cycle, and this novel concept could apply to any proliferating cell type and adult tissues.

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W-3108

FRUCTOLYSIS REGULATES SELF-RENEWAL EXPANSION AND ALPHA 2, 6-SIALYLATION OF PLURIPOTENT STEM CELLS

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Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can be grown in cultures maintaining their pluripotency and possessing the unlimited self-renewal capability. Recent studies imply pluripotent stem cells (PSCs) possess a unique metabolic features and require specific metabolites in maintaining their cell fate. We therefore intend to decipher the specific metabolites required for maintaining self-renewal expansion of ESCs/iPSCs. Utilizing RNA-Seq analysis, we showed that, in comparing with fibroblast, mouse and human ESCs/iPSCs were found to retain

the expression of critical enzymes involved in Krebs cycle. We then determined if the specific bioenergetics adaptation in cultures can affect pluripotency acquirement or maintenance. Oct4-GFP(+) iPSCs derived mouse fibroblast were seeded in medium containing either 4500mM Glucose (high glucose) with or without Oligomycin (mitochondrial ATPase inhibitor), 500mM Glucose (low glucose) and 0mM Glucose (no glucose) with or without pyruvate (to be utilized in TCA cycles). We found that depletion of glucose resulted in reduced growth of Oct4-GFP(+) iPSC colonies. In contrast, addition of pyruvate could rescue the growth of Oct4-GFP(+) iPSC colonies suggesting the retaining mitochondrial respiration may aid self-renewal expansion of pluripotent stem cells. The further analysis revealed that mouse and human ESCs/iPSCs had evaluated level of GLUT5 and KHK suggesting pluripotent stem cells can utilize fructose efficiently. We further validated that fructose substitution was sufficient to maintain the self-renewal growth of ESCs/iPSCs. Importantly, fructose substitution was found to enhance generation of iPSCs from Yamanaka factor mediated mouse fibroblast reprogramming possibly via selectively suppressing the growth of partial reprogrammed cells and upregulating alpha 2,6-sialylation as sialyltransferase ST6Gal1 is known to play an crucial role in regulating pluripotency. In summary, the current work elucidated that the specific bioenergetics adaptation in cultures linking to pluripotency maintenance and identified ESCs/iPSCs can utilize fructose efficiently which can therefore be used for maintaining the pluripotent cell fate.

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W-3110

GLOBAL HYPERACTIVATION OF ENHANCERS STABILIZES HUMAN AND MOUSE NAÏVE PLURIPOTENCY

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Pluripotent stem cells (PSCs) can transition between cell states in vitro, closely reflecting developmental changes in the early embryo. PSCs can be stabilized in their naïve state by blocking extracellular differentiation stimuli, particularly FGF5 MEK signaling. Here, we report that multiple features of the naïve state in human and mouse PSCs can be recapitulated without affecting FGF-MEK-signaling. Mechanistically, chemical inhibition of CDK8 and CDK19 kinases (CDK8/19i) removes their ability to repress the Mediator complex at enhancers. Thus CDK8/19i increases Mediator-driven recruitment of RNA Pol II to promoters and enhancers. This efficiently stabilizes the naïve transcriptional program, and confers resistance to enhancer perturbation by BRD4 inhibition. Moreover, naïve pluripotency during embryonic development coincides with reduction in CDK8/19. We conclude that global hyperactivation of enhancers drives naïve pluripotency, and this can be captured in-vitro by inhibiting extracellular FGF-MEK-signaling, or downstream, by CDK8/19i. These principles may apply to other contexts of cellular plasticity.

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W-3112

GENETIC BACKGROUND IMPACTS ON VARIABILITY OF GROUND STATE PLURIPOTENT STEM CELL LINES

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Variability between pluripotent stem cell lines is a prevailing issue that hampers not only experimental reproducibility but also large-scale applications and personalised cell-based therapy. Despite its importance, the mechanisms underlying this variability remain to be uncovered as both epigenetic and genetic factors could influence stem cell behaviour. Here, we address this question using mouse embryonic stem cell lines derived from distinct genetic backgrounds and grown in ground state conditions thought to universally reset the epigenetic state. We observed that these naïve cells display different capacities of differentiation confirming a major role of genetics in phenotypic variability. Furthermore, differences in Wnt signalling activity appear to be partially responsible while expression of Wnt pathway components are associated with specific genetic

variants. In conclusion, this study demonstrates that dampening the epigenome of pluripotent stem cells is not sufficient to erase their variability. This information is essential to deliver their clinical promises.

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W-3114

MUCIN-TYPE O-GLYCOSYLATION REGULATES PLURIPOTENCY IN MOUSE EMBRYONIC STEM CELL

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Embryonic stem cells are a powerful tool for both therapeutic applications and developmental biology research. In the past decades, mouse embryonic stem cells (ESCs) have been intensively studied to unravel the complex mechanisms underlying pluripotency. Nonetheless, glycosylation role within the pluripotency network has been overlooked. To address this, we performed a preliminary evaluation by screening approximately 100 glycosyltransferases using gene silencing, followed by the alkaline phosphatase activity assay as an indicator of the pluripotent state. As a result, the mucin-type O-glycosylation pathway was identified as a putative candidate. Mucin-type O-glycosylation is characterized by the initial addition of N-acetylgalactosamine (GalNAc) to serine or threonine residues and, together with N-glycosylation, is the most abundant form of glycosylation present on membrane-anchored and secreted proteins. Previously, our and other groups reported that mucin-type O-glycosylation is crucial during development. However, the function of mucin-type O-glycosylation and its relation with signaling in ESCs still remains undefined. In the present study, we identified T antigen as the most abundant mucin-type O-glycosylated structure in ESCs. Manipulation in the expression of C1GalT1, the enzyme involved in T antigen formation, resulted in the loss of ESCs' pluripotency. Lastly, we observed that ESCs' pluripotency loss was mediated by Wnt signaling, and characterized the mucin-type O-glycosylated components involved, unveiling a novel Wnt signaling regulatory mechanism. Here, we observed for the first time that mucin-type O-glycosylation regulates ESCs' pluripotent state modulating Wnt signaling. Our findings advance understanding of ESCs' pluripotency regulation accelerating the exploitation of embryonic stem cells in both regenerative medicine and developmental biology fields.

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PLURIPOTENT STEM CELL DIFFERENTIATION

W-3120

RECIPROCAL REGULATION BETWEEN ENDOTHELIAL AND PERICYTE-LIKE SMOOTH MUSCLE CELLS ENHANCES VASCULAR REPAVING OF ACELLULAR LUNG SCAFFOLDS

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Immune compatible bioartificial lungs can be created from recellularization of lung scaffolds. However, inadequate revascularization of these grafts is the main reason for poor in vivo function and survival. We posit that co-delivery of human iPSC-derived endothelial cells (iECs) and smooth muscle cells (iSMCs), will increase efficiency and robustness of revascularization due to synergistic cell-cell interactions. Presence of a-SMA and Calponin by immunostaining confirmed successful differentiation of iSMCs. The optimal SMC:EC ratio for scaffold seeding was determined by co-culture of iSMCs, or pulmonary artery SMCs (PASMCs), with human umbilical vein ECs in a Matrigel assay. While EC networks in monoculture collapsed after 12h, co-culture with either iSMCs, and to a lesser extent with PASMCs, improved EC-network persistence (e72h vs. d36h respectively), with an optimal EC:SMC ratio of 1:3. Immunofluorescence imaging revealed close pericyte-like apposition of iSMCs, and less so PASMCs, along the entire extent of EC networks, consistent with flow cytometry analysis showing higher pericyte marker expression (CD146, PDGFRb) in iSMC compared to PASMC (>95% vs. <15%, respectively). Following 24-72h of co-culture with iSMCs, ECs were isolated by CD144+ immunomagnetic-separation and Q-PCR performed. Compared to PASMCs, co-culture with iSMCs produced a more dramatic increase in Wnt5a, endothelial (eNOS, KLF2, TIE2) and pro-angiogenic (VEGF, ANGPT1) gene expressions in ECs. Fzd7 and Cdc42 expression also increased in co-cultured iSMCs, consistent with reports that the Wnt/Planar Cell Polarity (PcP) pathway mediates pericyte-EC interactions. Similarly, iSMC stabilized networks produced by CD34+CD31+CD144 iECs and increased their EC gene expression. Finally, co-delivery of iEC and iSMC (1:3 ratio) into acellular rat lung scaffolds led to more complete vascular coverage than iECs alone (n=4/group), as observed by H&E, and E-Cad immunostaining, together with greater proliferation of engrafted iECs (Ki67). Pericyte-like iSMCs interact with ECs to stabilize vascular networks and promote revascularization of lung scaffolds. Our data suggest that in addition to structural support, iSMCs mediate these effects by reciprocal Wnt/PcP pathway signaling and upregulation of pro-angiogenic EC genes.

W-3122

ANSWER ALS IPSC AND MOTOR NEURON REPOSITORY AT THE CEDARS-SINAI RMI IPSC CORE

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The Answer ALS project entails creating 1,000 induced pluripotent stem cell (iPSC) lines from amyotrophic lateral sclerosis (ALS) patients and controls. Combined with clinical data, these iPSCs can be used to model ALS in large scale data and omics investigations, identifying possible mechanisms underlying this fatal neurodegenerative disease. The iPSC Core at Cedars-Sinai Medical Center generates these iPSCs from samples collected from clinical sites across the United States. To date, the iPSC Core has collected over 900 unique peripheral blood mononuclear cell (PBMC) samples comprising 103 control and 833 ALS patients (40 of which harbor C9ORF72 mutations and 13 of which contain SOD1 mutations). We have reprogrammed over 469 of these samples into iPSCs using non-integrating episomal plasmids, and have verified pluripotency, quality, and genetic integrity via extensive testing and optimization. After all Answer ALS samples have been reprogrammed, the iPSC Core will have one of the largest biorepositories of ALS iPSC lines in the world, which, along with de-identified clinical data, will be available to researchers worldwide. Importantly, ANSWER ALS seeks to determine the extent to which motor neurons (MNs) differ between ALS and healthy patients. The iPSC Core has extensive experience in the differentiation of MNs from iPSCs, having previously developed MN differentiation protocols in support of the NIH-funded NeuroLINCS project. Presently, we have successfully differentiated over 162 ANSWER iPSC lines into MNs. Briefly, MN differentiation occurs over 18 or 32 days, beginning with dual SMAD inhibition of iPSCs to initiate neuroectodermal development and ending with the addition of neurotrophic factors, such as BDNF and GDNF, to enrich cultures for MNs. MNs are then collected, assayed for neuronal markers, and distributed to collaborating sites for characterization by RNA-seq, ATAC-seq, and proteomic analysis. The generation of ANSWER ALS MNs represents a novel and valuable resource to the study of human neurodegenerative disease. Indeed, when combined with the rich clinical data sets also collected as part of ANSWER, characterization of the ANSWER MNs will aid basic research into the cellular mechanisms driving ALS, as well as advance translational research into future therapeutic approaches.

W-3124

GAINS OF CHROMOSOME 12P13.31 RESULTS IN FAILURE TO EXIT PLURIPOTENCY AND REDUCED DIFFERENTIATION CAPACITY TO HEPATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Differentiated derivatives of hPSC hold great promise in regenerative medicine. However, a small subpopulation of cells, known as residual undifferentiated stem cells (rSC), frequently fail to differentiate and can jeopardize the safety of clinical applications through the formation of teratoma tumors. Determining the cause of rSC formation could help improve the safety of hPSC in the future. In this study we identified through a colony formation assay and subsequent re-differentiation a subline of VUB14, VUB14_rSC2, that failed to robustly undergo hepatic differentiation (HD) and retained a high proportion of OCT4+ cells after 8+ days of differentiation. aCGH of these cells revealed an 11.76Mb gain at 12p13.33p31. Two additional lines, ERB5 and VUB19_DM1, with similar gains on chromosome 12 demonstrated the same phenotype. Further analysis shows no deficiency in ectoderm differentiation or spontaneous differentiation, suggesting that the loss of differentiation capacity is specific to endoderm. Localized within the minimal region of gain available to us (2.5Mb at 12p13.31) are the pluripotency genes NANOG and GDF3, which are lowly and significantly upregulated, respectively. Knockdown of GDF3, a BMP4 inhibitor, results in a significant reduction of rSCs and improved HD differentiation. Concordantly, mutant lines have demonstrably fewer of pSMAD1+ cells early in differentiation, and addition of BMP4 at the onset of differentiation leads to a similar outcome as siGDF3.To our knowledge, this is this first description of a specific mutation and driver leading to the rSC phenotype. Further work is needed to fully elucidate the mechanism driving the work described here.

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W-3126

ISOLATION OF HEART FIELD SPECIFIC CARDIOMYOCYTES FROM DIFFERENTIATING HUMAN EMBRYONIC STEM CELLS, THE FIRST STEP TOWARDS SAFE REGENERATIVE THERAPY

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Cardiovascular disease (CVD) is the leading cause of death worldwide. Human embryonic stem cell (hESC)-derived cardiovascular progenitors (CVPs) or cardiomyocytes (CMs) represent a promising candidate for cell-based therapies to treat CVD. Myocardial infarction leads to extensive CM death mainly within the left ventricle, which is predominantly derived from the first heart field (FHF) during embryonic development. We postulate that the generation of chamber-specific CMs may play a key role in the development of safe and efficacious regenerative therapy. As a first step, we generated and characterized a FHF-specific TBX5-TdTomato+/W hESC reporter line. We show that TBX5+ cells represent an enriched population of FHF CVPs that can give rise to CMs, endothelial, and smooth muscle cells in vitro. Interestingly, we observed that TBX5- cells can also generate contractile CMs. Bulk RNA-sequencing analysis at different stages of development suggested that TBX5- cells are enriched for second heart field (SHF) CMs. To enable prospective isolation of FHF and SHF CMs, we generated a double transgenic TBX5-TdTomato+/W/NKX2-5eGFP/W hESC reporter line. We performed detailed electrophysiological, functional, and transcriptional studies to characterize the heart-field specificity of these hESC-derived CMs. Electrophysiological studies revealed that, despite the presence of atrial and ventricular action potentials (APs) in both FHF and SHF, there are significant differences in their AP duration and cycle length. In addition, both FHF and SHF CMs responded appropriately to adrenergic stimuli. Single-cell RNA sequencing analysis confirmed the absence of nodal genes within these populations and provided evidence of unique molecular signatures for isolating FHF- and SHF-like CMs. Finally, we identified CORIN as a novel cell surface marker for FHF CMs. Our studies provide a platform for investigating in vitro cardiovascular development, drug screening, and may facilitate a safe approach for cell therapy in heart disease.

W-3128

PRONEURAL FACTORS ASCL1 AND NEUROG2 CONTRIBUTE TO NEURONAL SUBTYPE IDENTITIES BY ESTABLISHING DISTINCT CHROMATIN LANDSCAPES

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Developmental programs that generate the astonishing neuronal diversity of the nervous system are not completely understood and thus present a significant challenge for clinical applications of guided cell differentiation strategies. We investigated the molecular mechanisms governing the divergent roles played by the proneural factors Ascl1 and Neurog2 during neuronal differentiation. Using direct neuronal programming of isogenic mouse embryonic stem cells, we found that Ascl1 and Neurog2 generate neurons by binding to largely different sets of genomic sites when expressed in similar chromatin and cellular contexts. Their divergent binding patterns are not determined by the previous chromatin state but are mediated by their DNA-binding domain's preference towards distinct E-box sequences. The initial divergent binding of Ascl1 and Neurog2 results in distinct regulatory landscapes that affect the binding pattern and the regulatory activity of shared downstream transcription factors in establishing shared (generic) and neuron-specific (subtype-specific) expression profiles. We speculate that the intrinsic differences in Ascl1- and Neurog2-induced neurogenesis increase the number of possible neuronal types generated during development by differentially altering the chromatin landscapes upon which the broadly expressed downstream TFs operate. Thus, in addition to the differentially expressed transcription factors and/or terminal selectors, the role of broadly expressed

transcription factors should also be considered in determining the aspects of neuronal subtype identity. This study provides a mechanistic understanding of how transcription factors constrain terminal cell fates, and it delineates the importance of choosing the right proneural factor in neuronal reprogramming strategies.

W-3130

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TOWARDS A NEURONAL TEST MODEL TO STUDY THE EFFECTS OF EXPOSURE TO VARIOUS WARFARE AGENTS

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The need for a human neuro-muscular test model to find new potential nerve agent antidotes is highlighted by the increasing global political uncertainty, such as the use of nerve agents especially in Syria in recent years. By the generation and differentiation of human induced pluripotent stem cells (hiPSCs) into neurons, in particular motoneurons, a high throughput screening is made possible. Thus avoiding the use of ethically highly discussed embryonic stem cells. For the generation of hiPSCs, the pluripotency-inducing Yamanaka factors (Oct3/4, Sox2, c-Myc, Klf4) were inserted into human fibroblasts by plasmid nucleotransfection. The cultivation of hiPSCs using complex culture media and their subsequent differentiation into neurons was determined by many success-determining parameters such as handling during cultivation and media additives that still need to be investigated. Since the efficiency of hiPSC-differentiation approaches to motoneurons varies greatly a naïve hiPSC-Line was taken along for comparison. Continuous morphological documentation, identification and characterization by means of immunohistochemical staining, PCR and flow cytometry and functionality testing via Multi-Electrode Array System are groundbreaking. The neuronal precursor structures showed a positive staining for PAX6 and Nestin. In the further course of cultivation Synapsin- and β-Tubulin-positive neuronal networks were formed. These cultures were subsequently grown on Polyornithine-Laminine-coated MEA-glass carriers for the measurement of spontaneous and induced excitatory postsynaptic potentials and inhibitory postsynaptic potentials. Using the Nanion Patchliner, a fully automated patch-clamp technique, further characterization on sodium, potassium and calcium channels was performed. First viability assays of Synpasin/β-Tubulin-positive neurons are currently conducted with VX concentration series by flow cytometry. The sensitive neuronal cultures show that further adaptation processes are necessary due to the time-consuming, three-month cultivation period. This human test model will provide new insights into the pathomechanism of nerve agents at the molecular level and might help to identify new therapeutic approaches.

W-3132

USING SINGLE-CELL ANALYSIS OF HUMAN IPSCS AND NEURAL LINEAGE ENTRY TO DISCOVER NOVEL MARKER GENES THROUGH A CONSENSUS PSEUDOTIME TRAJECTORY

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Single-cell RNA sequencing (scRNA-Seq) combined with pseudotime trajectory inference can illuminate the cell differentiation process of pluripotent stem cells. Currently, competing methods exist for constructing pseudotime trajectories based on expected topology (i.e. linear, cyclic, branching) and prior parameters (i.e. start cell, number of expected clusters). The researcher is left to judge which approach to select and no strategy exists that incorporates a statistical confidence measure in the predicted cell pseudotimes. Here, we performed controlled neural induction of human induced pluripotent stem cells (iPSCs) using dual-SMAD inhibition over six different timepoints (day 0-7) and carried out scRNA-Seq using the ddSeq platform. We selected the top four trajectory methods as ranked by the Dyno R package, which has benchmarked over fifty methods for accuracy against a gold standard, scaling, and quality control. The benchmark scores and the bootstrapped standard deviation of the mean cell-cell geodesic distances were used to weight cell pseudotimes. A consensus pseudotime was created from machine learning predictions and applied as a continuous phenotype in The Broad Institute's Gene Set Enrichment Analysis package to discover enriched genes in canonical KEGG, REACTOME pathways. Variance in each cell's prediction was estimated by stochastic bootstrapping of the machine learning model. Enriched gene sets were contrasted with those generated from the standalone Monocle 2 DDRTree pseudotime phenotype. The consensus outperformed this popular method in correlation strength of dynamic gene expression over pseudotime. Top endpoint genes along the pseudotime-ordered pluripotent vs. neuroectoderm states included PAX6, PRTG, TPBG, and other genes while preceding turning point genes at days 2-3 included TMSB4X and transient HESX1. The study shows that trajectory method

choice impacts resulting marker genes, which will be critical to measure as scRNA-Seq use may supersede bulk RNA-Seq. In summary, an aggregation framework based on comprehensive benchmarking may prove superior to single method choice as demonstrated here by identifying precisely regulated marker genes of human neural lineage commitment.

W-3134

AN IN-DEPTH ANALYSIS OF DISRUPTED MOLECULAR PATHWAYS IN PITT-HOPKINS SYNDROME PATIENT IPSC DERIVED NEURAL PROGENITOR CELLS

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Pitt-Hopkins Syndrome (PTHS) is a rare and relatively understudied autism spectrum disorder that is caused by an autosomal dominant mutation or deletion in the gene transcription factor 4 (TCF4). TCF4 is a basic helix-loop-helix (bHLH) transcription factor that plays a critical role in neuronal development through known interactions with other proneural bHLH proteins. Through these bHLH protein interactions, human TCF4 expression peaks during corticogenesis and patients with TCF4 mutations have profound developmental delays and autistic behaviors. To study the role of TCF4 in human neuronal development, we have developed a platform to differentiate PTHS patient and control induced pluripotent stem cells into human neural progenitor cells (NPCs). We have reprogrammed iPSCs and differentiated NPCs from 6 control patients and from 4 PTHS patients with a point mutation in the bHLH domain of TCF4 and 2 PTHS patients with large truncations of TCF4. Over the course of 20 days of differentiation, we have identified over 1,400 differentially expressed genes (DEGs) between PTHS and controls NPCs and validated some of these results with immunohistochemistry and Fluidigm qPCR. Gene ontology (GO) enrichment analysis of these DEGs has identified a upregulation in genes associated with axonal development along with additional down-regulation of genes associated with protein trafficking and ribosome function in PTHS NPCs. We have performed metabolomic analysis of PTHS and control NPCs to further assess the molecular pathways identified by our GO analysis. In addition, ChIP-seq analysis was performed to identify DEGs that may be directly regulated by TCF4. With this stem cell-based platform, we aim to better understand the role of TCF4 in cortical development in a human context, and to identify disrupted molecular events that underlie the pathophysiology in PTHS.

W-3136

POLYCOMB REPRESSIVE COMPLEX 1: ROLE IN NEURAL DIFFERENTIATION

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Chromatin modification is a crucial step to change the pluripotent stem cells (PSCs) into differentiated cells with more closed chromatin structure. There are different epigenetic activators and repressors which drive the differentiation process and thereby regulate development. The various epigenetic chauffeurs which aid in differentiation include DNMTs, nucleosome remodelling complexes and histone modifiers; one of the histone modifiers is polycomb group (PcG) proteins. Neurogenesis is a complex coordinated meshwork of signalling pathways, transcription factors, morphogens and epigenetic regulators. We are interested in understanding the role of PcGs during human neural differentiation; the two well researched protein complexes comprising of several PcGs include Polycomb repressive complexes 1 and 2(PRC1 and PRC2). PRC1 has core catalytic protein: RING1B which brings about H2AK119ub1 and BMI1 play a crucial role during this modification. We performed a directed differentiation of human pluripotent stem cells towards neural lineage by inhibiting TGFβ, WNT, ACTIVIN and BMP pathways in the initial stages followed by addition of morphogens like Sonic hedgehog(SHH), Retinoic acid(RA) and Fibroblast growth factors(FGF2 and FGF4) for a period of 20 days. We obtained neural rosettes which indicated our cells are in the neural stem cell stage and were characterised using NESTIN, PAX6 and TUJ1. After characterisation we assessed PRC1 levels during differentiation and we observed that there was no significant change in RING1B expression whereas there was a significant increase in BMI1 from day 12 to day 20 of differentiation; and the

expression of PRC1, H2AK119ub1 histone mark was checked. Although using the same differentiation protocol, the results showed subtle difference in expression of neural markers and PRC1 proteins between human embryonic and human induced pluripotent stem cells. We also observed that expression of PRC1 protein was significantly higher than the expression of PRC2 associated proteins during neural differentiation. It was also observed that with inhibition of all the pathways in the early days the levels of PRC1 goes down and then increases in differentiated cells. Our results signify that these PRC1 is crucial component of early human neuronal differentiation.

W-3138

USING DIFFERENTIATION BIAS OF HUMAN EMBRYONIC STEM CELL LINES AS A TOOL TO IDENTIFY REGULATORS OF DEFINITIVE ENDODERM SPECIFICATION

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Recently launched clinical trials aim to use human pluripotent stem cell (hPSC) mesendodermal (ME) derivatives to treat patients with heart failure and type 1 diabetes. As individual hPSC lines can significantly vary in their ME differentiation efficiency, acquiring a deeper understanding about regulators of early lineage specification is of great value to the field of regenerative medicine. In this study, we used five karyotypically normal human embryonic stem cell (hESC) lines to investigate molecular mechanisms of hESC differentiation bias towards definitive endoderm (DE) in a classic adherent culture and an in vitro model of early gastrulation-associated fate patterning in geometrically-confined micropatterned colonies. We identified VUB04 as a line with a very low DE differentiation efficiency, lacking upregulation of genes associated with primitive streak and endoderm formation upon WNT and Nodal signalling induction in both differentiation systems tested. Bulk mRNA sequencing at the undifferentiated stage showed a distinctive expression profile in VUB04 compared to the control hESC lines, with 417 significantly differentially expressed genes. Pathway enrichment analysis indicated JAK/STAT and MAPK signalling within the top enriched pathways in VUB04. Additional mRNA sequencing at 6-hour and 24-hour DE differentiation stages showed that the VUB04 expression profile continued to differ from the control lines with 552 and 637 significantly differentially expressed genes, respectively, and was depleted in genes regulating endoderm specification. We are currently modifying expression levels of candidate genes selected from a list of 120 genes differentially expressed in VUB04 at all three timepoints, and investigating if they have regulatory effects on early lineage specification. We also detected a significant upregulation of NANOG expression in VUB04 at the undifferentiated stage, and are investigating whether this difference can affect DE differentiation. Our work provides preliminary insight into the molecular mechanisms of how hESC may manifest DE differentiation bias and the experimental validation of an in vitro platform that can be employed for high-throughput screening of hPSC differentiation propensity.

W-3140

MODELLING HUMAN NEURAL CREST STEM CELLS and MELANOCYTE SPECIFICATION USING PLURIPOTENT STEM CELLS

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The neural crest (NC) is a transient embryonic population of multipotent stem cells originating between the neural tube and the future epidermis. Interestingly, these cells undergo an epithelial-to-mesenchymal transition and invade virtually the whole embryo, where they differentiate into several lineages, including skin melanocytes. Understanding human NC biology is of high importance, since many congenital diseases known as neurocristopathies are caused by an abnormal NC development. Moreover, recent studies have indicated that stem cells are defined by specific metabolic states and changes in metabolism are drivers of lineage specification. However, it is not known whether this is also a fundamental mechanism controlling NC cells (NCCs) and their differentiation into melanocytes. In this research project, we used human embryonic stem cells (hESCs) to investigate NC stemness maintenance and melanocytic specification in human. We have generated hESC-derived

NCCs and established protocols to derive human melanoblasts and melanocytes. In particular, we have targeted the TGF-β Superfamily and the Wnt/β-catenin pathway, and exploited the well-known roles of BMP4 and EDN3 to induce melanocyte progenitors. Noteworthy, these differentiation protocols are entirely chemically-defined, i.e. xeno-free and feeder-free, which makes them very attractive for potential clinical applications. We plan to perform single-cell RNA sequencing (scRNA-Seq) on the generated cell populations to better characterize early NC stem cells (NCSCs) vs. cells primed to the melanocytic lineage. We will combine results from the scRNA-Seq with metabolomics studies in order to define the metabolic landscapes that might be implicated in the NCSCs maintenance and melanocytic lineage specification.

W-3142

SOFTWARE FOR FOR USING HUMAN TFOME TO RAPIDLY DIFFERENTIATE STEM CELLS

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Many current efforts in the stem cell field are focused on the differentiation of human stem cells into other cell types represented in the human body. In general, common tools to perform this task include optimizing growth media conditions, manipulating surface growth conditions, and the over-expression of transcription factors (TFs). Some of the fastest and most efficient methods to date have emphasized the latter, and so the challenge has become how to identify the best set of TFs to convert stem cells to other cells types at maximal speed and efficiency. In attempt to solve this problem in high-throughput, our group has developed the “Human TFome” that is composed of a nearly exhaustive set of 1768 human TFs in genome-integrable vectors that can each induce the over-expression of each TF with doxycycline induction. While this library is intended to enable us to quickly explore combinations of TF over-expressions for conversion, there are too many possible combinations for us to explore them all. To reduce the library size to perform a reasonable screen, there are two general methods that are typically used - refining the library to those TFs currently described in the literature as relevant to the differentiation into a specific type of cell and building software tools to reduce this list based on analysis of data collected for various cell types. Here we present a method that uses epigenetics data to create rank-ordered lists of TFs relevant to particular cell types. These lists are used to perform multiplex differentiation experiments designed to explore a near exhaustive combinatorial screen of a small list of barcoded TFs. Those that yield the strongest amount of differentiation are then flow-sorted and sequenced. The software tool then analyzes these raw reads to determine which TFome TFs were present in cells with the greatest differentiation efficiency according to flow cytometry data and refines the TF list to enable construction of a stable cell line with high-efficiency conversion. As a proof of principle, we have applied this workflow to differentiate stem cells into a broad set of interesting types including types in hematopoetic, epithelial, and neuronal lineages. We believe that this method can yield rapid discovery of a large number of TF over-expression combinations to convert stem cells to other useful cell types.

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W-3144

IDENTIFICATION OF EARLY DEVELOPMENTAL BRANCHING IN HUMAN MEGAKARYOPOIESIS WITH IMPLICATIONS FOR MYOCARDIAL INFARCTION

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Haematopoietic stem cells (HSC) constitute the basis for blood formation and imbalances in their development and maturation may lead to a variety of disorders including cardiovascular disease and myocardial infarction. Here, we investigate the formation of megakaryocytes through single cell transcriptome sequencing of human HSCs and, for the first time, megakaryocytes (MK). We are able to find evidence of early priming of human HCS into MKs and our data suggests that two developmental pathways can be responsible for the generation MKs. By single cell RNA sequencing of MKs both from patients who has undergone coronary artery bypass surgery following heart attack and controls, we see possible impairment of one of these developmental trajectories, and also a tight link to the ability to increase ploidy, which is a hallmark of healthy platelet production. Our data gives the first transcriptomic delineation of

megakaryopoiesis at single cell level and furthermore suggests aberrant gene regulation in early stages of haematopoiesis as a key to explain the pathogenesis of the number one killer in western society.

W-3146

ENDOGENOUS IGF SIGNALING DIRECTS HETEROGENEOUS MESOENDODERM DIFFERENTIATION IN HUMAN EMBRYONIC STEM CELLS

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In a successful embryogenesis process, various cell types often emerge simultaneously from their common progenitors under the influence of intrinsic signals. In human embryonic stem cell (hESC) differentiation, mesoendoderm progenitor cells spontaneously generate multiple cell types, making them an excellent system to understand the molecular regulations that maintain the balance of different cell types in embryogenesis. In this report, we demonstrate that IGF proteins are endogenously expressed during differentiation, and contribute to the cell fate diversity from mesoendoderm progenitors. Small chemical LY294002 inhibits the IGF pathway to suppress epicardial and noncardiac cell fates, and promotes cardiomyocyte differentiation. LY294002 leads to distinct gene expression profile in cardiomyocytes, and provides a valuable addition to conventional differentiation methods. Further study shows that LY294002 inhibits CK2 pathway to induce cardiomyocyte cell fate, and CK2 inhibitor such as apigenin also efficiently promotes cardiomyocyte differentiation. This study elucidates the crucial roles of endogenous IGF in mesoderm differentiation, and the inhibition of IGF pathway is an effective approach to generate cardiomyocyte for hESC applications.

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W-3148

EFFICIENT KNOCKIN OF CARDIAC REPORTERS IN HUMAN PLURIPOTENT STEM CELLS ALLOWS FOR FAST FACTOR SCREENING AND CARDIAC PROGENITOR ISOLATION

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Cardiomyocytes differentiated from human induced pluripotent stem cells (iPSCs) hold promise for cardiac regeneration. iPSC reporter lines are instrumental for identifying novel factors that promote cardiac differentiation and also facilitating the enrichment of a pure population. Precise knockin efficiency in iPSCs is unfortunately notoriously challenging. Recently we reported that using a double-cut plasmid donor with transient BCL-XL delivery considerably increased CRISPR-Cas9 mediated editing efficiency in iPSCs (Genome Biology, 2017; Nucleic Acids Research, 2018). We now aim to create cardiac lineage-specific human iPSC reporter lines (MESP1, GATA4, and MYH6) using our editing system. RNA-seq analysis show no expression of these genes in iPSCs. To assess editing efficiency, we designed donors that add a 6 bp insertion at the STOP codon after successful homology directed repair (HDR). iPSCs treated in different conditions were harvested 3 days after electroporation. We conducted PCR with primers targeting the sequences flanking donor homology arms, followed by a second PCR targeting the editing site to have a final amplicon of 500-600 bp. Indel and HDR efficiency was analyzed with Inference of CRISPR Edits (ICE). After optimization, including BCL inhibitor treatment, up to 30% HDR editing was seen at MESP1, GATA4, and MYH6. We then designed donors that allow HDR integration of GFP. As expected, GFP was not initially expressed in edited iPSCs. In MESP1GFP iPSCs, ~1% GFP+ cells were seen on day 3 of cardiac differentiation induction. To further enhance directed differentiation, we electroporated iPSCs with a TBX6 expressing plasmid and observed ~3% MESP1-GFP+ cells after ~3 days of culture, indicating that TBX6 promotes cardiac mesoderm induction. Our approach of creating a reporter cell line and transfecting reprogramming factors in a single step can expedite identifying novel factors or combinations of factors for efficient directed differentiation. Higher levels of HDR in iPSCs will allow generation of reporter lines in several

days without tedious single cell cloning. This system also allows isogenic cardiac progenitors in various differentiation stages to be purified for further studies, e.g., genomic and epigenomic analyses, shedding light on cardiac differentiation mechanisms.

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W-3150

DIRECTED DIFFERENTIATION OF HUMAN STEM CELLS TOWARD PARATHYROID PROGENITORS

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Hypoparathyroidism is a disease that affects over 80,000 people in the United States. It most commonly occurs as a postoperative complication of thyroid surgery, although rare familial forms of the disease are known. The key biochemical hallmark of hypoparathyroidism is insufficient production of parathyroid hormone (PTH), which leads to hypocalcemia. Symptoms include mood changes, fatigue, tetany, and seizures. Patients take multiple daily pills containing calcium and active vitamin D or the recently-approved daily self-injections of PTH. However, physiological minute-to-minute adjustments of dosage is not an option as there is no method to sense changes in serum calcium concentration; a key function of healthy parathyroid cells. Stem cell-based therapy offers an appealing opportunity to replace missing parathyroid cells and restore regulation of serum calcium concentration without drugs. To this end, we created knock-in reporter stem cell lines (BJ-RiPS and HUES9) with mNeon-tagged GCM2, the parathyroid-specific master transcription factor. The reporter did not affect pluripotency, and its functional integrity was shown by the robust induction of GCM2-mNeon-positive cells (GCM2mNeon+) when targeting CRISPRa to the transcription start site of GCM2. We differentiated these reporter cells to definitive endoderm at 90% efficacy, followed by BMP and TGF inhibition to induce differentiation to anterior foregut endoderm (AFE), which was marked by robust induction of the transcription factors Tbx1, Pax9, and Eya1. To further mimic embryonic parathyroid development, we treated AFE cells with sonic hedgehog, retinoic acid, and BMP inhibition. This approach resulted in the appearance of up to 2.5% GCM2mNeon+cells, increasing to up to 5% when grown in 3D Matrigel; these cells generated PTH mRNA. Our results show that the use of a rigorous approach to recapitulate early steps in parathyroid development has the promise to produce parathyroid cells in vitro; initial steps toward the goal of regenerative medicine for patients with hypoparathyroidism.

W-3152

CUSTOMIZED TETRACYCLINE INDUCIBLE MYOD1 VECTOR IMPROVES TRANSGENE EXPRESSION AND ACHIEVES EFFICIENT AND SIMPLE MYOGENIC DIFFERENTIATION OF HUMAN IPSCS

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As patient derived human induced pluripotent stem cells (hiPSCs) become powerful tool for in vitro disease model, including muscular disease, the demand for efficient and reproducible differentiation methods has increased. Myogenic differentiation 1 (MYOD1) was reported as a master transcription factor of myogenic cells. In previous study, we developed piggyBac transposon based tetracycline inducible MYOD1 vector (Tet-MYOD1) and demonstrated inducible expression of MYOD1 in hiPSCs drives cells along myogenic cells efficiently. However, in this study, we also found the clones with low efficiency of myogenic cells differentiation, thus selection of clones was required. Recently, some of reports showed effect of vector construction on efficiency of transgene expression. Employing these findings, we focused on our Tet-MYOD1 construction and prepared 5 different new customized Tet-MYOD1 vectors. Flow cytometry analysis showed 60% of hiPSCs populations are positive for transgene expression with previous Tet-MYOD1 vector. Compare with this data, one of our new customized Tet-MYOD1 vector achieved higher transgene expression (90% of populations with transgene expression). Such improvement of transgene expression could be due to more uniform expression of the trans-activator after drug selection compared to the previous Tet-MTOD1 vector. Moreover, this new vector achieved more than 80% of myogenic cells differentiation efficiency from hiPSCs without clone selection. Importantly, 5 different hiPSCs cell line, including muscular disease patient derived hiPSCs, also achieved more than 80% of myogenic differentiation efficiency with new customized vector. In this study, we demonstrate customizing vector construction as a useful strategy for in vitro disease study of muscular disease by improving myogenic differentiation efficiency of hiPSCs compared to previous tetracycline inducible transgene expression system.

W-3154

HIGHLY SENSITIVE AND NON-DISRUPTIVE DETECTION OF RESIDUAL UNDIFFERENTIATED CELLS BY MEASURING MIRNAS IN CULTURE SUPERNATANT

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Recently, several types of therapeutic products derived from iPS cells are being developed for use in regenerative medicine and cell therapy. However, in order to use these products clinically, a major hurdle is the presence of residual undifferentiated cells that have tumorigenic potential. Although various methods for detecting undifferentiated cells have been devised, most of them are disruptive methods that involve irreversibly lysing a portion of the iPS cell-derived product. Here, we report a new method for detecting residual undifferentiated cells non-disruptively and with high sensitivity. In this method, we have employed measurement of miRNA copy numbers in cell culture supernatant to realize a low cost and high sensitivity alternative to existing methods. We prepared residual undifferentiated cell models by mixing iPS cells with differentiated cells such as RPE cells, HUVEC cells and MSC, in an arbitrary ratio. We measured miRNA copy numbers in the culture supernatant of these model cells and assessed their performance in the detection of undifferentiated cells, using intra-cellular levels of Lin28 as a reference. We were able to verify that our method was able to detect 0.01-0.001% of undifferentiated iPS cells in culture, demonstrating a performance equivalent to Lin28. In addition, our method could detect 0.01% residual undifferentiated cells even among liver cells, which are known to be difficult to detect by measuring Lin28. Our method bears high potential to contribute to clinical applications of iPS cells, especially in terms of monitoring the differentiation induction process as well as safety assessment of iPS cells-derived products.

W-3156

MAGNETIC MICROBEAD BASED ENRICHMENT OF HUMAN PLURIPOTENT STEM CELL-DERIVED BETA-CELLS

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Human pluripotent stem cell (hPSC)-derived insulin-secreting β² cells (SC-β²) can reverse hyperglycemia in animal models of Type 1 diabetes. Clinical translation of differentiation protocols for naked or encapsulated SC-β² cell human trials would benefit from the reproducible generation of a homogenous SC-β² cell product. Here, we demonstrate that an integrin (referred to as CD-I) is a cell surface marker suitable for enrichment of hPSC-derived SC-β² cells. Immunomagnetic enrichment of CD-I+ SC-β² cells is accomplished using a commercially available anti-CD-I antibody and a QuadroMACS LS positive selection column from Miltenyi. CD-I+ cell sorting can enrich SC-β² cells derived from two different hES cell lines (HUES8 and SEM01) at least 2-fold to over 70% (C-peptide+/ISL1+). Purified cells are stable in culture for up to 1 week as aggregates and secrete increased insulin levels in response to a 1-hour glucose challenge compared to non-sorted cells. Current efforts are focused on improving purification cell yields and scale-up. Thus, we demonstrate a CD-I-based sorting method for the reproducible enrichment of SC-β² cells for clinical β²-cell replacement therapy.

PLURIPOTENT STEM CELL: DISEASE MODELING

W-3158

TSG-6 IN EXOSOMES FROM CANINE ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELL ALLEVIATED INFLAMMATION IN INFLAMMATORY BOWEL DISEASE BY INCREASING REGULATORY T CELL IN MICE

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Mesenchymal stem cells(MSCs) have been shown to benefit patients with a variety of immune-mediated diseases by modulating immune cells. In particular, stem cell derived exosomes deliver immunoregulatory factors to recipient cells. In this study, we hypothesized that delivery of tumor necrosis factor-α-stimulated gene/protein 6 (TSG-6) in exosomes secreted from canine adipose tissue-derived (cAT)-MSCs is a key factor

influencing immunoregulation. In addition, we examined the therapeutic effects of TSG-6 in exosomes in an inflammatory bowel disease murine model and explored the mechanism underlying the immunomodulatory properties. Mice (C57BL/6) with dextran sulfate sodium-induced colitis were administered exosomes from cAT-MSCs intraperitoneally; colon tissues were collected on day 10 for histopathological, quantitative real-time polymerase chain reaction, and immunofluorescence analyses. TSG-6 in exosome ameliorated IBD and regulated colonic expression of pro-and anti-inflammatory cytokine such as tumor necrosis factor-α, interleukin-β, interferon-γ, interleukin-6, and interleukin-10. To investigate the effect of TSG-6 in exosome on population of regulatory T cells in vitro, exosomes were cocultured with canine lymphocytes. TSG-6 in exosome increased Tregs population. TSG-6 in exosome increased Tregs in the inflamed colon in vivo. Moreover, TSG-6 in the exosomes alleviated intestinal inflammation by increasing Tregs in the IBD murine model. In conclusion, TSG-6 in exosomes from cAT-MSCs showed immunoregulatory effect by increasing Tregs and alleviated dextran sulfate sodium-induced colitis by enhancing Tregs in mice.

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W-3160

SINGLE GENE KNOCKDOWNS IDENTIFY GENE-PHENOTYPE RELATIONSHIPS IN A HUMAN CELLULAR MODEL OF NEURODEVELOPMENTAL DISORDERS

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The 16p11.2 copy number variant (CNV), a deletion (16pdel) or duplication (16pdup) on chromosome 16, is one of the leading causes of neurodevelopmental disorders including autism, schizophrenia, language impairment, intellectual disability and sei-zures. In addition, CNV carriers also manifest opposing phenotypes such as macro-cephaly and obesity in 16pdel carriers and microcephaly and underweight in 16pdup carriers. We have previously shown that induced pluripotent stem cell (iPSC)-derived neurons from 16p11.2 CNV carriers show opposing cellular phenotypes – soma size and total dendritic length are increased in 16pdel neurons while they are decreased in 16pdup neurons – in line with the contrasting head size differences. To understand how the CNV may affect head size, we used small hairpin RNAs (shRNAs) to knock-down 16p11.2 genes in control neurons and assessed soma size and dendritic growth. We found that knockdown of single 16p11.2 genes can recapitulate distinct cellular phenotypes. Neurons treated with the KCTD13 shRNA had significantly larger somas while the PAGR1 knockdown neurons had a significantly longer dendrites compared with control shRNA-treated neurons. In contrast, expressing the shRNAs in 16pdup neurons rescued the cellular phenotypes. These data have important implications - i) KCTD13 and PAGR1 can regulate neuronal size via cell growth in iPSC-derived neu-rons. This is intriguing because previous studies have shown Kctd13 to regulate brain size in zebrafish via proliferation and apoptosis, but not neuronal size and PAGR1 acts in obesity and seizure-related pathways but not cell growth. Also interesting will be to determine whether the mechanisms mediating brain size might influence com-mon behavioral deficits, e.g. autism, seizures. ii) the CNV harbors ~29 genes, many of which are involved in neurodevelopment suggesting overlapping roles. However, our data point toward key drivers of neurodevelopmental phenotypes. iii) we provide evidence that expression change in opposite direction of the same gene(s) in the 16p11.2 CNV result in opposing neuronal phenotypes, suggesting gene dosage as a causative mechanism. Our findings open up research avenues that will help elucidate the nature of gene expression patterns in the CNV, which is crucial to identify gene-phenotype relationships.

W-3162

HUMAN INDUCED PLURIPOTENT STEM CELL-BASED IN VITRO MODELLING OF OCULOCUTANEOUS ALBINISM

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Individuals with oculocutaneous albinism (OCA) have low or no ocular pigmentation and decreased best-corrected visual acuity due in part to foveal defects. Since fovea development starts prenatally and continues through the early postnatal years, interventions in childhood to improve pigmentation in the eye may prove efficacious in rescuing vision defects, if fovea development is even partially restored. In adults, increased pigmentation in the eye may result in improved symptoms associated with glare and photosensitivity. The purpose of our study is to develop a disease-in-a-dish model for OCA and to identify drugs that can improve ocular pigmentation. Fibroblasts from two unrelated OCA1A and two OCA2 individuals were reprogrammed to induced pluripotent stem cells (OCA-iPSC) using Cyto-tune II™ and validated. All OCA-iPSCs lines were differentiated to retinal pigment epithelium (OCA-RPE) using a developmentally guided differentiation protocol and characterized at the morphological, molecular and functional level by immunostaining, trans-epithelial resistance (TER) measurements, phagocytosis assay, and electron microscopy analysis. All OCA-iPSCs expressed

the stem cell markers OCT4, SOX2, TRA-1-81 and TRA-1-60, formed embryoid bodies that could be differentiated to multiple cell types and gave rise to teratomas when implanted in vivo. OCA-RPE were cultured on semi-permeable membranes for eight weeks to obtain a functionally mature and polarized monolayer tissue that was similar to RPE derived from unaffected control individuals (control-RPE) based on morphology, expression and localization of cell-cell junctional markers like ZO1, β-CATENNIN, and cell surface proteins EZRIN and COLLAGEN. Functionally, OCA-RPE displayed TER and photoreceptor phagocytosis comparable to control-RPE. By electron microscopy, we observed significantly reduced and degenerating melanosomes in OCA-RPE compared to control-RPE. CRISPR/CAS9-mediated mono-allelic correction resulted in rescue of the pigmentation defects in OCA-RPE derived from OCA2 patients. A fully functional RPE can be derived from OCA patient iPSCs that faithfully recapitulate the patient pigmentation phenotype in vitro in a retinal cell culture system.

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W-3164

BALANCED BRAIN: DEVELOPING HUMAN CORTICAL NEURAL NETWORKS TO MODEL STXBP1 HAPLOINSUFFICIENCY

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Mutations in STXBP1 cause a neurodevelopmental disorder in approximately 1 in 91,000 children. STXBP1 encephalopathy includes developmental delays, infantile epilepsy, and cognitive impairment. The extent and severity of phenotypes varies, but the degree of intellectual disability is classified as severe to profound in 88% of cases. Current medications are able to control the seizures in a subset of patients; however, there is no established treatment for minimizing or preventing intellectual disability. To date, mouse models have been critical in advancing our understanding of STXBP1 encephalopathy. Interestingly, mice heterozygous for Stxbp1 in GABAergic inhibitory neurons (GINs) alone showed impaired viability with surviving animals showing stronger epileptic activity. Importantly, interspecies differences may be especially pronounced in GINs, which are more heterogenous and represent a greater proportion of cortical neurons in humans in comparison to rodents. Due to the combination of interspecies differences and lethality of subtype specific mutants, human induced pluripotent stem cell (hiPSC) based neural networks will be critical to understanding, and eventually treating, STXBP1 encephalopathy. No existing methods yield human cortical neural networks (hcNNs) with physiological proportions of both excitatory and inhibitory neurons. Independent generation of late-stage excitatory and inhibitory neuronal progenitors followed by coculture at physiological ratios establishes a human neural network with widespread and coordinated activity that can be used to interrogate network development and activity. Here we present hcNNs with physiological proportions of excitatory and inhibitory subtypes from hiPSCs derived from an infant with a de novo frame shift mutation in STXBP1, alongside familial controls, in order to model STXBP1 haploinsufficiency. Phenotypes seen in STXBP1+/- hcNNs are quantified alongside network dysfunction in Stxbp1+/- mice, providing a cross-species comparison of the functional effects of STXBP1 haploinsufficiency. Together, these two models provide a platform to assess potential targeted therapies for STXBP1 patients.

W-3166

MOLECULAR CHANGES IN PRADER-WILLI SYNDROME DPSC-DERIVED NEURONAL CULTURES REVEALS SUBTYPE SPECIFIC EXPRESSION SIGNATURES AND CLUES ABOUT INCREASED ASD INCIDENCE

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Prader-Willi syndrome (PWS) is a neurodevelopmental disorder characterized by hormonal dysregulation, obesity, intellectual disability, and behavioral problems. Most PWS cases are caused by paternal interstitial deletions of 15q11.2-q13, while a smaller number of cases are caused by maternal uniparental disomy (UPD) where only maternal copies of the PWS critical region are present. The PWS critical region is imprinted, meaning the genes on the maternal chromosome are silent, resulting in a phenotype that is milder than, but equivalent to PWS cases where the paternal copy is deleted. There are, however, notable phenotypic differences between PWS Deletion and PWS UPD. PWS UPD tends to be a milder phenotype and is associated with an increased risk of developing autism spectrum disorder (ASD). Understanding the molecular differences between PWS Deletion versus UPD may provide clues underlying the increased ASD incidence in PWS UPD cases. We have established a large collection of dental pulp stem cell (DPSC) lines derived from the dental pulp of subjects with various neurodevelopmental diseases. We regularly differentiate these stem cell lines into cortical-like neuronal cultures consisting of both neurons and glia. Using neuronal cultures derived directly from DPSC of affected individuals and neurotypical controls, we preformed RNAseq studies to investigate differential gene expression changes associated with PWS subtypes and increased ASD risk. We found key molecular differences between PWS Del and

UPD both within and outside of the 15q region. The expression of critical PWS genes such as SNRPN, SNURF, MAGEL2 were absent from all PWS neurons. In addition to genes that are specific to each class, there are 20 shared differently regulated transcripts between Del and UPD. These transcripts may be responsible for the PWS phenotype. In the UPD subgroup, we found 74 genes differentially expressed in the ASD+ versus ASD- UPD subgroup. In addition, enrichment analysis revealed a consistent decrease in mitochondrial-associated transcripts in the ASD+ subgroup, which may underlie an overall dysregulation of energy metabolism in affected individuals. We are currently in the process of validating these changes at both the transcript and protein level using real-time quantitative PCR and immunofluorescence.

W-3168

MODY3 HUMAN IPSC-DERIVED β-LIKE CELLS EXHIBIT REDUCED GLUCOSE UPTAKE ABILITY

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Maturity onset diabetes of the young 3 (MODY3) results from heterozygous mutations in the transcription factor HNF1A, resulting in early age onset of diabetes. It is characterised by a progressive loss of insulin secretion that results in hyperglycaemia. In this study, we investigated a novel coding-non-synonymous mutation in the DNA-binding domain of HNF1A, which results in a change in amino acid (pos.126) from histidine to aspartic acid (H126D). The mutation was identified in a pair of Singaporean Chinese siblings who have MODY3. We hypothesized that the H126D mutation may affect HNF1A protein's binding affinity to its target genes, hence affecting the regulation of its downstream targets, to cause β-cell dysfunction. While human cadaveric MODY3 islets are not readily available for study, differentiated pancreatic cells generated from human pluripotent stem cells (hPSCs) can offer a good alternative platform for mechanistic studies. We aimed to use MODY3 patient-derived induced pluripotent stem cells (iPSCs) that were differentiated into β-like cells as an experimental model to characterize the mechanisms that link a human genetic variant to the diabetic phenotype observed in the patients. We reprogrammed the patients' fibroblasts into iPSCs, and then differentiated them into endocrine progenitors and pancreatic β-like cells to investigate the effects of the mutation on pancreatic development and function. HNF1A expression was found to peak at the endocrine progenitor stage before decreasing during the maturation into β-like cells. RNA-Seq analysis revealed differential gene expression between wild-type and mutant endocrine progenitors. Glucose-stimulated insulin secretion assays demonstrated reduced insulin secretion from the mutant β-like cells. In addition, glucose uptake assays also showed that the mutant β-like cells exhibited a reduced glucose uptake ability. These results suggest that HNF1A mutations may disrupt the regulation of genes to diminish the glucose uptake and impair the insulin secretory function of β-cells in MODY3 patients. Future efforts are focused on elucidating mechanisms that could provide potential therapeutic targets for MODY3 treatment.

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W-3170

DEVELOPMENT OF AN IN VITRO HUMAN NEUROMUSCULAR JUNCTION

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Botulinum neurotoxins (BoNTs) inhibit acetylcholine release at the neuromuscular junction (NMJ), preventing muscle contraction. An in vitro model of the human NMJ, would aid study of BoNTs and development of novel BoNT therapeutics. We have developed a co-culture of human induced Pluripotent Stem Cells derived motor neurons (hiPSC-MN) and human muscle cells that forms functional NMJ suitable for studying BoNT. Human muscle cells

were cultivated as described in Braun S., et al J. Neurol. Sci. 1996 in 96 well plates. hiPSC-MN were purchased from CDI or BrainXell and plated onto the muscle cells cultures. hiPSC-MNs were characterised by qrtPCR and immunofluorescence and NMJ were evaluated by Alexa 488-alpha bungarotoxin labelling. After 8-10 days of co-culture, movies were recorded using an In Cell 2200 microscope in a thermostatic chamber, before and after tetrodotoxin (TTX) or α-bungarotoxin (α-bung) treatment. Calcium wave frequency was measured with Fluo-4 before and after glutamate, TTX or α-bung treatment. hiPSC-MNs expressed markers of neurons (MAP2), MN progenitor (OLIG2) and MN (ISLET1, HB9, AchE, ChAT). The percentage of MN increased over time, with MN and MN progenitor marker expression levels remaining stable. Co-cultivating hiPSC-MN with human muscle cells led to contractions after only 7 days and stable for several days. Contraction frequency and calcium mobilization were both dose—dependently reduced using TTX and α-bung. In conclusion, a coculture system has been developed using hiPSC-MN and human muscle cells in a 96-well plate format. This system is sensitive to TTX and α-bung and thus offers a relevant in vitro model to study BoNT activity at the NMJ.

W-3172

INDIRECT MTOR INHIBITION ATTENUATES FIBRODYSPLASIA OSSIFICANS PROGRESSIVA (FOP) IDENTIFIED BY USING PATIENT-DERIVED IPSCS

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Fibrodysplasia ossificans progressiva (FOP) is a rare and intractable disorder characterized by extraskeletal bone formation through endochondral ossification. Patients harbor gain-of-function mutations in ACVR1 (FOP-ACVR1), a type I receptor for bone morphogenetic proteins. Despite numerous studies, no drugs have been approved for FOP. Here, we developed a high-throughput screening (HTS) system focused on the constitutive activation of FOP-ACVR1 by utilizing a chondrogenic ATDC5 cell line that stably expresses FOP-ACVR1. After HTS of 5,000 small-molecule compounds, we further identified two hit compounds that are effective at suppressing the enhanced chondrogenesis of FOP patient-derived induced pluripotent stem cells (FOP-iPSCs) and suppressed the heterotopic ossification (HO) of multiple model mice, including FOP-ACVR1 transgenic mice and HO model mice utilizing FOP-iPSCs. Furthermore, we revealed that one of the hit compounds indirectly inhibits mTOR signaling during chondrogenic induction. Our results indicate a new inhibitory mechanism of FOP. Moreover, the hit compounds could contribute to future drug repositioning and the mechanistic analysis of mTOR signaling.

W-3174

DEVELOPMENT AND VALIDATION OF POTENCY ASSAY FOR MESENCHYMAL STEM CELLS IN THERAPEUTIC APPLICATIONS

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Fibroblast are key modulators of the structural framework in many tissues and initiate signaling mechanisms to many cell types in the tissue microenvironment. Under inflammatory conditions, fibroblasts upregulate the production of pro-inflammatory cytokines and contribute to tissue degeneration and fibrotic damage. MSCs exhibit anti-inflammatory properties that benefit the viability and growth of cells. We hypothesize that potent MSC products will exert an anti-inflammatory effect on fibroblasts when co-cultured in transwells in the presence of the pro-inflammatory agent LPS. We also hypothesize that potent MSC products will decrease T-cell activation in the presence of a T-cell stimulatory agent. Preliminary results showed an induced expression of pro-inflammatory cytokines IL-6 (140-Fold), IL-1a (20-Fold), and TNF-alpha (5.8-Fold) after 6 hours of LPS treatment. We then aimed to test the influence of MSC co-culture treatments on the observed LPS response. A total of 800,000 fibroblast cells were seeded in 6 well culture plates and treated with LPS for 6 hours. After the induction of a pro-inflammatory phenotype, 800,000 MSCs were seeded on the top layer of the trans-well plate and cultured for 24 and 48 hours. Treated fibroblasts with no co-culture treatment. We found that expressions of pro inflammatory cytokines had blunted after 48 hours of co-culture with MSCs when compared amongst groups of co-culture vs control (IL6: 10.3-fold increase in control vs 6.82 increase in co-cultures, IL1a: 12.3-fold increase in control vs 10.2 increase in co-culture, TNF-a: 1.84-fold increase vs 0.3 fold-increase in co-culture). We found our MSCs to induce a variable suppression of T-cell activation. Although preliminary, this result suggests the variable result of different products to be an indication of immunological potency. In this case, MSC expanded in hPLT showed the greatest immune-suppressive potency (47% CD69+ with co-culture vs 76.1% CD69+ activation in control) whereas MSC expanded in FBS had the lowest potency (75.1% CD69+ vs 76.1% activation in the control). This preliminary data suggests the anti-inflammatory and immunomodulatory properties can be analyzed in this model for potency analysis. We plan to repeat this result to fully develop and adopt this assay into our final product criteria.

W-3176

FRAGILE X SYNDROME: FROM DRUG SCREENING IN HUMAN PLURIPOTENT STEM CELLS TO VALIDATION IN HUMANIZED ANIMAL MODELS

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Fragile X syndrome (FXS) is caused most commonly by a CGG repeat expansion in the FMR1 gene that triggers its transcriptional inactivation. We have previously modeled FXS in human induced pluripotent stem cells (iPSCs). In order to investigate the regulatory layers involved in FMR1 silencing, we tested a collection of chromatin remodeling compounds for the ability to reactivate FMR1 expression in FXS-iPSCs. While DNA methyltransferase (DNMT) inhibitors induced the highest levels of FMR1 expression, a combination of a DNMT inhibitor and another epigenetic agent potentiated the effect of reactivating treatment. To better assess the rescue effect observed following direct demethylation, we have characterized the long-term and genome-wide effects of FMR1 reactivation in FXS-iPSC derived neural progenitor cells. Since to date there is no animal model that recapitulates the molecular pathogenesis of FXS, we have established two humanized mouse models for evaluation of candidate FXS therapies. Systemic drug treatments in mice carrying differentiated human FXS iPSC-derived transplants robustly induced FMR1 expression in the affected tissue, which was sustained for a prolonged period of time. Finally, we show a proof-of-principle for FMR1 reactivating treatment in the context of the central nervous system.

W-3178

INDUCED PLURIPOTENT STEM CELLS FOR LESCH-NYHAN DISEASE

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Lesch-Nyhan Disease (LND) is an X-linked inherited disorder with a characteristic phenotype that includes neurobehavioral abnormalities, megaloblastic anemia, and overproduction of uric acid. LND is caused by mutations in the HPRT1 gene, which encodes the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HGprt). Mechanisms responsible for the clinical features are unclear, and appear to vary according to cell and tissue type. Six induced pluripotent stem (iPS) cell lines were prepared from 3 unrelated LND cases along with 6 healthy controls. Two of the LND cases carried nonsense mutations c.151C>T or c.508C>T; the third carried a frame-shifting insertion 371insTT. All lines were characterized for pluripotency markers, morphology, and karyotype. RNAseq analysis was performed and a total of 144 differentially expressed genes between LND and healthy controls at nominal $p<0.001$ were identified. After correcting for multiple comparisons, 16 genes remained significantly differentially expressed (FDR<0.1), including the HPRT1 gene. Gene Set Enrichment Analysis (GSEA) analysis revealed a number of biological pathways significantly altered. Proteomic studies revealed only 4 proteins significantly altered at nominal $p<.001$, but only one protein reached statistical significance at FDR <0.10: HGprt itself. Metabolic studies revealed 10-20x increase in hypoxanthine, consistent with the enzymatic defect. These studies are currently being differentiated into cells responsible for the phenotype including neurons, marrow derivatives and liver cells. This resource of iPS cells can now be used as models for exploring the mechanisms of pathogenesis in Lesch-Nyhan Disease.

W-3180

PATIENT-SPECIFIC IPSC MODELS REVEAL A SUBSET OF MUTATIONS AMENABLE TO GENE AUGMENTATION IN AN AUTOSOMAL DOMINANT MACULOPATHY

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Best vitelliform macular dystrophy, commonly known as Best disease (BD), is an inherited disorder that leads to progressive and irreversible loss of central vision in affected individuals. BD is caused by mutations in BESTROPHIN1 (BEST1) gene, which encodes a homo-pentameric calcium activated chloride channel (CaCC) in retinal pigment epithelium (RPE) in the retina. Over 200 mutations associated with BD are known, majority of which are dominantly inherited. While canine models for autosomal recessive BD are available, similarly relevant animal models of autosomal dominant BD (adBD) do not exist. Therefore, we utilized induced pluripotent stem cell-derived RPE (iPSC-RPE) based disease model to assess therapeutic strategies for adBD. CaCC currents measured from adBD patient-specific iPSC-RPE were found to be significantly diminished when compared to wildtype and isogenic controls. Gene augmentation via viral expression of wildtype BEST1 restored CaCC to normal levels in iPSC-RPE where adBD was caused by mutations in predicted calcium clasp or chloride ion binding regions, but not when caused by a mutation predicted to be localized to a structural region of the channel. This restoration of CaCC correlated with improved rhodopsin degradation, an important function of RPE. As an alternative approach for adBD mutation unresponsive to gene augmentation, we tested gene editing via CRISPR-Cas9 to silence mutant allele in iPSC-RPE. Post-gene editing, all tested adBD iPSC-RPE lines showed increase in CaCC current. Overall, our results using patient-specific iPSC-RPE show that GA is a feasible strategy for some adBD patients depending on the role of the mutated amino acid. Those missense mutations that are unresponsive to gene augmentation are candidates for gene editing. Importantly, in absence of suitable animal models, iPSC-RPE based BD model could be utilized as a preclinical testing platform.

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W-3182

IMAGE-BASED HIGH-THROUGHPUT SCREENING OF IPSC-DERIVED NEURAL STEM CELLS FOR IDENTIFICATION OF LEAD COMPOUNDS FOR NIEMANN PICK DISEASE TYPE C

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Niemann-Pick disease type C (NPC) is a rare, genetic neurodegenerative lysosomal storage disorder characterized by defective cholesterol transport and lysosomal lipid accumulation leading to premature neuronal cell death. While NPC possesses a wide range of clinical onset time, disease presentation and severity, all NPC patients share neurodegenerative progression and premature death; most early-onset patients do not survive past the age of 20. There are currently no curative or FDA-approved disease-specific treatments for patients with NPC. High-throughput screening (HTS), combined with induced pluripotent stem cell (iPSC) technology, offers a uniquely powerful tool for early stage drug discovery. Because neuronal abnormalities and cell death are the primary drivers of NPC neurological disease progression, it is critical that we screen potential compounds in a physiologically relevant model. To achieve this, we have generated several stable NPC-patient iPSC lines derived from individual patient's skin fibroblasts and differentiated them into neural stem cells (NSCs). Neural stem cells have comparable disease phenotypes compared to differentiated mature neurons, while being easily expandable in large quantities necessary for high-throughput screening. The NPC-patient derived NSCs accurately recapitulate significant intracellular cholesterol accumulation correlating with increased cholesterol accumulation that is a hallmark of patient fibroblasts. Using these NPC-patient derived NSCs, we have screened a library of over 3,600 FDA-approved or pharmacologically active compounds for independent, additive, or synergistic activity with low dosage phase IIb/III clinical trial NPC compound, 2-hydroxypropyl-β-cyclodextrin (HPβCD). We used high-content imaging based on direct cholesterol staining to identify lead compounds. We chose to explore synergistic compounds for a potential co-therapy approach with HPβCD, as a synergistic approach may alleviate ototoxicity and other side effects associated with current effective HPβCD clinical doses. Here, we report some of the lead compounds we identified for further development and potential mechanisms of action of the newly identified compounds.

Funding Source: NIH

W-3184

MODELING DYSFUNCTIONAL EPITHELIAL DIFFERENTIATION RELATED TO IDIOPATHIC PULMONARY FIBROSIS USING HUMAN IPSC-DERIVED ALVEOLAR EPITHELIAL PROGENITOR CELLS

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Idiopathic pulmonary fibrosis (IPF) is a fatal disease with no known cure that is characterized by progressive fibrotic remodeling of the lung. While the exact pathophysiological mechanisms remain unknown, growing evidence suggests that a specific pro-fibrotic lung environment and aberrant alveolar epithelial repair might play key roles in the pathogenesis of IPF. A process often observed in IPF lungs is bronchiolisation. It refers to the abnormal emergence of airway epithelial like cells within the alveolar compartments of the lung and a loss of functional alveolar epithelial cells. It is not clear if this is a result of proximal-to-distal migration of cells from the airways or of aberrant trans-differentiation of resident alveolar epithelial stem cells. We aimed to investigate if a pro-fibrotic milieu, similar to that found in an IPF lung, could potentially skew alveolar epithelial progenitor cell differentiation towards airway epithelial like cells. We therefore developed an air-liquid interface system to model human alveolar type II (ATII) cell differentiation from iPSC-derived lung progenitor cells in vitro and treated the cells with an IPF-relevant cocktail (IPF-RC). NKX2.1+ lung progenitor cells were derived via directed differentiation of iPSCs, followed by maturation at air-liquid interface. The ATII like phenotype of the resulting cells was confirmed using qRT-PCR, RNAseq, IF and TEM. We then designed an IPF-RC of cytokines, previously reported to be elevated in IPF lungs, and added it into the culture medium for the last two weeks of differentiation. IPF-RC stimulated cells displayed a reduction in ATII-specific SFTPC expression, while the expression of several airway related genes including KRT5, SCGB1A1, MUC5B and BPIFB1 was upregulated. In addition, treatment with IPF-RC resulted in elevated MMP-7 and MMP-10 concentrations in the culture medium. Thus, we have demonstrated using an iPSC-derived model of alveolar epithelial progenitor cell differentiation that a pro-fibrotic environment has the potential to impair ATII differentiation by driving a shift towards an airway epithelial like expression signature and to induce IPF biomarker secretion. These results suggest that aberrant alveolar epithelial progenitor cell differentiation in the IPF lung could play a role in bronchiolisation.

W-3186

MODELING ALS WITH IPSC-DERIVED MOTOR NEURONS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease caused by the selective loss of both spinal and upper motor neurons with no effective treatment. Approximately 90% of the ALS cases are sporadic and the remaining 10% are familial. The most prevalent ALS mutations are the SOD1 (superoxide dismutase 1); C9ORF72 (chromosome 9 open reading frame 72); TARDBP (TAR DNA-binding protein 43 or TDP-43) and FUS (fused in sarcoma). Understanding the effect of mutations in ALS and the underlying disease mechanisms are necessary for developing therapeutics. Patient-specific induced pluripotent stem cells (iPSC) gives the opportunity to model the disease in a dish and make it possible to recapitulate some of the hallmark pathology leading to motor neuron (MN) degeneration. Further, they may also serve as a renewable source for drug discovery, genetic testing, and cell replacement therapy. Efficient differentiation of motor neurons from human pluripotent stem cells is very critical to understand motor neuron development and modeling the disease in vitro. We have established a combinatorial small molecule-mediated protocol for the differentiation of motor neurons from human pluripotent stem cells (hPSC) under xeno-free and feeder free conditions. After 4 weeks of differentiation, hPSC derived motor neurons showed the expression of key markers (HB9, ISL1, ChAT). In this study, we plan to use patient specific iPSC lines harboring different ALS mutations – C9ORF72 hexanucleotide, SOD1, TARDBP mutations. We are differentiating these lines into motor neurons and characterizing them to examine the associated pathophysiology specific for each of the mutations and typical of ALS including: alteration in soma size, reduced neurite length, mitochondrial dysfunction, TDP43 protein

aggregation, Neurofilament inclusions etc. Owing to the heterogeneity of the disease, our study will provide insight into the disease mechanism, unique or shared among the different mutations leading to MN degeneration.

W-3188

CHRONIC INTESTINAL PSEUDO-OBSTRUCTION: DISEASE MODELING and THERAPEUTIC APPROACHES

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Chronic Intestinal Pseudo-Obstruction (CIPO) is a rare monogenic disorder that affects the peristaltic movement of luminal content in the gastrointestinal tract (GI), which results in a severe life-long disabling condition. Most patients with the monogenic form of CIPO present clinically as neonates severe vomiting, constipation, abdominal distension and pain. Those with the severe form are unable to meet their normal caloric requirements through enteral nutrition, and usually rely on life-long parenteral nutrition, or allogenic intestinal transplantation. Our laboratory and others have found that the most common gene associated with CIPO is smooth muscle gamma actin, or ACTG2, which is required for normal intestinal peristalsis (muscular contractions and relaxation). We believe that mutations of ACTG2 affect the function of smooth muscle cells (SMCs), resulting in abnormalities of intestinal contractility. Restoration of impaired SMC function is a potential therapy for children with CIPO and it is our long-term goal. The current goal of this study is to use advanced genetic methods to create and rescue human SMCs with ACTG2 mutations in induced pluripotent stem (iPS) cell lines. To reach this goal, we chose to develop an iPS cell line from a patient containing the ACTG2 p.Arg178Ser mutation due to its unique phenotype – muscularis mass is dramatically thicker than a normal or other CIPO intestine. We have designed several CRISPR gRNAs that target the Arg178Ser variant, which is located in exon 6, and have assessed their cutting efficiency on HEK293T cells. From this, we identified that the optimal gRNA sequence (GCCCCATGCCATCATGCGCC) has a cutting efficiency of 19%, relatively higher than the other sequences tested. This gRNA is being used for CRISPR/Cas9 experiments in combination with HDR donor sequences to correct the Arg178Ser variant in patient specific iPS cells, followed by creating the variant in ES cells. We will then evaluate whether specific mutations of ACTG2 alters the cell cycle including proliferation and survival, and contraction of SMCs following in vivo implantation in NOD-SCID-IL2Rgnull mice. We believe that this approach may improve our understanding the role of ACTG2 in CIPO, and set the foundation for cell-based therapeutics for patients with CIPO.

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W-3190

CARDIAC DISEASE MODELING USING 3D MICRO-TISSUE OF HIPSC-DERIVED CARDIOMYOCYTES CARRYING DISEASE SUSCEPTIBLE GENETIC VARIATIONS

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Human genetic validation increases a medicine's likelihood of success. In this study, we combined a variety of technologies, including stem cell biology, CRISPR genome editing, tissue engineering, and automated microscopy, to build a systematic approach that enables deciphering the cardiac disease biology resulting from genetic variants. We used CRISPR genome editing to introduce genetic mutations previously identified in patients in human induced pluripotent stem cells (hiPSCs). The genetically engineered hiPSCs cells were then subjected to a 14-day monolayer-based, chemically-defined cardiac differentiation protocol that generates functional cardiomyocytes with 85-95% purity. Molecular, pharmacological, and electrophysiological properties of differentiated cardiomyocytes were examined to verify their resemblance to human cardiac cells. Edited, hiPSC-differentiated cardiomyocytes were then mixed with cardiac fibroblasts in ECM and seeded into custom-engineered 96 well cardiac micro-wire (CMW) plates. Within 7 days, cells formed 3D circular micro-tissue wrapping around flexible posts that deflect upon tissue contraction; post deflection, directly related to contractile force generation, was then measured by automated video microscopy. We have successfully automated labor intensive steps including cell seeding, image acquisition, and image analysis. The assay was further optimized to detect changes in contractile force with positive and negative inotropes. We are currently focusing on exploring potential disease phenotypes exhibited by hiPSC-cardiomyocytes carrying disease susceptible genetic variations using this 3D model system.

REPROGRAMMING

W-3192

DYNAMIC ERASURE OF GENE SILENCING DURING IPSC REPROGRAMMING AND X CHROMOSOME REACTIVATION

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Successful development relies on the establishment, maintenance and reversal of silent chromatin. Although the formation of facultative heterochromatin has been extensively studied, almost nothing is known about how stable gene silencing can be erased. Here, we use X chromosome reactivation (XCR) as a paradigm to study the mechanisms that orchestrate the reversal of stable gene silencing during reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). In order to define the precise dynamics of chromosome-wide gene silencing erasure, we generated allele-resolution transcriptome and epigenome maps during reprogramming to iPSCs. We show that transcriptional reactivation from the inactive X chromosome is a hierarchical and protracted process. We reveal that gene activation is initiated before the upregulation of late pluripotency genes and prior to silencing of the long non-coding RNA Xist, but completed only late during reprogramming. We then interrogated the relationship between the timing of transcriptional activation and genomic and epigenomic features. We will present results showing that early reactivating genes tend to reside in regions closer to genes that escape X chromosome inactivation and might be differentially targeted by pluripotency transcription factors. Furthermore, toward better understanding of the mechanisms underlying the prolonged nature of XCR during reprogramming, we employed epigenetic drug screens and identified histone deacetylation as a barrier to gene reactivation from inactive X chromosome. Our data suggest that continuous removal of active histone modifications by histone deacetylases might restrict transcriptional activation until the entry into pluripotency. Altogether, we report the dynamics of chromosome-wide transcriptional activation on the inactive X chromosome during reprogramming to iPSCs and provide insights into possible mechanisms behind the stability and reversal of gene silencing.

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W-3194

IN VIVO PARTIAL REPROGRAMMING OF PARENCHYMAL GLIA INTO NEURAL PROGENITOR CELLS IN THE MOUSE BRAIN

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Although the concept of adult neurogenesis has important implications for regenerative medicine, the formation of new functional neurons from progenitors during adult life is rare and occurs only in confined areas of the mammalian brain. Because adult neurogenesis is limited, the regenerative capacity of the brain is restrained and the possibilities of recovery from damage are almost absent. This project, focuses on a novel approach to engineer neurogenesis in vivo, based on nuclear cell reprogramming technology, to induce regeneration of damaged areas of the brain. The aim is to generate new neurons in regions naturally devoid of neurogenesis. The approach involves the overexpression of the Yamanaka factors (OCT4, SOX2, KLF4 and MYC: OSKM) directly in parenchymal glia, with the purpose of rejuvenating these cells back in development in order to recover their stem cell potential lost during specification. We hypothesise that this "rewinding" to a neural progenitor-like state may rearrange the local environment and remodel it towards a stem cell niche that help instruct and integrate new neurons within the preexisting circuits. Partial reprogramming through OSKM has been employed to convert fibroblasts into neural progenitor cells in vitro. To investigate the process of partial reprogramming in vivo, we use a combination of viral and transgenic mice approaches to drive the OSKM expression in specific gliaL cell types of the brain in a time and dose controllable manner.

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W-3196

GENERATION OF HUMAN CLINICAL GRADE AUTOLOGOUS IPS CELL LINES

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The Boston Autologous Islet Replacement Therapy (BAIRT) program seeks to generate pancreatic ß-cells from induced Pluripotent Stem (iPS) cell lines for replacement therapy in diabetic patients. We developed a GMP-compliant protocol to manufacture autologous clinical grade iPS cell lines. The process for reprogramming, iPS cell expansion and selection uses defined media throughout, without animal products. T cells were chosen as the starting material since the T cell receptor (TCR) rearrangement in every mature T cell creates a unique genetic marker of each individual iPS cell line and all subsequent differentiated cells derived from this line. The manufacturing process was performed in a dedicated negative pressure clean room. Peripheral blood mononuclear cells were isolated from two patients and T cells were activated with soluble CD3/CD28 antibodies and IL-2 for 3 days. Activated T cells were reprogrammed using cGMP Sendai virus Cytotune 2.1 (Thermo Fisher). Twenty iPS lines from patient 1 and 5 iPS lines from patient 2 were selected for further expansion. Several assays were used to evaluate the quality and safety of each iPS line including, transgene elimination, genetic integrity, pluripotency state and clonal TCR rearrangement. qRT-PCR testing for transgene elimination revealed that 54% of the iPS lines were transgene-free by passage 8. Five transgene-free iPS lines from each patient are subsequently tested for chromosomal abnormalities by G-banding karyotype. DNA from these lines are evaluated by targeted sequencing of 447 cancer-related genes (OncoPanel). Results are compared to donor T cell DNA to identify any mutations that might have arisen during the reprogramming process and subsequent expansion of individual iPS lines. TCR clonality assay is used to confirm that these lines are generated from individual T cells. Finally, iPS lines are confirmed to express pluripotency genes (Oct4, Sox2, Lin28, SSEA4, TRA-1-81, TRA-1-60) by qRT-PCR and/or flow cytometry. Using this rigorous GMP-compliant protocol, individual iPS Master cell banks will be stored for potential clinical use to generate autologous islet cells for transplantation in patients with diabetes. The same manufacturing protocol can be used to generate autologous iPS for patients with other indications.

W-3198

SINGLE CELL RNA SEQUENCING COMBINED WITH EPIGENOMIC PROFILING REVEALS DISTINCT ROLES FOR TRANSCRIPTION FACTORS DURING CARDIAC REPROGRAMMING

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Direct lineage conversion, whereby a somatic cell assumes a new identity, can be driven by ectopic expression of transcription factors. To understand the explicit transcriptional and epigenetic dynamics associated with direct cardiac reprogramming from a fibroblast to an induced cardiomyocyte driven by Gata4, Mef2c and Tbx5 (GMT), we evaluated RNA expression dynamics using single-cell RNA sequencing. Evaluation of the entire population on a single cell level indicated that a reprogramming trajectory was acquired within 48 hours of GMT introduction and that few transcriptional changes were detected beyond seven days, suggesting a mature reprogrammed cardiomyocyte can be acquired rapidly. We additionally found that genes associated with proliferation were not expressed during this process, suggesting cell division was not necessary for the reprogramming event to occur. This analysis also revealed that successful transduction with GMT was the rate-limiting step in reprogramming in this context as ectopic expression of these three factors was only detected in the reprogramming trajectory based on a pseudotime analysis. To better understand the molecular regulators that facilitate this process, we used a random-forest classifier that integrated the observed gene expression dynamics with regions of dynamic chromatin accessibility. Surprisingly, this analysis suggested that Mef2c and Tbx5 but not Gata4 were robust drivers of this cell fate conversion process and that Tbx5 in particular was likely involved in gene expression dynamics. DNA binding

profiles generated by ChIP-sequencing indeed revealed that sites bound by Mef2c and Tbx5 exhibited gains in chromatin accessibility in the absence of the other factors, supporting their role in driving the acquisition of the new cell fate. However, Gata4 binding sites exhibited limited chromatin dynamics as expected based on our expression analysis and predictions. Furthermore, ChIP-sequencing revealed that GMT were infrequently bound at the same genomic regions, supporting the prediction that GMT work independently during the early stages of reprogramming. Collectively, these results reveal novel mechanisms by which transcription factors promote cell fate decisions.

W-3200

FUNCTIONAL REJUVENATION OF AGED INTESTINAL STEM CELLS BY METABOLIC INTERVENTION AND DIRECT REPROGRAMMING

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Intestinal stem cells play an essential role in maintaining epithelial homeostasis and driving regeneration following damage. Aging impairs these processes and leads to a decreased ability to recover following injury. Here, we investigated both intestinal stem cells and Paneth niche cells to uncover age-associated functional, metabolic and molecular changes. Using in vivo regeneration and ex-vivo organoid assays, we defined a decline in intestinal stem cell function with age. This was accompanied with specific transcriptional and epigenetic changes associated with reduced Wnt signaling and a decrease in metabolic activity. Importantly, only partial rescue was achieved by elevation of Wnt signaling, while administration of a Nicotinamide adenine dinucleotide precursor to old mice restored metabolism, Wnt signaling, transcriptional profile and organoid formation frequency to levels found in young animals. Finally, using the predictive algorithm Mogrify, we unveiled Egr1, Irf1 and Fosb as key drivers of a “young” transcriptional network. Expression of these factors in organoids reprogrammed aged cells to a young state as evidenced by rescue of regenerative defects and re-establishment of a youthful metabolic phenotype. Our data demonstrate that changes in the epigenome and the associated transcriptional network of aged stem cells results in signaling and metabolic alterations. Notably, these changes can be efficiently reversed, which has direct implications for future aging intervention strategies.

W-3202

BRCA1 IS REQUIRED FOR REPROGRAMMING DUE TO ITS ROLE IN DOUBLE STRAND BREAK REPAIR DURING DNA REPLICATION

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BRCA1 tumor suppressor maintains genome integrity through multiple processes, including double-strand DNA break repair by homologous recombination (HR) and the protection of stalled replication forks (SF). It was recently shown that Brca1-deficient fibroblasts reprogram poorly, suggesting that Brca1 is also required for efficient iPS cell generation. To ascertain how BRCA1 contributes to iPS formation, here we examined reprogramming in mouse embryonic fibroblasts (MEFs) bearing well-defined separation-of-function mutations in the BRCA1 pathway. In particular, we studied MEFs with a Brca1 mutation that abrogates both homologous recombination and stalled fork protection (the HR–SF– phenotype), mutations in the Brca1-associated protein Bard1 that abolish SF but not HR (the HR+SF– phenotype), or loss of SMARCAL1, a DNA translocase which catalyzes replication fork reversal, that restores stalled fork protection in Brca1-mutant cells (the HR–SF+ phenotype).

Leveraging these genetic interactions, we observed markedly reduced reprogramming efficiencies in MEFs with the HR–SF– and HR–SF+ phenotypes, but not those with the HR+SF– phenotype. These results point to a critical role of Brca1 in the repair of double strand breaks by HR during reprogramming, rather than protection of stalled replication forks.

W-3204

DIRECT REPROGRAMMING OF MOUSE ASTROCYTES TO INDUCED-NEURONS USING MIRNAS AND SMALL NEUROGENIC MOLECULES: STUDY OF THE MOLECULAR MECHANISM AND IN VIVO POTENTIAL THERAPEUTIC EFFECT

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Direct neuronal reprogramming of glial cells has emerged as a promising approach for neuronal replacement using resident brain cells in order to alleviate neuronal loss due to neurodegeneration or trauma. Accordingly, astrocytic reprogramming to induced-neurons has been well-established by several studies in vitro and to some extent in vivo using a combination of transcription factors (TFs) or chemical cocktails, however little is known about the mechanisms that govern the reprogramming process. Additionally, miRNAs have emerged as critical post-transcriptional modulators of gene expression during neurogenesis and thus appear as attractive candidates to instruct direct astrocytic reprogramming, offering also the possibility of a non-viral delivery method for future in vivo therapy. Here, we have investigated the role of the brain enriched miRNA miR-124 and the small molecule ISX9 in inducing neuronal reprogramming of mouse cortical astrocytes, focusing on the elucidation of the core transcriptional mechanisms that instruct the reprogramming process. Our in vitro data indicate that forced expression of miR-124 potently reprograms cortical astrocytes into βIII-tubulin+/MAP2+ neurons, but with medium efficiency, which can be significantly improved by the addition of the small neurogenic molecule ISX9, leading to the acquisition of more mature neuronal phenotypes. Molecular characterization of induced cells with RNA-seq analysis, real time RT-PCR and immunofluorescence revealed that the major TFs upregulated early during the reprogramming process by miR-124 are the proneural bHLH TF, Mash1 and the homeobox TF, Gsx2 and to a lesser extent the bHLH TF Neurogenin2. On the other hand ISX9 greatly enhances the intermediate progenitor cells' TF, Tbr2, as well as the Neurogenin2/NeuroD1 transcriptional network. Interestingly, ISX9 also highly upregulates a set of TFs and other genes implicated in midbrain dopaminergic neuron development, including Lmx1b and Foxa1, possibly by upregulating components of the Shh signaling pathway, such as Gli1. Finally, miR-124 efficiently reprograms astrocytes to induced-neurons in vivo in a mouse model of cortical trauma, which is further facilitated by ISX9, highlighting its in vivo direct reprogramming capacity and potential therapeutic value.

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W-3206

THE FUNCTIONS OF CYTOPLASMIC M6A READERS YTHDFS IN SOMATIC CELL REPROGRAMMING

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N6-methyladenosine (m6A) is the most abundant internal modification present in the messenger RNAs of higher eukaryotes, and plays important roles in RNA metabolism, such as translation, RNA processing, and decay, via the recognition by the m6A readers. In the cytoplasm, there are three main readers with YTH domain called YTHDFs. The YTHDF1 promotes translation efficiency of mRNA with m6A modification, and YTHDF2 accelerates m6A modified transcripts decay. Recently, YTHDF3 has been reported functions in facilitating translation and also RNA decay by interacting with YTHDF1 or YTHDF2. Here, we report that the YTHDFs have different effects during somatic cells reprogramming. Deletion of YTHDF2 and YTHDF3 impair the generation of induced pluripotent stem cells, but not YTHDF1. In addition, YTHDF2 and YTHDF3 have different RNA decay pathway. YTHDF2 affects reprogramming through CCR4-NOT complex pathway, but YTHDF3 is through PAN2-PAN3 pathway. Knockdown of YTHDF2 and YTHDF3 inhibit the degradation of MEF related gene mRNAs in the early stage of somatic cell reprogramming, resulting in the loci of chromatin were wrong open and impair cell fate transition.

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W-3208

A SIGNALING-DIRECTED EPIGENETIC PATHWAY CONTROLS ACETYLATION SHIFT AND SOX2 NUCLEAR CYTOPLASMIC TRAFFICKING IN REGULATING NEURAL STEM CELL REPROGRAMMING AND DIFFERENTIATION

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Understanding direct signals that control epigenetic regulation to determine cell fate provides fundamental knowledge to develop pharmacological approaches to regenerate the injured brain. Here we report that an atypical protein kinase C (aPKC)-mediated Ser436 phosphorylation of CBP, a histone acetyltransferase, coordinates an acetylation shift between Sox2 and histone 2B (H2B) and Sox2 nuclear-cytoplasmic trafficking, thus modulating neural stem cell (NSC) reprogramming and differentiation. Using an ischemic stroke model, we first identified Sox2 protein nuclear import as an important feature for reprogramming of ischemia-activated pericytes (i-pericytes) into multipotent neural stem cells (i-NSCs) in culture. Subsequently, we show that inactivation of aPKC-CBP pathway using a phospho-null murine strain (CbpS436A) or an AMPK inhibitor, compound C, accelerates Sox2 nuclear import to facilitate reprogramming of i-pericytes into i-NSCs. This increased Sox2 nuclear import was also associated with reduced Sox2 acetylation while enhanced H2B acetylation during the reprogramming process. In contrast, activation of the aPKC-CBP pathway by an AMPK activator, metformin, enhances Sox2 acetylation while reducing H2B acetylation, elevating Sox2 nuclear export to promote neuronal differentiation of NSCs. Together, this study elucidates that pharmacological approaches targeting the aPKC-CBP pathway control acetylation shift and Sox2 nuclear cytoplasmic trafficking to modulate both NSC reprogramming from ischemia-activated pericytes and differentiation of the induced-NSCs into newborn neurons. This discovery provides fundamental knowledge to develop therapeutic strategies targeting in vivo cellular reprogramming/differentiation to promote local regeneration at the site of brain injury.

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TECHNOLOGIES FOR STEM CELL RESEARCH

W-3210

RESTORATION OF THE FVIII EXPRESSION BY TARGETED GENE INSERTION IN THE FVIII LOCUS OF THE HEMOPHILIA A PATIENT-DERIVED IPSCS

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Target-specific genome editing, using engineered nucleases ZFN, TALEN, and CRISPR/Cas9, is considered a promising approach to correct disease-causing mutations in various human diseases. In particular, hemophilia A can be considered an ideal target for gene modification via engineered nucleases because it is a monogenic disease caused by the mutation of coagulation factor VIII (FVIII), and a mild restoration of FVIII levels in plasma can prevent disease symptoms in patients with severe hemophilia A. In this study, we describe a universal genome correction strategy to restore FVIII expression in induced pluripotent stem cells (iPSCs) derived from patients with hemophilia A by the human elongation factor 1 alpha (EF1α) mediated normal FVIII gene expression in patient's FVIII locus. We used CRISPR/Cas9 mediated homology directed repair (HDR) system to insert the B-domain deleted from of FVIII gene with the human EF1α promoter. After gene targeting, we obtained the FVIII gene correctly inserted in iPSC lines at a high frequency (81.81%) and these lines retained pluripotency after knock-in and the neomycin resistance cassette removal. More importantly, we confirmed that endothelial cells from the gene-corrected iPSCs could generate functionally active FVIII protein from the inserted FVIII gene. This is the first demonstration that the FVIII locus is a suitable site for integration of normal FVIII gene and can restore FVIII expression by the EF1α promoter in endothelial cells differentiated from hemophilia A patient-derived gene-corrected iPSCs.

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W-3212

A SYSTEMATIC CRISPR/CAS9-MEDIATED ENDOGENOUS GENE TAGGING AND QUALITY CONTROL PIPELINE REVEALS STEM CELL ORGANIZATION AND GENOMIC, CELL BIOLOGICAL, AND STEM CELL INTEGRITY

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The Allen Institute for Cell Science has created a collection of fluorescently tagged hiPS cell lines to illuminate cell organization. To date we have edited ~40 unique loci including single- and dual-tagged lines targeting the major cellular structures. Recently we have tagged and RNA/DNA-binding protein that localizes to condensed phase compartments (RNA-binding protein FUS), a pluripotency transcription factor (sox-2), a signaling molecule (beta catenin), and dual tagged the nuclear envelope protein (lamin B1) with an ER marker (sec61 beta). The resulting clonal lines are carefully assessed for genomic and cell biology fidelity and then used for high resolution live cell imaging to visualize fusion protein localization and cell organization. Here, we present our methods for mono and bi-allelic editing of expressed genes, a novel Cas9-excisible selection strategy to tag silent genes in iPSCs, and the methods and workflows used to enrich the edited cell population and generate clonal lines with high rates of survival. Among our extensive quality control procedures, we have developed standardized PCR assays to screen clones for precise editing to ensure incorporation of the tag, absence of donor template insertion, and absence of NHEJ-mediated errors. After the genetically correct clones are identified, a subset of clones is further assayed for cell biological and stem cell integrity before a final clone is chosen for our imaging pipeline. We performed off-target analysis with Sanger sequencing for predicted sites as well as transcriptomic and exome sequencing for final clones. We have also developed PCR-based assays to evaluate genetic instability and other common mutations that arise during stem cell passaging. Using this suite of gene edited lines, we have found no off-target editing. However, we do find mutations that are associated with long term culture of hiPSCs. We will present our editing and QC pipeline and off-target analysis of the edited clones used to create the Allen Cell Collection. These cell lines, plasmids used to generate them, and a database of 3D images are available to the research community (www.allencell.org).

W-3214

COMPUTER-AIDED DESIGN AND 3D PRINTING OF POLYCAPROLACTONE SCAFFOLDS

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3D printing of scaffolds has lately been widely used with promising results due to the ability to precisely decide and design the internal micro and macrostructure of the scaffolds. Polycaprolactone (PCL) is one of the materials reported to be used for the fabrication of scaffolds due to its reasonable cost, thermoplastic characteristics and the ability to fabricate structures by Fused Deposition Modeling technology (FDM). Internal porosity and ability of the differentiated cells to adhere to the lattice structure is a crucial factor in the outcome of the fabricated construct. Increasing the internal porosity – which enables better cellular adhesion – unfortunately produces scaffolds with lower compressive and tensile moduli. The proposed designs of scaffolds all followed an orthogonal internal structure where the different printed layers are placed at a right-angle orientation to each other. Although shown to allow acceptable cellular differentiation and function, this design does not mimic the complex cellular orientation and extracellular matrix present in-vivo. Non-orthogonal internal structure is where the consecutive layers are not perpendicular to each and that is the orientation present in vivo and was reported to positive influence on the cellular behavior. The aim of this study is to evaluate the designing and printing of orthogonal and non-orthogonal pcl scaffolds using FDM printing technology. Two pore designs were used the conventional square -rectangular mesh and a honeycomb-like structure. Orthogonality was achieved by stacking the layers such that they were perpendicular to each other. The non-orthogonal architecture was achieved by orienting the consecutive layers at 45 and 90 angles to the first layer. The designing was made on AutoDesk computer software. Both structures were then printed in PCL material (filaments of 1.75mm diameter) with a nozzle dimeter of 20µ. The printed scaffolds were assessed under a microscope to assess the printed accuracy and evaluate the internal architecture of the fabricated scaffolds.

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W-3216

A HIGH-THROUGHPUT, NANOPATTERNED MEA PLATFORM FOR ASSAYING STRUCTURE-FUNCTION RELATIONSHIPS IN HUMAN PLURIPOTENT STEM CELL-DERIVED EXCITABLE CELLS

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Excitable cells derived from human induced pluripotent stem cell (hiPSC) sources have the potential to revolutionize current in vitro screening technologies as they make direct analysis of healthy and diseased human cells possible at scale. However, the inconsistent ability of such cells to recapitulate the structural and functional characteristics of their native counterparts has raised concerns regarding their ability to accurately predict the phenotypic impact of chemical or pathological insults on human tissues. Furthermore, the lack of biomimetic cytoskeletal organization within conventional cell culture environments prevents analysis of how structural changes in hiPSC-derived excitable cells affect their function. In this study, we used multiwell, nanotopographically-patterned microelectrode arrays (nanoMEAs) to investigate the effect of structural organization on hiPSC-derived cardiomyocyte and neuronal function. We found that nanoscale topographic substrate cues mimicking the size and orientation of native extracellular matrix fibers promote the development of more ordered cardiac and neuronal monolayers while simultaneously enhancing cytoskeletal organization, protein expression, and electrophysiological function. We then demonstrated that these phenotypic improvements act to alter the sensitivity of hiPSC-derived cardiomyocytes to treatment with arrhythmogenic and conduction-blocking compounds that target structural features of the cardiomyocyte. Similarly, we established that the sensitivity of hiPSC-derived neuron populations to synaptic blockers is increased when cells are maintained on nanotopographically-patterned surfaces. The improved structural and functional capacity of hiPSC-derived cardiomyocyte and neuronal populations maintained on nanoMEAs may have important implications for enhancing the predictive capacity of stem cell-based preclinical electrophysiological screening technologies in the near future.

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W-3218

ASSESSMENT OF DNA DOUBLE-STRAND REPAIR MECHANISMS IN HUMAN NAÏVE PLURIPOTENT STEM CELLS REGULATED BY THE TANKYRASE INHIBITOR XAV939

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Preserving genomic stability is critical during early embryonic development. Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are the main mechanisms that eukaryotic cells use for double-strand DNA break (DSB) repair and preservation of genomic integrity. Pre-implantation embryos and mouse embryonic stem cells (mESC) favor the more accurate HDR rather than the fast, but error-prone NHEJ. In contrast, lineage-primed post-implantation mouse epiblast stem cells (mEpiSC) and conventional, primed human pluripotent stem cells (hPSC) both predominantly utilize NHEJ for DSB repair. Functional naïve human pluripotent stem cells (N-hPSC) with improved HDR could greatly impact developmental biology and regenerative medicine. The Zambidis lab recently established that LIF and triple chemical inhibition of WNT, MEK and tankyrase (XAV939) pathways (LIF-3i) stably revert primed hPSC to a functional human naïve state that recapitulates molecular and epigenetic signatures of the human pre-implantation epiblast. More importantly, LIF-3i-reverted N-hPSC maintained normal karyotypes and epigenomic imprints. The study of HDR/NHEJ mechanisms may functionally validate the predominant DSB repair strategy utilized by N-hPSC. Herein, we investigated the HDR machinery of LIF-3i reverted N-hPSC and their isogenic, primed hPSC counterparts (E8 culture). We detected DSBs in hPSC by quantitating p-H2AX protein levels via Western blotting following DNA damage induction using the radiomimetic agent neocarzinostatin (NCS). N-hPSC accumulated less p-H2AX in LIF-3i on feeders or in feeder-free conditions relative to isogenic primed hPSC controls, but responded robustly to NCS. Interestingly, LIF-3i appeared to interrupt tankyrase auto-PARylation-mediated proteolysis. Elevated tankyrase protein in N-hPSC correlated with stabilization of the non-cleaved isoform of its partner MDC1 and reinforcement of RAD54 and BRCA1. Functional HDR machinery in N-hPSC was validated using a HDR/NHEJ transgenic reporter system. We propose that modifying the balance between NHEJ and HDR events by manipulating naïve vs. primed pluripotent states may enhance genome editing strategies (e.g., CRISPR-Cas9, plasmid-based HDR), and allow more facile gene targeting of hPSC.

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W-3220

USE OF THE NANOBRIDGE SYSTEM FOR RAPID EXPANSION OF HUMAN PLURIPOTENT STEM CELL AGGREGATES AND DIFFERENTIATION TO NEURAL PROGENITOR CELLS AND CARDIOMYOCYTES

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Here we demonstrate the reproducible expansion of human pluripotent stem cells (hPSC) and their differentiation to neural progenitors (NPC) or cardiomyocytes using the Nanobridge system. The Nanobridge system utilises a thermo-responsive poly N-isopropyl acrylamide (PNIPAM) polymer decorated with extracellular matrix protein fragments to bind to and bridge between adjacent cells and form aggregates at 37°C. A temperature shift from 37°C to 32°C causes the PNIPAM to become water soluble weakening the bonding between adjacent PNIPAM chains and allowing the aggregates to be broken down to smaller aggregates through increased shear forces. Repeating this cycle each passage allows for rapid expansion of cell numbers. In addition, the ability to vary the concentrations and ratios of the two components in the Nanobridge system, coupled with the temperature shift procedure during passage, allows for tight control over aggregate diameters at all stages of the expansion process. In this work, we use the Nanobridge system to reproducibly expand pluripotent stem cells in 3D environments coupling this process to ectoderm or mesoderm directed differentiation. Using the system for the rapid expansion of hPSC we show a consistent expansion of aggregates to an average diameter of 348 µm and reproducible passage to an average of 139 µm after temperature shift. Over 5 passages, there was a 500 fold increase in cell number, with 90% viability and maintenance of pluripotent markers NANOG, OCT3/4 and SOX2. Differentiating these cells to NPCs, cells demonstrated upregulation and subsequent maintenance of neural-associated markers (PAX6, SOX1, NCAM) in aggregate culture up to 96 days and neurite outgrowth representing maturation. Passaging resulted in an overall seven fold increase in the number of cells expressing the neural-associated markers. Over long term culture there was no evidence of necrotic cores or diffusion limitations. For differentiation to cardiomyocytes, cells expressed typical markers for all cardiomyocyte subtypes with the highest percentage being ventricular cardiomyocytes. We have demonstrated that the Nanobridge system has the potential to facilitate the scale-up of pluripotent cells and differentiated progeny in bioreactors for applications in regenerative medicine and pharmacological testing.

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W-3222

A FLUORESCENT REPORTER OF CELL CYCLE SPEED

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Cell fate transitions are often accompanied by profound changes in cell cycle dynamics. The ability to identify and isolate live cells with divergent proliferation rates can benefit the study of cell fate control. Currently available strategies to analyze proliferation in live cells focus on specific cell cycle phases; require invasive labeling procedures; lack sensitivity/resolution; or are not tractable for use in vivo. We have developed a genetic reporter that measures the relative cell cycle speed of live cells in a single measurement using two fluorescent wavelengths. This reporter is based on the color-changing Fluorescent Timer (FT) protein, which emits blue fluorescence when newly synthesized before maturing into a red fluorescent protein. Its ability to report cell cycle speed exploits the different half-life of the blue vs. red form of the same molecule, as predicted by mathematical modeling. When a Histone H2B-FT fusion protein is expressed at steady-state in heterogeneously dividing cells, faster-cycling cells can be distinguished from slower-cycling ones by the intracellular ratio between blue and red fluorescent wavelengths. Cell cycle perturbation experiments validated the H2B-FT transgene as a bona fide reporter of cell cycle speed in a variety of cultured mammalian cell lines. Additionally, the

color profile of virally expressed H2B-FT faithfully tracked with previously known proliferation kinetics of blood stem and progenitor cell types in vivo. We targeted an inducible H2B-FT allele into the endogenous HPRT locus, which was crossed with a model of acute myeloid leukemia driven by the MLL-ENL fusion oncogene. This allowed us to assess cell cycle length heterogeneity in normal and malignant hematopoiesis. As the H2B-FT is compatible with flow cytometry, it provides a strategy to physically separate subpopulations of live cells cycling at different rates for downstream analysis. We anticipate this system to be useful in diverse cell types and tissue contexts for dissecting the role of cell cycle speed in development and disease.

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W-3224

INFLUENCE OF THREE-DIMENSIONAL MICROENVIRONMENTAL PRIMING OF HUMAN MESENCHYMAL STEM CELLS IN HYDROGEL SYSTEMS ON RETROVIRAL TRANSDUCTION

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There are numerous approaches to improve the low transduction efficiency of retroviral vectors on two-dimensional (2D) cell culture substrates. However, the effect of a three-dimensional (3D) microenvironment, which better mimics in vivo conditions, is unknown. Cytocompatible hyaluronic acid (HA) hydrogels are a good candidate to study this issue. Here, photocrosslinkable HA hydrogels with an elastic modulus of 1.0–2.7 kPa are successfully prepared by varying the degree of methacrylation in the HA backbone. The culture of human adipose-derived stem cells (hASCs) in a 3D microenvironment significantly reduces the amount of time required for retroviral gene transduction compared with a 2D conventional method and maintains a high transduction efficiency. This acceleration of retroviral gene transduction correlates with the rate of cell cycle synchronization. hASCs cultured in a 3D microenvironment have a shorter G1 phase and total cell cycle length than hASCs cultured using a 2D conventional method. This cell cycle regulation is dependent on cyclin D1 expression. In summary, the prior culture of hASCs in a 3D microenvironment accelerates retroviral gene transduction by regulating cyclin D1 expression and accelerating cell cycle synchronization. We conclude that priming via culture in a 3D microenvironment facilitates efficient and rapid retroviral gene transduction of hASCs without inducing apoptosis.

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W-3226

ROBOTIC CELL CULTURE, MULTI-LINEAGE DIRECTED DIFFERENTIATION, AND TRANSLATIONAL APPLICATION OF HUMAN IPSCS

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Clinical translation of human induced pluripotent stem cells (iPSCs) critically depends on implementing robust, scalable, and standardized methods for quality-controlled cell lines and their optimized long-term growth and functional differentiation. Currently, culturing iPSCs is variable and labor-intensive and these process development and manufacturing limitations pose major challenges for many downstream applications of patient- and disease-specific cell lines. Here, we established robotic workflows under defined feeder-free conditions that fully automate and industrialize all essential steps of iPSC culture, thereby allowing parallel-processing of multiple cell lines and directed differentiation into various cell types. Importantly, single-cell transcriptomic analysis of different pluripotent cell lines demonstrated that robotic cell culture can produce highly homogenous and pure populations of ectodermal, mesodermal, and endodermal precursors. These multi-lineage precursors were further differentiated into functional cells (e.g.

neurons, cardiomyocytes, hepatocytes) and utilized for assay development enabling high-throughput screening and Zika virus experiments. Taken together, this work demonstrates how automation can help to overcome translational challenges in the stem cell field and facilitate standardization and scalability of the iPSC technology for disease modeling, drug discovery, and cell therapeutics.

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W-3228

THE ROLE OF NOTCH2NL IN HUMAN DEVELOPMENT AND EVOLUTION

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Autism prevalence has continued to rise in the past few decades, now approaching 2% of the American population. Efforts such as Genome Wide Association Studies (GWAS) to understand the genetic causes of autism have come up largely empty. Autism is an extraordinarily hard disease to study because it is caused by complex interactions of multiple genes. Despite failing to find specific causal genes, studies have identified a handful of genomic regions as hotspots for copy number variation that is associated with disease. Interestingly, these regions are enriched for genes known to play a role in neurodevelopment, so, in recent years groups have shifted to studying autism from the angle of brain development. iPS and ES cell lines have revealed numerous genes expressed in the developing cortex that affect the total number of neurons. One such gene family, NOTCH2NL, exhibits copy number variations that have been associated with diagnoses of autism as well as other related neurodevelopmental disorders. This gene family is particularly interesting because it is only expressed in humans and our closest ancestors. Recent work of ours has shown the gene emerged after our common ancestor with chimpanzee. The goal in this project was to better understand the population genetics of NOTCH2NL by sequencing and assembling its paralogs in several people. We hypothesized that there would be overall similarity between paralogs and that copy number would be stable because of the known relationship of deletions with autism. To date, we have sequenced and assembled NOTCH2NL paralogs for 20 neurotypical and 10 patient samples. Contrary to our hypothesis, we have found substantial diversity in these paralogs, both within and between individuals. We also found that only two of the three NOTCH2NL paralogs in this gene family never had deletions. Together, these results suggest that the functional role of NOTCH2NL may rely on only a subset of the paralogs, or that the resulting protein does not substantially change with the predicted amino acid substitutions.

W-3230

INTEGRATING CA2+ INDICATOR WITH LUMINESCENT REPORTER TO ADVANCE INSULIN SECRETAGOGUE IDENTIFICATION AND β-CELL DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) have been recognized as a potential source for generating therapeutic β-cell for treating diabetes. However, despite recent improvements in the β-cell differentiation process, the efficiency of generating mature and functional iPSC-derived β cells remains low. Voltage-dependent Ca2+ channels play a crucial role in stimulus-secretion coupling in β cells. As such a reliable method that enables the simultaneous measurement of Ca2+ flux and insulin secretion is crucial to facilitate the high-throughput identification of insulin secretagogue as well as compounds that can enhance β-cell differentiation. By combining a Ca2+ indicator together with insulin-luciferase fusion protein, we were able to rapidly detect secretion of insulin into the supernatant upon the addition of luciferin, as well as visualize Ca2+ flux, which can be measured by red fluorescent protein (RFP) signal at a single cell level. To identify new targets that may enhance β-cell differentiation, we utilized our platform to assess a number of drugs, including Nateglinide and 3-isobutyl-1-methylxanthine (IBMX) that target various pathways associated with β-cell function. To date, we have shown that the use of IBMX as a cAMP-raising agent significantly increases the expression of pancreatic markers including NGN3 and PDX1. This also induced elevated Ca2+ influx through voltage-dependent Ca2+ channels, which enhanced C-peptide expression and could improve the efficiency of iPSCs to differentiate into β cells. Taken together, our current progress demonstrated the feasibility of combining Ca2+ indicator together with luminescent reporter in facilitating insulin secretagogue identification and research in β-cell differentiation.

W-3232

OPTIMIZED MEDIA AND WORKFLOW FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN SUSPENSION CULTURES

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3D suspension culture enables scale-up of human pluripotent stem cell (hPSC) manufacturing. However, media and methods optimized for 2D adherent cultures can lead to low volumetric productivity and laborious workflow in suspension culture. To overcome these limitations we developed fed-batch media based on either mTeSR™1 (BSA-containing) or TeSR™-E8™ (animal component-free) for hPSC expansion as aggregates in suspension culture. Fed-batch feeding protocols are more efficient and cost-effective than batch media changes because only exhausted components are replenished. Optimization studies were performed using human embryonic (H7 and H9), and human induced pluripotent (WLS-1C and STiPS-M001) stem cell lines. Suspension cultures were fed daily using either 50% medium exchange of standard 2D media, or fed-batch optimized media and protocols. hPSC aggregate diameter must be kept below 350 µm to maintain cell viability and phenotype. With observed growth rates, aggregates required passaging every 3 or 4 days into clumps of 5-10 cells with Gentle Cell Dissociation Reagent. Clumps were re-seeded into fresh test medium plus 10 µM Y-27632. Passaging and feeding cycles were repeated for at least 5 passages. Optimization was performed by iteratively modifying the feed solution to maintain consistent nutrient levels and maximal growth rate while maintaining cell quality. The concentration of feeding solutions were optimized to minimize volume changes during feeding cycles which can negatively impact mixing during cell culture. To enable fed-batch protocols to be applied to a wide variety of cell lines with differing growth rates, feeding schedules with 3 day passaging cycles, alternating 3 / 4 day cycles and 3 day / 4 day with 50% media exchange on day 4 have been developed and tested. By selecting the appropriate feeding schedule robust and repeatable cell expansion can be achieved during scale-up. Control and optimized fed-batch formulations demonstrated between 1.4 and 1.8-fold expansion per day, >90% viability, Oct4 and TRA-1-60 expression >90%, in vitro trilineage differentiation, and normal karyotype (n=8 independent cultures). Suspension culture optimized mTeSR™-3D or TeSR™-E8™3D fed-batch media enables cost-effective production of hPSCs as aggregates with efficient workflow and high cell quality.

W-3234

PHAGE DISPLAY FOR DISCOVERY OF NOVEL SPECIFIC TARGETING PEPTIDE IN HUMAN DENTAL PULP STEM CELLS (DPSCS)

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Phage display peptide libraries have enabled the discovery of peptides that selectively target specific organs. Identification of organ-specific peptides is mediated through interaction between peptide displayed on phage coated protein with adhesion molecules expressed in targeted organs. Dental-Derived Mesenchymal Stem Cells are great source for future applications in regenerative dentistry. Dental pulp stem cells (DPSCs) are having an unique phenotype of MSCs residing in the pulp tissue of teeth. Our research aim is to identify a novel dental pulp stem cell-specific targeting peptides (DPSC-SPs) to suggest a new method to stimulate adhesion and differentiation for dental pulp stem cells. Peptide library screening in vivo was performed on human dental pulp stem cells with Ph.D.™-12 phage display peptide libraries. Three specific peptide sequences (SHAVTKHTGARS, SYLHNFNAVRS, and RGPQPDRTS) which were enriched in dental pulp stem cells, were screened, and respectively, named DPSCSP-SH, DPSCSP-SY, and DPSCSP-RG) through three rounds of biopanning. SPs were compounded and labeled with fluorescein isothiocyanate (FITC). The specificity and affinity of FITC-SPs were evaluated in human dental pulp stem cell lines in vitro by immunofluorescent staining. Results showed that only DPSCP-SH specifically bound to the cell membrane DPSCs in vitro. In conclusion, the novel 12-residue peptide DPSCSP-SH peptide is a special biomaterial for human dental pulp stem cells. We propose that this material can be used in regeneration and therapy of dental pulp stem cells (DPSCs).

W-3236

TOWARDS CLINICAL-GRADE MESENCHYMAL PROGENITORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human mesenchymal stem cells (MSCs) are a strong candidate for cell therapies owing to their regenerative potential, paracrine regulatory effects, and immunomodulatory activity. Human induced pluripotent stem cell-derived mesenchymal progenitor (iPSC-MP) cells closely resemble adult MSCs, but in contrast can be produced in large numbers from every patient, strengthening their potential for personalized clinical applications. For therapeutic applications, human iPSC-MP cells must be produced without animal-derived components (i.e., xeno-free) and in accordance with Good Manufacturing Practice (GMP) guidelines. In the present study, we have studied the effects on fitness and function of culturing human iPSC-MP cells for ten passages in medium supplemented with human platelet lysates (HPL, xeno-free) and in the high-performance GMP-compatible medium (Unison Medium). We find that long-term culture in xeno-free and GMP-compatible media had minimal effects on most iPSC-MP phenotypes relative to culture in traditional medium supplemented with fetal bovine serum, with only slight alterations in the morphology, expansion potential, gene expression and cytokine profile. The findings shed new light on the biology of human iPSC-MP cells, and support the potential to manufacture large numbers of clinical-grade human iPSC-MP cells for use in personalized regenerative medicine.

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W-3238

REGULATION OF INTRACELLULAR CALCIUM CONCENTRATION IN HUMAN MESENCHYMAL STEM CELLS DURING CHONDROGENIC DIFFERENTIATION

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Articular cartilage is an avascular, dense tissue with poor regenerative capacity. Therefore, human mesenchymal stem cells (MSCs) are of great interest in cartilage regeneration due to their high potential to differentiate into chondrocytes. MSCs can be isolated from almost all human tissues, including menstrual blood (MenSCs). Although MenSCs isolation has many advantages as compared to classical bone marrow MSCs (BMMSCs), due to low cost and ease of access, these cells are little investigated so far. During MSCs chondrogenic differentiation, calcium ions (Ca2+) play a crucial role in regulating cell functions and improving cell differentiation potential. The aim of this study was to evaluate differences in intracellular Ca2+ levels in MenSCs and BMMSCs and its regulation mechanisms, focusing on chondrogenic differentiation potential. The cells were treated with different types of Ca2+ channel regulators, including voltage operated calcium channel (VOCC) inhibitors (nifedipine, etc.) and agonists, TRPV4 channel inhibitor and endoplasmic reticulum Ca2+ channel inhibitors. Cell proliferation capacity was analyzed using cell proliferation kit 8 (CCK-8) (spectrophotometry). Intracellular Ca2+ levels were measured using fluorescent dye Cal-520 (flow cytometry). Chondrogenic differentiation was evaluated by Safranin, Collagen II antibody staining (immunohistochemistry) and gene analysis (RT-PCR). Different Ca2+ channel inhibitors downregulated proliferation capacity in both cell types, whereas agonists stimulated it. Intracellular Ca2+ concentration was remarkably higher in MenSCs, as compared to BMMSCs. However, chondrogenic differentiation capacity was similar in MenSCs and BMMSCs, according to Collagen II staining and SOX9 gene expression. Furthermore, VOCC regulators improved chondrogenic differentiation potential in both cell types, as well as indirectly increased intracellular Ca2+ levels through endoplasmic reticulum Ca2+ stores. In addition, different Ca2+ channel regulators had different effects on intracellular Ca2+ levels in MenSCs and BMMSCs. In conclusion, intracellular Ca2+ levels are different in MenSCs and BMMSCs, which may lead to better understanding of calcium signaling during chondrogenesis and to the development of new therapies for cartilage defects.

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W-3240

TECHNIQUES FOR CRISPR/CAS9-FACILITATED GENOME EDITING AND SINGLE-CELL CLONING OF HPSCS

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Induced pluripotent stem cells (iPSCs) are versatile in vitro model systems for studying human disease mechanisms directly in patient-derived cells. CRISPR/Cas9-faciliated genome editing has enabled efficient genetic manipulations for many mammalian cell types. Together, these two technologies have synergistic potential to create a vast number of new discovery tools for biomedical research. Yet genome editing in human iPSCs still poses many challenges, such as ensuring consistent delivery of CRISPR/Cas9 reagents into iPSCs, optimizing screening workflows to conserve reagents, and maintaining pluripotency and genomic stability in edited clonal cell lines. We found that iPSCs adapted to single-cell passaging transfect more efficiently and demonstrate high rates of indel formation using sgRNA-Cas9 ribonucleoprotein (RNP) complexes. Edited clones retained a normal karyotype after screening. We used a combination of various commercially available reagents and analyzed genomic DNA sequencing results using free online bioinformatics software. Finally, we saw greater viability and continued growth of iPSCs that were sorted and cloned as single cells using a microfluidics chip-based cell sorter. In sum, our results show that efficient genome editing in human iPSCs is possible using currently available reagents and techniques.

Funding Source: Advanced Technology Cores at Baylor College of Medicine

W-3242

HUMAN MESENCHYMAL STROMAL CELL EXTRACELLULAR VESICLE MOLECULAR PROFILING USING PROTEOMICS SHOWED IMPORTANT MEDIATORS OF CELL-CELL INTERACTIONS

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Extracellular vesicles (EVs) are considered to be a major paracrine effector in therapeutic responses produced by human mesenchymal stromal/stem cells (hMSCs). As the hMSC-EV regenerative capacity is mainly ascribed to the transfer of proteins and RNA composing the EV cargo and to the activity attributed by the protein surface markers, we sought to profile the protein composition of hMSC-EVs using a quantitative proteomics analysis. hMSC-EVs were produced from 5 MSC donors following a 48h culture in exosome depleted medium followed by steps of centrifugation and filtration. Nanoparticle Tracking Analysis showed no differences in the hMSC-EV concentration and size among the 5 donors (1.83 x 1010 ± 3.23 x 109/mL), with the mode particle size measuring at 109.3 ± 5.7 nm. Transmission Electron Microscopy confirmed the presence of nanovesicles with bilayer membranes. Flow cytometric analysis identified EVs expressing exosomal (CD63/81/9) and hMSC (CD105/44/146) markers and western blot analysis confirmed an enriched expression of MMP-2 in hMSC-EVs. Quantitative proteomic analysis performed using Tandem Mass Tag labeling combined to LC-MS/MS identified 5108 proteins in parental hMSCs versus 782 proteins in hMSC-EVs, of which 270 proteins were enriched by at least 2-fold (FDR p-value <0.05) in hMSC-EVs vs hMSCs. The dataset showed 65 proteins in common with the top 100 proteins from the EV Exocarta database. Proteomic analysis also confirmed the presence of known exosomal tetraspanins (CD63/151), integrins (alpha 5/CD49e and beta 1/CD29), and adhesions molecules such as Cadherin 5 type 2, as well as novel surface proteins (currently under investigation) involved in cellular movement pathways important in migration and invasion of cells, as well as chemotaxis and vasculogenesis. Our hMSC-EV production workflow and proteomics profiling of the protein composition from multiple MSC donors has yielded not only commonly reported exosomal and hMSC markers, but also novel mediators of cell-cell interactions which may help to unravel hMSC's mechanism of action.

Funding Source: Canadian Genomics Research and Development Initiative (GRDI) Phase VI

LATE-BREAKING ABSTRACTS

W-4002

ENHANCED EXOSOME SECRETION FROM MESENCHYMAL STEM CELLS BY HYPOXIC 3-DIMENSION CULTURE, THROMBIN AND CYTOKINE COCKTAIL

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Islet transplantation is most efficient therapy of type 1 diabetes. However, islet engraftment failure by immune rejection is the unsolved problem of islet transplantation therapy. MSC derived exosomes are one of cell signaling factors mediated cell-to-cell communication by carrying miRNAs, proteins and lipids in their cargo. Therefore, MSC derived exosomes have potential as cell-free-therapy adjuvants through immunomodulatory effect of MSCs. We aimed to promote exosome secretion from MSCs by hypoxic 3-dimension (3D) culture, thrombin and cytokine treatment, and analyze which exosomal contents improved islet viability. MSCs spheroids forming by 3D culture secrete more exosomes and have more functional effect than 2D monolayer culture. Therefore, we compared exosomes secretion isolated from 2D, 3D culture MSCs with or without hypoxic condition and treatment Thrombin and cytokines are known for exosome secretion enhancing materials. MSCs are isolated from pancreas exocrine cells from 10~12 weeks C57BL/6 mice and under PN 5 cells are cultured by 2D, 3D culture method. 2D and 3D cultured MSCs are treated with Thrombin (50U, 100U/ml), cytokine (TNFα, INFγ 40ng/ml), Thrombin (50, 100U/ml) with cytokine. Cells were incubated in hypoxia (O2 1%) and normoxia (O2 20%) during 72hr, then conditioned media was collected. Exosome was isolated from MSC-conditioned media by precipitation method using total exosome isolation reagent. The quantity of isolated exosomes was measured by protein quantification and exosomal marker (CD81, CD9, TSG101) was confirmed by western blotting. Protein profiling assay was progressed for analyzing exosomal proteins that have therapeutic effects. Through this experiment, MSC-derived exosome secretion was enhanced in hypoxia than normoxia and observed the highest quantity in 3D-hypoxia-100U/ml of Thrombin with cytokine condition. Further study, we expect to confirm immunomodulatory reaction of 3D-hypoxia- MSC enhanced-exosomes to islet viability in vivo and in vitro.

W-4004

DEVELOPMENT OF A CENTRALLY VASCULARIZED TISSUE ENGINEERING BONE GRAFT WITH THE UNIQUE CORE-SHELL COMPOSITE STRUCTURE FOR LARGE FEMORAL BONE DEFECT TREATMENT

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Great effort has been spent to promote the vascularization of tissue engineering bone grafts (TEBG) for improved therapeutic outcome. However, the thorough vascularization especially in the central region still remained as a major challenge for the clinical translation of TEBG. Here, we developed a new strategy to construct a centrally vascularized TEBG (CV-TEBG) with unique core-shell composite structure, which is consisted of an angiogenic core and an osteogenic shell. The in vivo evaluation in rabbit critical sized femoral defect was conducted to meticulously compare CV-TEBG to other TEBG designs (TEBG with osteogenic shell alone, or angiogenic core alone or angiogenic core+shell). Microfil-enhanced micro-CT analysis has been shown that CV-TEBG could outperform TEBG with pure osteogenic or angiogenic component for neo-vascularization. CV-TEBG achieved a much higher and more homogenous vascularization throughout the whole scaffold (1.52-38.91 folds, $p < 0.01$), and generated a unique burrito-like vascular network structure to perfuse both the central and peripheral regions of TEBG, indicating a potential synergistic effect between the osteogenic shell and angiogenic core in CV-TEBG to enhance neo-vascularization. Moreover, CV-TEBG has generated more new bone tissue than other groups (1.99-83.50 folds, $p < 0.01$), achieved successful bridging defect with the formation of both cortical bone like tissue externally and cancellous bone like tissue internally, and restored approximately 80% of the stiffness of the defected femur (benchmarked to the intact femur). It has been further observed that different bone regeneration patterns occurred in different TEBG implants and closely related to their vascularization patterns, revealing the potential profound influence of vascularization patterns on the osteogenesis pattern during defect healing.

W-4006

HPSCREG'S CLINICAL STUDY DATABASE FOR HPSC-DERIVED CELL THERAPIES

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The generation of human pluripotent stem cell (hPSC) lines at multiple sites, such as different kinds of research facilities (e.g. core facilities, individual research laboratories, biobanks) lends to a high degree of variability in the availability of donor information and the characterization and production process generating PSC lines. Coupled with additional variability introduced by cross-border regulatory regions and cultures, it is difficult to compare and evaluate lines that originate from diverse sources. To make hPSC data FAIR (Findable, Accessible, Interoperable and Re-usable), the Human Pluripotent Stem Cell Registry (hPSCreg; https://hpscreg.eu) collects a wide range of PSC-related data in standard formats, including ethical provenance, evidence of pluripotency, and genetic constitution. The standardized collection of these key data enables an informed assessment of registered hPSC lines for their applications in research and clinical translation by academia, regulatory bodies, and industry. To further monitor the success of hPSC-based cell therapies from their source hPSC cells, hPSCreg has created a clinical study database specifically for clinical applications of hPSC-derived cells (https://hpscreg.eu/browse/trials).

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W-4008

CELLULAR PROTECTIVE EFFECT OF ONCOGENESIS ASSOCIATED DJ-1 GENE IN CANINE MESENCHYMAL STEM CELLS

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As cancer and neurodegenerative disorder involved protein, DJ-1 have already been studied in order to confirm that putative cellular functions induce the human diseases. Especially DJ-1 involves the response against oxygen species (ROS) and protective role to cells from oxidative stress. However, the cellular protective mechanism of DJ-1 is not fully understanding, and we need to be further study their functions in novel organism. In the present study, we initially investigated the cellular functions of DJ-1 activated by oxidative stress in the established gene modified canine mesenchymal stem cells. On the basis of these experiments, canine amniotic fluids stem cells isolated and DJ-1 gene modified cell lines were established. The results showed that DJ-1 overexpressed cells were up-regulated cell viability under oxidative stress, whereas loss of DJ-1 cells were down-regulated the cell survival activity. Additionally, overexpression of DJ-1 cells increased cell resistance to oxidative stress and inhibited the elevation of cell death and cellular ROS-induced apoptosis. On the contrary to this, DJ-1 null cells show defective cellular protection against oxidative stress conditions. The results showed that DJ-1 protein significantly promoted cell viability and survival activity antioxidant against the oxidative damage by attenuates cellular apoptosis and ROS generation. Through these ontology results, we searching the candidate gene related to the cell protective function of DJ-1. Present studies were conducted into the cellular functions of canine were investigated in the first time focus on DJ-1 gene. Furthermore, canine mesenchymal stem cells are investigated to the current approaches for therapeutics application before clinical trials. These findings would be used to support the basic framework of disease research of cancer and neurodegenerative disorder in human and dogs.

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W-4010

DELINEATING THE TRANSCRIPTIONAL REGULATORY ROLES OF RETROTRANSPOSONS IN HUMAN PLACENTA

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Approximately 40% of the human genome consists of retrotransposons, which are transposable elements that replicate via an RNA-mediated “copy-and-paste” mechanism. They are further divided into two classes: long terminal repeats (LTR) retrotransposons, also termed endogenous retroviruses (ERVs), and non-LTR retrotransposons. If left unchecked, these sequences can potentially dysregulate gene expression, affect genome stability and induce mutations. Given their potential deleterious effects, retrotransposons are generally repressed by epigenetic mechanisms. Intriguingly, a subset of elements has been co-opted to become integral parts of our genomes, including functioning as cis-regulatory elements or as coding sequences. Interestingly, the placenta is particularly permissive to retrotransposon activities. Perhaps due to this feature, the best-studied example of ERV domestication is found in placental cells. The ERV-derived protein SYNCYTIN play critical roles in the differentiation of syncytiotrophoblasts. Previous studies have also found retrotransposons to serve as placenta-specific enhancers or alternative promoters in both mouse and human genomes. These studies hint at the potential regulatory functions of retrotransposons in shaping placental transcriptomes and their putative involvement in placental disorders. Here we use state-of-the-art techniques to generate epigenomic and transcriptomic profiles from human placenta samples. We then perform integrative analysis to delineate the regulatory roles of retrotransposons in human placenta. We discovered that those elements contribute to placenta-specific splicing events. Moreover, we identified a specific AluJb element as a placenta-specific promoter for the LIN28B gene. Importantly, dysregulation of this gene was reported in placenta samples of preeclampsia patients. Taken together with findings from previous studies, we further investigate whether this novel transcript contributes to preeclampsia. Our study provides insights into the role of retrotransposon in placental development and placental diseases.

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W-4012

REAL-TIME MONITORING OF CYTOTOXIC EFFECT OF ANDROGRAPHOLIDE ON HUMAN PRIMARY GLIOBLASTOMA CELLS

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Andrographolide, an active diterpenoid compound extracted from the leaves and stem of andrographis paniculata, shows some pharmacological activities, such as anti-inflammatory, and especially the anti-cancer effects. In the test of the animal model, it is also known as a nearly non-toxic compound. In recent studies, the main causes of cancer are chronic inflammation and high blood glucose level. The purpose of this study was to investigate the cytotoxic effect of andrographolide, and the effects on cellular morphology, proliferation, and migration on human primary glioblastoma cells (U-87MG). The heart of this study was the use of electric cell-substrate impedance sensing (ECIS), which monitored subsequent changes of the overall impedance of the cell monolayer and of cell micromotions in responds to different levels of andrographolide for 72 hours. Our ECIS data indicated that the exposure of U-87MG cells to andrographolide (10, 30, or 100 mM) for 48hrs caused the dose-dependent decrease of the overall measured resistance at 4 kHz. The reduction of impedance fluctuations due to cellular micromotion and wound-healing recovery rate were also observed. Parallelly, biochemical assays such as trypan blue, alamar blue cell viability assay, and western blot showed the apoptotic effect of andrographolide on U-87MG cells. In summary, our results suggest ECIS, a useful tool to real-time monitor the cell growth, and andrographolide, might be used for potential cancer treatment in the clinic. The clinical use of andrographolide on brain tumor awaits further investigation.

W-4014

CHARACTERIZATION OF INTEGRIN α6 SPLICED FORMS IN HUMAN IPSC AND CANCER STEM CELLS

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Induced Pluripotent Stem Cells (iPSC) have great promise in regenerative medicine, however they have malignant potential. Cancer is characterized by cell heterogeneity including a subpopulation of cancer stem cells (CSC) that self-renew and maintain the tumor. Interestingly, the maintenance of stemness in both iPSC and CSC is associated with specific integral membrane proteins of the integrin family, mainly integrin alpha 6 (ITGA6) and integrin beta 1 (ITGB1), which heterodimerize with

each other. Intregrin beta 4 (ITGB4) can also heterodimerize with ITGA6, but usually is expressed after cells have differentiated. ITGA6 has alternative splice variants that have been linked to differentiation, which are often overlooked in RNA seq analysis. In this project, we aimed to characterize ITGA6 splice variants in relation to changes in differentiation states and phenotypes of iPSC and breast cancer cell lines. In order to induce cell differentiation and phenotype changes we challenged iPSC and cancer cells with differing microenvironment conditions including hypoxia, serum containing and serum free media, and conditioned media from iPSC or cancer cells. Levels of ITGA6 splice variants were measured by RT-PCR after each treatment. Further cell characterization was done through flow cytometric analysis, using established stemness CD markers (CD44, CD24, and CD49f). Breast cancer cells exposed to iPSC conditioned media for three weeks showed reduced CSC (CD44+/CD24-) and increased ITGA6 variant A expression. iPSCs exposed to cancer conditioned media showed increased ITGA6 variant A and ITGB4 expression. Interestingly, the molecular changes described above were reversible after removing the corresponding conditioned media, confirming the plasticity of our model. Altogether, our results suggest that the analysis of ITGA6 splice variants is a useful tool in tracking changes in cell differentiation and phenotype of iPSC and CSC, with the potential to be used as a marker of malignancy.

W-4016

TRANSGENIC HUMAN EMBRYONIC STEM CELLS OVEREXPRESSING FGF-2 STIMULATE REGENERATION FOLLOWING RAT SPINAL DORSAL RHIZOTOMY

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Spinal dorsal rhizotomy (DRZ) leads to loss of primary afferent inputs, combined with degeneration of the respective synapses within the spinal cord. It triggers strong glial reaction, with formation of glial scar. Loss of afferents greatly affects behavior causing a significant deficit of motor coordination. In this context, we proposed a new surgical approach to treat DRZ, partially restoring sensorimotor integration. For that, platelet-rich plasma (PRP), an adhesive and inductive element for nerve regeneration, was used to reconnect the roots on the spinal surface. We also combined such scaffold with transgenic human embryonic stem cells (hESCs) overexpressing fibroblast growth factor 2 (FGF-2) in order to overcome the degenerative effects of DRZ. Thus, female adult Lewis rats were submitted to unilateral rhizotomy (DRZ), and hESCs/FGF-2 were applied to the injury site using the PRP scaffold. The animals were divided into the following groups (n=10/group): (1) Control without lesion; (2) DRZ without repair; and (3) DRZ followed by root repair with PRP; and (4) DRZ followed by root repair with PRP+hESCs. The reflex arc recovery upon ipsilateral hind limb plantar stimulation was evaluated weekly through the electronic von-Frey method, for eight weeks. Also, eight weeks post-lesion, animals were euthanized, and the spinal cords were processed for glutamatergic input (VGLUT1) and glial reaction (GFAP and Iba-1) immunolabeling. FGF-2, VEGFA, NGF, BDNF, and GDNF mRNA levels were evaluated with qRT-PCR. The results indicate that the combination of hESC overexpressing FGF2 with PRP induces regeneration after dorsal rhizotomy, restoring the paw withdrawal reflex, significantly enhancing VGLUT1 immunoreactivity in deeper spinal cord laminae. Also, PRP + hESC therapy did not exacerbate glial reactivity. We hypothesize that local hESC + PRP immunomodulation at the site of injury overcomes the effects of growth inhibitory molecules. In conclusion, the present data demonstrate that root reimplantation combined with cell therapy at the lesion site may be considered an effective therapeutic approach. Moreover, modified hESCs therapy further improves the intraspinal regeneration of axons from primary afferents resulting in sensorimotor restoration.

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W-4018

SINGLE-CELL SPATIAL RECONSTRUCTION REVEALS DIFFERENT ROLES OF DIPLOID, TETRAPLOID AND OCTAPLOID HEPATOCYTES IN THE LIVER

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The mammalian liver consists diploid, tetraploid, octaploid and higher polyploid hepatocytes. However, the functional roles of mature hepatocytes with different ploidy are still unclear. Here, we perform single-cell transcriptomics to map the spatial distribution of mouse diploid, tetraploid and octaploid hepatocytes. Single-cell analysis reveals that hepatocytes with different ploidy are zonally distributed, and comprise several cell populations with different functions. Tetraploid hepatocytes express metabolic genes in a higher level and are functionally mature. Intriguingly, a pericentral octaploid hepatocyte population is active in cell cycle and expresses several liver stem cell marker genes, including Axin2 and Lgr5. Our study reconstructs the zonation profiles of various hepatocyte populations, and provides insights into the functional differences among these populations.

W-4020

RESTORATION OF NOTCH1 INTRACELLULAR DOMAIN REPRESSES MALIGNANT PHENOTYPE IN OSCC BY REGULATING THE TEL2 - SERPINE1 AXIS

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Mutational landscape of Oral Squamous Cell Carcinoma (OSCC) is predominated by frequent inactivating mutations in EGF- like ligand binding domain of NOTCH1. However, the role of NOTCH1 signalling in tumorigenesis is highly context and cell type dependent. In this study, we investigated the phenotypic effect of NOTCH1 mutations in a primary line derived from OSCC biopsy. Based on gain-of-function assays in NOTCH1 mutant line we demonstrated regulation of cell behaviour and morphology, by NOTCH1 intracellular domain (NICD) leading to changes in cell proliferation, migration, clonal growth and differentiation. Importantly, overexpression of NICD results in upregulation of ETV7/TEL2 which negatively regulated SERPINE1 expression and conferred malignant cells with less aggressive phenotype. Further knockdown of SERPINE1 expression simulated the phenotypes observed upon rescue of NICD. In accord with our cell-based model we observed strong correlation between NOTCH1, ETV7 and SERPINE1 expression in OSCC primary tumours indicating similar deregulated mechanisms during oral carcinogenesis. Overall this study suggests a tumour suppressive role of NOTCH1 in OSCC and highlights possible mechanisms leading to changes in cell behaviour and morphology in absence of NICD potentially by regulating TEL2 and SERPINE1 expression. NOTCH1 signalling pathway plays a vital role in development and is involved in a wide array of key cellular processes such as the government of cell fate, the maintenance of stem cells, cell survival, proliferation and apoptosis. Our study gives a better understanding of the complexities of the functional consequence of NOTCH signalling in HNSCC, which is essential before the pathway could be targeted therapeutically.

W-4022

REPROGRAMMING OF CANCER CELLS INTO INDUCED PLURIPOTENT STEM CELLS QUESTIONED

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It has been first reported in 2007 that reprogramming factors, OCT4, SOX2, KLF4, and C-MYC, can reprogram human somatic cells into human induced pluripotent stem cells (iPSCs). Several recent studies have claimed that even cancer cells can be reprogramed into iPSC like cells. However, novel pluripotency was not fully confirmed. Therefore, we used either retroviral or episomal reprogramming methods to see if pluripotency can be fully achieved in human cancer cells (MCF10A and MCF7). Whereas we could reprogram human fibroblasts (BJ) into iPSCs, interestingly, we were not able to obtain iPSCs from the cancer cells. To understand why, we performed time-course gene expression analysis using qPCR to examine the induction of endogenous pluripotent markers OCT4, SOX2, and NANOG in BJ, MCF10A, and MCF7 cells. In addition, we conducted RNA-sequencing analysis on day 5 (at an early stage) to find the genes associated with the inability of tumorigenic cells to be reprogrammed into iPSCs. We found that the expression patterns in the genes related to the induction of pluripotency in cancer cells differed from those in BJ cells. These results suggest that, in cancer cells, the gene expression machinery required for the induction of pluripotency cannot be operated by the reprogramming factors that can be used to reprogram all human somatic cells, and provide new insights into cancer-specific cell properties.

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W-4024

THERAPEUTIC EFFECTS OF TRAIL-SECRETING MESENCHYMAL STEM CELLS WITH FOCUSED ULTRASOUND-INDUCED TEMPORARILY OPENING OF BLOOD-BRAIN BARRIERS IN THE BRAIN TUMOR

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Mesenchymal stem cell (MSC)-based gene therapy is a promising tool for the treatment of various neurological disease including brain tumor. The tumor necrosis factor-related apoptotic ligand (TRAIL) is believed to have promise as a cancer the therapy. For stem cell therapy in the brain tumor, therapy efficiency of stem cells by non-invasive method is extremely limited due to blood brain barrier (BBB). Noninvasive pulsed focused ultrasound (FUS) can temporarily opening the BBB of specific areas in the brain. Herein, we investigated the tumor-targeted BBB

temporarily opening by FUS and therapeutic efficiency of NIR fluorescence-labeled MSCs-TRAIL by intravenous injection in brain tumor. In addition, we observed the inflammatory changes of microglia cells by FUS using PET-CT and histological analysis. As a results, In vivo survival experiments showed that FUS treated-MSC-TRAIL group has greater therapeutic efficacy than FUS untreated-groups. The number of MSCs accumulated specifically site in the tumor region. MSC migration toward FUS treated-tumor site greater compared to the FUS untreated-tumors. Furthermore, the changes of microglia activity by FUS was no difference between the other groups. Collectively, these results suggest that therapeutic efficacy of MSC-TRAIL increases by temporarily opening the BBB using the FUS, which may be a more useful strategy for cancer therapy.

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W-4026

AUTOMATED MASS PRODUCTION OF INDUCED PLURIPOTENT STEM CELLS WITH THE CLOSED CELL CULTURE EQUIPMENT

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For the spread of regenerative medicine, reduction in cost by mass production is necessary in addition to high-quality cellular production. To solve this problem, we develop automated cell culture equipment (iACE1) which can automatically perform the expansion of iPS cells and the differentiation to dopaminergic progenitors for the Parkinson's disease treatment. This equipment has a closed flow path and vessels, which prevents entry of bacteria from the outside and can cultivate safely. In this closed system culture, 5% CO2 gas is supplied into the closed flow path and vessels, and therefore, it is important to control the internal pressure of the closed system (differential pressure from the atmospheric pressure). We investigated the influence of internal pressure on automated cell seeding and automated culture in closed systems, and realized closed cell culture of iPS cells under unaffected conditions. Furthermore, as we repeatedly expanded iPS cells, the expansion of the iPS cells cultured by the automated cell culture equipment tended to proceed faster than that of manual culture. As a result of measuring the culture temperature which is a factor influencing the proliferation, since the medium replacement work is carried out in the incubator in the apparatus, there is almost no temperature decrease while the temperature is greatly decreased in the manual culture. Therefore, the stability of the temperature of the automated culture equipment seems to contribute to the proliferation of iPS cells, and the possibility that the culture period can be shortened by automatic culture was shown.

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W-4028

ENGINEERING A MULTICELLULAR PATTERNING PLATFORM TO CONTROL CELL SIGNALLING FOR STEM CELL APPLICATIONS

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Tissue engineering strategies based on in vitro stem cell differentiation and organoid formation suffer from variable efficiency and disorganization of developing anatomical domains. Whereas a significant body of work has focused on understanding the molecular and cellular components of stem cell niches in vivo, bottom-up efforts in engineering synthetic niches in vitro have been limited. Here, we present our work in generating cell-based, self-organizing platforms to control cell signalling using synthetic biology. First, we program pattern-formation in mammalian cells, so that integrin-mediated heterotypic adhesions drive the formation of hyperuniform cell distributions. CRISPR genome editing was used to generate three transgenic HEK-293 lines each expressing heterophilic ICAM-1/MAC-1/LFA-1 integrins and dedicated fluorescent reporters in a drug-inducible manner. Upon transgene activation, high levels of integrin peptides accumulate on the plasma membrane of transgenic cells. Cell adhesion assays reveal that transgenic cells overexpressing ICAM-1 possess enhanced adhesion for peripheral mononuclear blood cells expressing MAC-1/LFA-

1, while transgenic cells overexpressing MAC-1/LFA-1 show enhanced adhesion for human umbilical vein endothelial cells stimulated to express ICAM-1. We observed the arrangement and patterning of cells by fluorescence microscopy at various cell ratios. Moreover, we utilize a pre-established HEK-293-based phase-separation system to drive organized production of Wnt3A from patterned multicellular islands. Molecular and cell-based assays reveal that Wnt3A is successfully produced from engineered cells and functionally activates the β-catenin pathway in receiver mouse embryonic stem cells (mESCs). We co-cultured patterned Wnt3A producers with mESCs during early stages of differentiation, and evaluated the spatial arrangement of β-catenin activation and mesoderm induction via immunofluorescence. Through this work, we aim to provide proof-of-concept that patterned multicellular systems can be used to impose a layer of organization in contemporary stem cell differentiation protocols, contributing to the development of synthetic stem cell niches.

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W-4030

FOXA2 INHIBITS PROLIFERATION OF RAT HEPATIC PROGENITOR CELLS VIA SUPPRESSING GLYCOLYSIS BY PI3K/AKT-REGULATED HK2 ACTIVITY

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Hepatic progenitor cells serve as a key cell compartment for liver regeneration in persistent chronic liver injury-induced liver fibrosis/cirrhosis when proliferation of mature hepatocytes is compromised. It is well known that energy balance and metabolic status determine cell fate, and forkhead box protein A2 (FoxA2) is an essential transcription factor controlling liver specification and cell proliferation of stem cells. The aim of this study was to investigate the role of FoxA2 in the metabolic regulation of hepatic progenitor cells and the underlie mechanism. We found that hepatic progenitor cells expressed FoxA2 both in human cirrhotic liver and in the liver of rats fed with a choline-deficient diet supplemented with ethionine (CDE). Using rat hepatic progenitor cells isolated from CDE rats, we found that knocking down FoxA2 via FoxA2 shRNA significantly accelerated proliferation of these cells. RNA sequencing data revealed that FoxA2 suppression markedly increased the gene transcription of metabolic pathway and PI3K/Akt signal pathways. Among the metabolic pathway, FoxA2 suppression increased the gene transcription, protein expression, enzyme activity of hexokinase 2 (HK2), the first enzyme in glycolytic pathway. Although there was no significant difference of non-glycolytic acidification, FoxA2 knocking-down cells had a much higher glycolysis, glycolytic capacity, and glycolytic reserve capacity than control shRNA cells, indicating FoxA2 suppresses glycogenesis in hepatic progenitor cells. Suppression of glycogenesis by 2-deoxy-glucose (2-DG), a glycolysis inhibitor, significantly reduced proliferation of FoxA2 knocking-down cells, suggesting glycolysis contributes to cell proliferation of hepatic progenitor cells. Furthermore, knocking-down FoxA2 significantly increased the PI3K transcription and Akt phosphorylation at the sites of Thr308 and Ser 473. Blocking PI3K/Akt signal pathway by Ly294002 significantly reduced cell proliferation by inhibiting HK2 activity and glycolysis. Taken together, our study indicates that FoxA2 is the transcription factor controlling hepatic progenitor cell proliferation by inhibiting glycolysis via PI3K/Akt-regulated HK2 activity.

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W-4032

PROINFLAMMATORY MACROPHAGES TRIGGERS DDC-INDUCED HEPATOCYTE REPROGRAMMING AND LIVER REGENERATION

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Liver is a pivotal organ possessing remarkable regenerative capability. By employing mouse liver injury models and lineage tracing strategy, recent studies have shown that differentiated hepatocytes undergo reprogramming to hepatic progenitor-like cells (HPC), and serve as a totally new cell source for mammalian liver regeneration. Inflammation is often observed in patients with various liver diseases. DDC-induced hepatocyte reprogramming, which simulates cholestatic liver diseases in patients, is also accompanied by inflammation. However, the role of inflammation in hepatocyte reprogramming and liver regeneration is unknown. Here we showed that hepatocyte reprogramming was accompanied by activation of M1 macrophages. Depletion of macrophages, but not T cells, B cells or NK cells, led to reduced hepatocyte reprogramming and liver regeneration. Through dissecting the molecular dynamics of hepatocytes and macrophages during hepatocyte reprogramming process, we found that proinflammatory cytokines were critical for hepatocyte reprogramming. Moreover, CCL2-mediated macrophage recruitment promotes hepatocyte reprogramming and liver regeneration. Our findings reveal that proinflammatory macrophages and proinflammatory signals are essential for the induction of hepatocyte reprogramming and liver regeneration. The study improves our understanding of the role of inflammation in hepatocyte reprogramming and liver regeneration and provides new strategy in promoting regeneration of injured livers.

W-4034

TREND IN REGENERATIVE MEDICINE AND GENE THERAPY TRIALS FOR RETINAL DEGENERATION AS SEEN FROM CLINICAL TRIAL REGISTRIES, AND THEIR PUBLICATION OF CLINICAL TRIAL RESULTS

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Previously, we reported on clinical study trends in regenerative medicine in four disease areas using clinical trial registries. This study offers an analysis of retinal degeneration (RD). Clinical trial registries offer basic information on each trial's research plan, but results are rarely included. However, results published elsewhere with an abstract containing an associated Study ID can be found through literature search. ClinicalTrials.gov and the International Clinical Trial Repository Platform were used to identify 51 regenerative medicine (RM) and six gene therapy (GT) studies targeting RD. We found published results for 5 of 28 RM trials and 3 of 3 GT trials that were completed before 2018. For GT, this represented a 100% report rate while RM was less than 20%. We discovered that RM studies reported results with lower frequency than GT studies. Of the five RM studies, one involved fetal tissue transplantation, two involved ESCs, one involved iPSCs, and one involved donor-derived retinal progenitor cells. On the other hand, most trials with results that were not reported used bone marrow, umbilical cord or adipose tissue as materials. In RM, autologous transplantation results tended to be published less frequently than allogeneic transplantation results. A report from a different group, not the corresponding group, was withdrawn in 2017 when it was reported that a trial autologous adipose tissue transplantation caused an accidental vision loss. This suggests that results for clinical trials focusing on RD in RM tend to be reported less frequently if researchers have low motivation and less awareness. The trend towards RM report rates below 20% was also identified in some research for disease areas such as spinal cord injury and Parkinson disease. This indicates the need for more thorough investigation of all RM research. Clinical trial results are useful tools that help participants avoid known dangers and prevent waste of resources due to duplication of research. Therefore, clinical researchers should promptly report and share the results of their research. In addition, clinical trial registries should not be used for commercial advertising.

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W-4036

HUMAN MESENCHYMAL STEM CELLS MEDIATED ANTI-HYPERTROPHIC EFFECTS ON INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES OF HYPOPLASTIC LEFT HEART SYNDROME PATIENTS

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Hypoplastic left heart syndrome (HLHS) is a complex, multifactorial congenital heart disease affecting 0.02% of the newborns. Surgical interventions have improved the survival of HLHS patients. Meanwhile, these patients get exposed to chronic volume-pressure overload on the right ventricle after few years. In such abnormal loading conditions, the right ventricle undergoes pathological hypertrophy. To explore potential intervention of pathological hypertrophy by HLHS patient, we differentiated induced pluripotent stem cells (iPSCs) of 3 healthy controls and 3 HLHS patients into cardiomyocytes (iPSC-CMs) as an in vitro disease model for cardiac hypertrophy. After replating iPSC-CMs at a density of 50,000 cells per well in a 250 µL xeno-free and serum-free RPMI media with B27 supplement, the cells were treated with a hypertrophic agonist, endothelin-1 for 48 h to induce hypertrophy. The hypertrophic properties were assessed by the relative mRNA expression of ANF, BNP, GDF15 and β-MHC. Immunocytochemistry was performed for WGA and sarcomeric α-actin to estimate the relative cell size and myofibrillar disarray. Human mesenchymal stem cells (MSCs) and its total conditioning media (TCM) were used to treat hypertrophic iPSC-CMs of healthy control and HLHS patients. Our results showed that endothelin-1 effectively stimulates hypertrophy in both HLHS iPSC-CMs and control iPSC-CMs by demonstrating increase in the expression of hypertrophic markers (such as ANF, BNP and β-MHC) and cell size. Interestingly, treatment of iPSC-CMs with MSCs or its TCM illustrated significant reduced hypertrophic stresses in HLHS iPSC-CMs. Our preliminary results suggest the potential role of MSCs and its TCM as a therapeutic target for cardiac hypertrophy in HLHS paitents.

W-4038

INDUCTION OF PLURIPOTENCY BY ALTERNATIVE FACTORS

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Reprogramming somatic cells to pluripotency represents a paradigm for cell fate determination. A binary logic of closing and opening chromatin provides a simple way to understand iPSC reprogramming driven by both Yamanaka factors or chemicals. Here we apply this logic to the design a seven factor combination, Jdp2, Jhdm1b, Mkk6, Glis1, Nanog, Essrb and Sall4 (7F), that reprogram MEFs to chimera competent iPSCs efficiently. RNA- and ATAC-seq reveal differences between 7F and Yamanaka induced pluripotency, 7FIP and YIP, in transcriptomic and chromatin accessibility dynamics(CAD). Sall4 emerges as a dominant force that can close and open chromatin with the help of Jdp2 and Glis1 in resetting somatic chromatin to a pluripotent state. These results reveal a previously unknown path between somatic and pluripotent states, open a door for cell fate control.

W-4040

COMPARISON OF MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN UMBILICAL CORD TISSUE BY EXPLANT AND DIGESTION

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Umbilical cord (UC) tissue, which can be collected at delivery in a noninvasive manner, is rich in mesenchymal stromal cells (MSCs) of considerable research and therapeutic potential. A growing portion of newborn stem cell banks offer cryopreservation of umbilical cord tissue, but considerable breadth of approaches for preparing and cryopreserving UC tissue exists among institutions. We compared characteristics of MSCs derived from UC tissue by the two primary methods, enzymatic digestion and tissue explant. 10 umbilical cords were obtained for research from consenting mothers. Each tissue was divided in half with one half cut into small pieces cryopreserved as whole, composite material, and the other half enzymatically digested using a previously-validated protocol to obtain a heterogeneous cell suspension that was cryopreserved. After at least 1 week, thawed composite tissue was explanted in MSC-selective medium for 2 weeks while cells from digested tissue were seeded directly into culture. Cells from both isolation methods were expanded to the end of the third passage (P3), and cell characteristics were compared. Viabilities of MSCs at the end of P3 from explanted and digested tissue were 97.1%(±3.6) and 97.4%(±2.4) by trypan blue exclusion staining, respectively, with no significant difference (p=0.83). The cell populations expressed surface markers CD73, CD90, and CD34/45 at 99.0%(±1.4%), 94.2%(±5.1%), and 0.8%(±1.4%) vs. 98.8%(±1.4%), 94.0%(±3.0%), and 1.0%(±1.4%), respectively, with no significant differences (p=0.7, p=0.9, p=0.7). Average maximum doubling time during P3 growth was 1.21(±0.27) days for cells from explant and 1.22(±0.40) days for cells from digest, with no significant difference (p=0.9). No difference in levels of IL-6 (p=0.5) and FGF-2 (p=0.5) secreted were seen. Average P1 culture yield per gram was higher for cells from explant than cells from digest (1.7x10^7 ±8.1x10^6 vs. 4.5x10^5 ±2.0x10^5), presumably due to higher initial purity of MSCs from explant isolation compared to the heterogeneity of cells derived from digest. Thus, while initial yields post-thaw favor storage of UC tissue as a composite material, once established in culture MSCs isolated by explant and digest are comparable, suggesting that either method of storage is acceptable for maintaining MSC properties.

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W-4042

COMPARATIVE ANALYSIS OF SECRETORY FACTOR PROFILES OF HUMAN STROMAL VASCULAR FRACTION BY BODY REGION

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Stromal vascular fraction (SVF) is increasingly being used for therapeutic treatments; however, there is little data showing the characteristics of these cells. To the best of our knowledge, this is the first paper to investigate and compare the secretory factor profiles of SVF from subcutaneous adipose tissues from different body regions. Fifteen samples of adipose tissue were harvested from the abdomen, flanks, and thighs (five samples of each), of fifteen female donors ranging from 22 to 77 years of age. The SVF cell characteristics were then analyzed. Specifically, we looked at cell counts, viability, population doubling time, cell density, time to confluency, and secretory factor profiles. While no significant differences were found in cell viability or proliferation between body regions, levels of some secretory factors differed from the various body regions. These novel findings suggest that there are cytokine level variations in SVF cells depending on the body region the cells are harvested from. These variations may be considered when SVF is being used as a therapeutic treatment.

W-4044

THE KYNURENINE PATHWAY OF TRYPTOPHAN METABOLISM IS INDUCED BY INTERFERON-GAMMA IN HUMAN ADIPOSE STEM CELLS

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Adipose stem cells (ASCs) are multipotent mesenchymal cells, capable of differentiation into a broad variety of cells including adipocytes, bone cells and neurons. The Kynurenine pathway (KP) critically regulates bioavailability of the essential amino acid Tryptophan (Trp), and is highly induced by interferon-gamma (IFN-g). In Multiple Sclerosis (MS) the KP is dysregulated, producing high levels of metabolites including the potent neurotoxin Quinolinic acid (QUIN). In bone marrow MSCs, we showed IFN-g significantly reduced proliferation and mediated

differentiation to adipogenic and osteogenic lineages. Here, we investigated the hypothesis that modulating the KP using IFN-g would drive changes in ASC proliferation and KP metabolite secretion in healthy control cells. ASCs were isolated from abdominal lipoaspirates with informed consent (a minimally invasive procedure), purified and cultured in DMEM/10% FBS for 5 passages prior to analysis. Secreted KP metabolites were quantified by HPLC and GC/MS. After 72 hours, 500IU/mL IFN-g treatment significantly depleted extracellular Trp in culture media (1.12+/-0.14uM vs. Untreated 20.17+/-0.08uM, $p<0.0001$, $n=3$ patient cultures in triplicate), suggesting the activity of tryptophan 2,3-dioxygenase (TDO) and/or indoleamine-2,3-dioxygenase 1 (IDO-1) which perform this metabolic role. Downstream metabolite Kynurenine was significantly upregulated with IFN-g (32.57+/-0.36uM vs. Untreated 0.43+/-0.007uM, $p<0.0001$); neuroprotective Picolinic acid, and QUIN were minimally altered. IFN-g caused no significant difference in confluency at any timepoint however cell cycle parameters remain to be evaluated. Image analysis showed phase bright cells with small processes (possible stochastically differentiated neuroblasts) sharply declined with IFN-g (Time 0, 4.33+/-0.33 cells/field; no cells at 48/72 hour timepoints) vs. Untreated (Time 0, 2.33+/-0.88 cells/field and 0.67+/-0.33 cells (72 hours). This study is the first characterization of KP in ASCs and will be extended in future e.g. by assay of KP enzymes and comparison to MS patient cells. Owing to their capacity for self-renewal as well as differentiation into a variety of cell types, assays of ASCs may serve as a minimally invasive means of understanding the impact of MS on these stem cells.

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W-4046

AN UNBIASED PROTEOMICS APPROACH TO IDENTIFYING THE SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) derived from bone marrow have the ability to aid skeletal tissue repair and regeneration owing to their self-renewal and differentiation abilities, and trophic functions such as their secretion of growth factors and cytokines. With aging, MSCs undergo dramatic changes due to onset of the senescence-associated secretory phenotype (also known as SASP) which may largely contribute to age-related bone loss, including osteoporosis. Functional consequences of senescence in MSCs could affect their therapeutic potential, if processes such as their paracrine effects, immunomodulatory activity, differentiation potential, and cell migration ability are compromised. With the aim of defining the SASP in MSCs, this study undertook a mass spectrometry (MS)-based proteomics approach. MSCs were characterized based on surface marker profiles, proliferation, and differentiation capacities. Replicative senescence was achieved by exhaustive in vitro sub-cultivation (passaging) in chemically-defined xeno-free and serum-free conditions and confirmed by standardized proliferation criteria. Conditioned media from young and senescent MSCs were prepared for MS. Proteomics and bioinformatics analyses enabled the identification of 415 proteins in young MSCs and 480 proteins in old/senescent MSCs, of which 151 proteins were expressed exclusively in senescent MSCs, including matrix metalloproteinases, cathepsins, collagens, and cell adhesion markers. Peroxidasin (PXDN) and lamins (e.g. LAMA2) were among the most highly expressed proteins in senescent MSCs. Protein ontology analysis revealed enrichment of proteins linked to extracellular matrix, lysosome, cell adhesion, and calcium ion binding. In addition, 329 proteins were found to be expressed in common between young and old MSCs but at variable levels. Functional enrichment analyses revealed MSC-SASP proteins to be related to biological processes, functions and pathways linked to protein metabolism, catalytic activity, metallopeptidase activity, and extracellular functions. This unbiased, comprehensive analysis of changes in the MSC secretome with aging has identified the unique protein signature of the SASP and holds potential for identifying new therapeutic targets for the treatment of age-associated bone loss.

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W-4048

A NOVEL APPROACH FOR GENTLE SORTING OF ADULT NEURAL STEM CELLS FROM WILDTYPE MOUSE BRAIN USING THE MACSQUANT® TYTO®

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Neural stem cells (NSCs) in the adult subventricular zone and the dentate gyrus have the capacity to self-renew and generate new neurons throughout lifetime. Their ability to react to brain injury by generating new neural cells makes them a valuable cell source for endogenous repair in the adult brain. As NSCs are a very rare and sensitive cell population and a complex marker combination is necessary to distinguish them from other cells, their purification is still challenging. Here, we show a new approach to purify NSCs from wildtype mouse brain. First, an optimized automated dissociation protocol was applied, which ensures high viability and epitope integrity of the resulting single

cell suspension. Then, NSCs were identified by labeling the positive markers GLAST, PlexinB2, and EGFR and the negative markers CD24, TER119, and CD45. Subsequently, purification of NSCs was carried out with the MACSQuant® Tyto®, a new multi-parameter cell sorting device that uses a micro-chip based sorting technology for sterile and gentle cell isolation. Unlike conventional droplet sorters, cells do not experience high pressures and no charge is applied, ensuring high viability and functionality. This experimental setting resulted in highly pure (>95%) and viable NSCs (>90%) in less than 4 h. Neurosphere assays led to formation of a high number of neurospheres, that gave rise to secondary neurospheres and differentiated into different neural cell types. In summary, we present a novel approach for isolation of NSCs by combining elaborated cell preparation methods with an optimized marker combination and a sophisticated cell sorting Technology using the MACSQuant® Tyto®.

W-4050

SPATIAL TRANSCRIPTOMIC SURVEY OF HUMAN EMBRYONIC CEREBRAL CORTEX BY SINGLE-CELL RNA-SEQ ANALYSIS

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The cellular complexity of human brain development has been intensively investigated, although a regional characterization of the entire human cerebral cortex based on single-cell transcriptome analysis has not been reported. Here, we performed RNA-seq on over 4,000 individual cells from 22 brain regions of human mid-gestation embryos. We identified 29 cell sub-clusters, which showed different proportions in each region and the pons showed especially high percentage of astrocytes. Embryonic neurons were not as diverse as adult neurons, although they possessed important features of their destinies in adults. Neuron development was unsynchronized in the cerebral cortex, as dorsal regions appeared to be more mature than ventral regions at this stage. Region specific genes were comprehensively identified in each neuronal sub-cluster, and a large proportion of these genes were neural disease related. Our results present a systematic landscape of the regionalized gene expression and neuron maturation of the human cerebral cortex.

W-4052

NOVEL HUMAN GAPTRAP REPORTER AND MODULATORY HPSC LINES REVEAL DISTICNT PATTERNS OF NEURAL GRAFT-GRAFT CONNECTIVITY IN VIVO

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The directed differentiation of human pluripotent stem cells (hPSCs) to specific neuronal subtypes presents unique opportunities for treating and modelling human neurological conditions. Here, we utilized novel GAPTrap reporter and channelrhodopsin (ChR2) hPSC lines to generate cortical grafts to investigate the capacity of human graft-graft connectivity in vivo. RFP and GFP GAPTrap reporter hPSC lines were differentiated to cortical progenitors and transplanted into the left and right hemisphere respectively, of the motor cortex in mice. Robust and long-term reporter expression enabled detailed analysis of graft fiber patterns revealing an extensive axonal growth and reciprocal innervation of each graft, largely via the corpus callosum reminiscent of callosal projection neurons (CPN). Imunnohistochemical labelling showed an uneven distribution of fiber innervation from the contralateral graft. Areas of dense innervation were rich in glutamatergic fibres with co-localization of human synaptophysin. Increased innervation tended to correspond to graft regions that were highly populated with upper layer cortical cell identity (Brn2+). To test the functional connectivity of human cortical grafts in vivo, cortical progenitors were generated using the ChR2 GAPTrap hPSC line and transplanted contralateral to cortical RFP grafts. In vivo electrophysiological recordings of RFP graft activity after optical stimulation of the contralateral ChR2 graft showed enhanced neuronal spike activity shortly after the onset of ChR2 graft stimulation, suggesting functional graft-graft connectivity. Overall these results demonstrate the capacity for human grafts to form functional neuronal circuits in vivo.

W-4054

LOCAL DELIVERY OF FLAVOPIRIDOL REPAIRS RAT SPINAL CORD INJURY BY REGULATION OF ASTROCYTES AND INFLAMMATION

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The repair of spinal cord injury (SCI) is closely related to inflammatory cytokines, among which quite a few have been demonstrated detrimental or beneficial to repair. The sequential changes of local inflammatory cytokine protein levels after rat SCI are still not clear. Flavopiridol has been reported to significantly improve motor recovery and decrease reactivity of astrocytes which are the important source of inflammatory cytokines. But its high systemic dose may cause strong side-effects. The mini-osmotic pump used for intrathecal flavopiridol delivery is costly and may cause problems with histocompatibility. Biodegradable and injectable Poly (lactic-co-glycolic acid) (PLGA)-based methylprednisolone nanoparticles (NP) have been used in SCI repair, and the NP-enabled local delivery is significantly more effective than systemic delivery. So ①we studied the sequential changes by multiplex immunoassay and found 4 cytokines that might be beneficial to repair decreased after SCI, and 9 cytokines that might be detrimental to repair increased. ②We found that flavopiridol inhibited proliferation, scratch-wound healing, and inflammatory factor synthesis in astrocytes, while permitting the survival of neurons. ③We fabricated flavopiridol NP and found that they improved the functional recovery of injured rats. They also increased the integrity of spinal cord gross tissue structure, inhibited the glial scarring and cavitation, and facilitated neuronal survival and regeneration. Flavopiridol NP decreased the cell-cycle related protein expressions of astrocytes, neurons and macrophages in vivo. Multiplex immunoassay showed that flavopiridol NP affected local inflammatory cytokine profile. They increased GM-CSF while decreased IP-10. We confirmed in vitro that they indeed significantly decreased the pro-inflammatory factor synthesis by astrocytes, while the IL-10 expression was elevated. These findings demonstrated that local delivery of flavopiridol in PLGA NP improves recovery from SCI by regulation of astrocytes and inflammation. Future studies may aim to develop personalized strategies of locally-delivered therapeutic agent cocktails for effective and precise regulation of inflammation, and substantial functional recovery from SCI.

W-4056

SINGLE CELL RNA SEQUENCING ANALYSIS OF LIZARD NEURAL PROGENITOR CELL POPULATION DYNAMICS DURING TAIL SPINAL CORD REGENERATION AND NEUROSPHERE CULTURE

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Lizards are the only amniotes able to regrow amputated tails, but replacement lizard tails exhibit “imperfect” regenerative outcomes. For example, regenerated tail spinal cords (SCs) consist of ependymal tubes that lack other neural cell populations. We have shown that differences between original and regenerated SCs stem from neural progenitor cell (NPC) lineage restrictions that favor radial glia/ependymocyte maintenance at the expense of neuron, astrocyte, and oligodendrocyte differentiation. These differentiation limitations are mirrored by NPCs cultured in vitro as neurospheres. The goal of this project is to identify NPC populations selected for during tail regeneration and neurosphere culture and to determine how they relate to one another. Lizard original and regenerated SC and neurosphere cells were analyzed by single cell RNA sequencing using the 10X Genomics platform, and cell clustering analysis was performed used SPRING. Identified population markers were validated with histology/immunostaining. Original tail SC samples exhibited three distinct neural cell clusters based on differential marker expression: neurons (NEFL, MAP2); oligodendrocytes (SOX10, OSP/CLDH11, MBP); and astrocytes (GLUL). In addition, three NPC populations (SOX2, FABP7) exhibited distinct expression profiles and clustering properties: A SOX8+, SOX10+, SOX11+ group clustered with astrocytes/oligodendrocytes (NPC-OA); a GATA2+ group clustered with neurons (NPC-N); and a divergent GFAP+, CDH2+ group co-localized with ependymocytes in Sox2-immunostainted cryosections (NPC-E). Regenerated SC samples did not exhibit any neural cell clusters and contained a single NPC group (NPC-R). The NPC-R population clustered with NPC-E over NPC-OA and NPC-N groups, and was distinguished from NPC-E by increased expression of SHH, EGF, and WNT16. Neurosphere samples also exhibited a single NPC population that clustered with NPC-E and NPC-R cells. In conclusion, original tail SCs include multiple NPC populations with divergent lineage biases, and SC regeneration and neurosphere culture selects for NPC populations restricted to ependymocyte identities. Future work will investigate the mechanisms regulating NPC population selections during lizard tail regeneration.

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W-4058

EFFECT OF GLUCOCORTICOID ON MITOPHAGY INHIBITION IN HIPPOCAMPAL NEURONS AND SUBSEQUENT PROGRESSION OF DEMENTIA IN STRESS-INDUCED MOUSE VIA REPRESSING PGC1A-NIX AXIS

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Excessive glucocorticoid is a key pathogenic factor of dementia inducing synapse damage and cell death in hippocampus via impairing mitochondria quality control (MQC) system. Even though glucocorticoid prominently triggers perturbed bioenergetics and altered morphology in mitochondria, it is poorly understood how glucocorticoid suppresses mitophagy machinery in neuronal cells. Here, we investigated the inhibitory effect of glucocorticoid on mitophagy machinery including mitophagosome formation and degradation via downregulating mitophagy receptor NIX expression, the core activator of receptor-mediated mitophagy. In our results, glucocorticoid hampered mitophagosome formation and transport from distal neuron to soma in a glucocorticoid receptor (GR) dependent pathway, resulting in synaptic dysfunction in hippocampal neurons. We found that GR directly binds to PGC1α promoter, the key molecule of mitochondria biogenesis, repressing its expression and nuclear translocation. Among many mitophagy regulators, NIX upregulated by PGC1α was decreased upon glucocorticoid treatment. Thus, NIX overexpression enhanced mitophagy process enhancing mitophagosome formation in axons and transport into soma, which was finally degraded by lysosomes. Furthermore, mitochondria dysfunction followed by excessive mitochondrial ROS, altered dendrite lengths, decreased synaptic density, and ATP production impairment was recovered by NIX overexpression even under excessive glucocorticoid. In the stress-induced mouse model, the treatment of NIX enhancer led to elevated mitophagy function and hippocampal cell survival, followed by recovered memory function. In conclusion, we demonstrated that NIX could be a potential therapeutic target against detrimental effect of glucocorticoid on mitochondrial dysfunction which impairs both mitophagosome formation and intracellular trafficking.

W-4060

IDENTIFICATION OF NEURODEVELOPMENTAL ABNORMALITIES THAT MAY UNDERLIE C9ORF72 ALS AND FTD PATHOLOGY

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The most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is a (GGGGCC)n repeat expansion in a gene called C9ORF72. Previous studies have shown that presymptomatic carriers of the repeat expansion have smaller brain regions decades before disease onset. This implies that age-dependent or genetically induced neuronal loss may result in an increased vulnerability towards disease. It is well-known that reductions in proliferation and/or premature differentiation of the neural stem cell (NSC) population during development can lead to these decreases in brain size. To this end, we hypothesize that there may be a neurodevelopmental defect underlying C9ORF72 pathology. In our study we investigated the effects of the repeat expansion in human iPSC-derived NSC proliferation and differentiation, as well as, in vivo using a C9-BAC mouse model. Our in vitro results revealed C9-ALS/FTD patients have a 30% reduction in their NSC population, a significant reduction in proliferation (control: 61.07% ± 12.14, n = 4; C9-ALS/FTD: 41.84% ± 6.35, n = 3; p-value = 0.0078), and a significant increase in differentiation (control: 5.13% ± 1.75, n = 4, C9-ALS/FTD: 10.02% ± 3.10, n = 3; p-value = 0.0097) compared to controls. Interestingly, lentiviral overexpression of the repeat expansion by-products or ablation of C9ORF72 expression in controls did not result in a patient-like phenotype, but removal of the repeat expansion via CRISPR-Cas9 in our patient lines rescued the observed proliferative and differentiation defects. This suggests that the repeat expansion itself is enough to disrupt NSC proliferation and differentiation. Lastly, we used magnetic resonance imaging to determine the effects of the repeat expansion on total and region-specific brain volumes in E18.5 C9-BAC mice. Our results revealed no differences in total brain volumes but identified a significant reduction in thalamic volume (WT: 0.021 mm3 ± 0.005, n = 4; C9-BAC: 0.012 mm3 ± 0.001, n = 4; p-value = 0.046). This study could lead to a paradigm shift in thought by suggesting late-onset neurodegenerative diseases may begin during early neurodevelopment. New understanding of this relationship may be harnessed for early diagnoses and identify new molecular pathways for therapeutic interventions.

W-4062

MACHINE LEARNING FOR DEEP ELECTROPHYSIOLOGICAL PHENOTYPING OF HUMAN DOPAMINERGIC NEURONS

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Neuronal cultures derived from induced pluripotent stem cells (iPSCs) provide an attractive avenue to study neurological disorders in living human tissue. iPSC-derived neurons retain the unique genetic signatures of their donors and, hence, can help to elucidate mechanisms of diseases that the donors may carry. Here, we used high-density microelectrode array (HD-MEA) recordings to systematically study subcellular and cellular characteristics, as well as network phenotypes of human iPSC-derived dopaminergic neurons. We present a machine learning approach to infer which electrophysiological features, or combinations of features, inferred from high-density electrophysiological recordings are most predictive to differentiate neurons carrying a genetic mutation associated with Parkinson's disease (PD) from healthy ones. Although there are previous studies on the electrical activity of iPSC-derived neurons, the latter did not infer the morpho-electric properties of individual neurons or attempted to characterize their synaptic connectivity. Here, we demonstrate that HD-MEA-based extracellular mapping of iPSCs, including the analysis of detailed spatiotemporal resolved electrophysiological profiles of single neurons, provides a unique opportunity for deep electrophysiological phenotyping. After spike sorting of neuronal network recordings, we inferred single- and multi-channel features and estimated functional connectivity among single units. Applying classification techniques, such as support vector machines (SVMs), we demonstrate that sub-cellular and cellular features are reliable predictors of whether neuronal networks are from controls or carriers of the Parkinson's disease (PD) mutation. The most predictive metrics include classical action potentials waveform features, multi-channel features and network properties. Combining human iPSC technology with HD-MEA recordings yields a state-of-the-art phenotypic screening platform, which will accelerate in vitro drug discovery and help personalize treatment strategies.

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W-4064

TRENDS IN PARKINSON'S REGENERATIVE MEDICINE AND GENE THERAPY TRIALS AS SEEN FROM CLINICAL TRIAL REGISTRIES AND A STUDY OF CLINICAL TRIAL RESULT REPORTS

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We have previously reported analyses of regenerative medicine clinical study trends in four disease areas using clinical trial registries. This study offers an analysis of the field of Parkinson disease (PD). Clinical trial registries offer basic information on each trial's research plan, although trial results are rarely registered. However, results published elsewhere with an abstract containing an associated Study ID can be found through literature search. We surveyed clinical trials of regenerative medicine (RM) and gene therapy (GT) targeting PD from ClinicalTrials.gov (CTG) and International Clinical Trial Registry Platform (ICTRP), 23 RM and 14 GT studies were obtained. Among these, we selected the studies that were completed by 2018, counted the studies whose results were published, and found that RM was 2 out of 11 studies whereas GT was 7 out of 7 studies. For GT, this represented a 100% report rate while RM had less than 20%. We discovered that even though both categories target PD, the results of gene therapy treatments were reported with high frequency, whereas results of regenerative medicine treatments were low. The two RM cases involved fetal tissue transplantation and parthenogenetic NSCs (ISC-hpNSCs). However, most trials without published results used bone marrow or adipose tissue as materials. We also found that in regenerative medicine, autologous transplantation results were reported less frequently than allogeneic transplantation results. This suggests that PD regenerative medicine clinical trial results tend to be reported more frequently if investigators have high awareness, for example, if a featured material is rarely used and/or if there are unknown risk concerns. Other diseases area studies in RM tend to have report rates less than 20%. This indicates that further detailed research on regenerative medicine in other disease areas is needed. In conclusion, clinical trial outcomes are valuable resources that should be shared accordingly to help new trial subjects avoid known dangers and to prevent unnecessary duplication of research. Clinical researchers therefore have a duty to report their findings. We hope that this situation will improve sometime in the near future.

Funding Source: This study was supported by the Highway Program for Realization of Regenerative Medicine of The Japan Agency for Medical Research and Development (AMED) under Grant Number JP18bm0504009.

W-4066

GLOBAL GENE EXPRESSION ANALYSIS OF IPSC- AND IMMORTALIZATION-DERIVED HUMAN DOPAMINERGIC NEURONS

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Dopaminergic neurons enable voluntary movements and behavioral processes in mammals, and their loss causes Parkinson's disease (PD) in humans. Two cellular models for human dopaminergic neurons were compared, one derived from iPSC (iCell-Dopa neurons from Cellular Dynamics International Inc.), and another from a conditionally-immortalized fetal human mesencephalic cell line (LUHMES). After differentiation in vitro, these two cell lines manifest common characteristics of dopaminergic neurons: both cell models express tyrosine hydroxylase (TH), Dopamine Transporter (SLC6A3) and dopamine receptors; both exhibit electrophysiological synaptic and pacemaker-like neurotransmission activities; and both respond to alpha-synuclein (α-SYN) gene/protein mutation by exhibiting neurodegeneration. To assess their neurogenesis and transcriptional properties, we examined gene expression profiles in the fully differentiated iCell- and LUHMES- dopaminergic neurons by RNA-Seq. Genes encoding transcription factors and other neurogenesis markers were revealing. The majority of the relevant genes showed robust mRNA expression in both cell lines, e.g. TH, NURR1/NR4A2, LMX1A/B, PITX3, FOXA2, NGN2, EN1/EN2.). However, several genes were differentially expressed. For example, expression of NEUROD1, NEUROG1, HEYL and OLIGO2 was very low in iCell-neurons (<1 reads per million, RPM) but higher in LUHMES neurons (20 to1,200 RPM), whereas the expression of BMP2, ALK, BDNF and NTN1 was high in iCell neurons (5 to 300 RPM) but low in LUHMES neurons (< 4 RPM). Additionally, the LUHMES neurons expressed only DRD2 (140 RPM), whereas iCell neurons exhibited low-level expression of both dopamine receptors DRD1 and DRD2 (0.23 and 3.8 RPM, respectively). This suggested that iCell neurons were indeterminate or mixed regarding identity as activating (DRD1) or inhibitory (DRD2) dopaminergic neurons. Thus these two Dopa neuron models exhibited different transcriptional activities.

Funding Source: This study was supported by Intramural Research Program and Tox21 Program at NCATS/NIH.

W-4068

EXPLORATION OF EFFICACY OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS ON PARKINSON'S DISEASE

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Parkinson's disease (PD) is a disease developed by lack of dopamine, a neurotransmitter, upon disappearing dopaminergic neuron in the brain. PD has been increasing exponentially with entering aging society and lowers the quality of life significantly. Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSC) is a valuable cell that has been recently used as an effective cell therapy in multiple diseases such as atopic dermatitis, osteoarthritis, and so on. The objective of this study is to investigate the therapeutic efficacy of hUCB-MSC in the mouse PD model. Animal experiments were approved by the Institutional Animal Care and Use Committee of Seoul National University. To do so, the PD model was produced upon injecting 6-hydroxydopamine (6-OHDA) into substantia nigra region in ICR mice. After emergence of behavioral phenotypes on PD, hUCB-MSC was injected once (1 x 10^4 / head) in the injection area of 6-OHDA, intrastriatally. Since cell administration, behavioral recovery was checked using a variety of methods such as beam test for 6 weeks. Behavioral scores were significantly lowered in about 1 week after administration of 6-OHDA, while control group maintained the low behavioral scores. In addition, decline of tyrosine hydroxylase (TH) positive cells in the area of 6-OHDA administration was found by immunohistochemistry. In 6 weeks after administration of the cell, behavioral recovery was found significantly compared to the control group. Although human nuclei positive cell was not detected in the area of cell administration, some TH positive cells were found. In conclusion, single intrastriatal injection of hUCB-MSC with 1x10^4 / head showed the therapeutic effect in the mice PD model. This effect is predicted by paracrine effect of hUCB-MSC. Further studies are required on the detailed mechanism of action for the causes of therapeutic efficacy and also, it is required to develop the study clinically upon using non-human primate model.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2016K1A3A1A61006001).

THURSDAY, JUNE 27, 2019

POSTER II - ODD
18:00 – 19:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

T-2001

EGF DERIVED FROM HUMAN PLACENTAL MESENCHYMAL STEM CELLS IMPROVES PREMATURE OVARIAN INSUFFICIENCY VIA NRF2/HO-1 ACTIVATION

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Human placental mesenchymal stem cells (hPMSCs) are a potential therapeutic option for clinical applications because of their ability to produce cytokines and their capacity for trilineage differentiation. To date, few researchers have investigated the relevance of hPMSCs in the treatment with premature ovarian insufficiency (POI) at the oxidative stress level. A POI mouse model and human ovarian granule cells (hGCs) collected from individuals with POI were prepared to assess the therapeutic effects and illuminate the mechanism of hPMSCs in curing POI. Our results showed that hPMSCs displayed therapeutic activity on ovarian function in the POI mouse model, including recovered follicular numbers and increased marker expression. Furthermore, the yields of hPMSC-secreted EGF (epidermal growth factor) were higher than those of other growth factors. FACS and Western blot showed that EGF promoted the proliferation rate and inhibited the apoptosis rate in hGCs. FACS and ELISA method indicated that the hPMSCs and EGF inhibited oxidative stress. In addition, protein assays demonstrated that EGF suppressed oxidative stress by dose-dependently upregulating the expression of the NRF2/HO-1 pathway and inhibited the apoptosis rate by regulating the PTEN/PI3K/AKT pathway. These findings demonstrate for the first time the molecular cascade and related cell biology events involved in the mechanism by which EGF derived from hPMSCs improves ovarian function during POI via the reduction of oxidative stress by activating the NRF2/HO-1 signaling pathway. Moreover, this discovery suggests that EGF may serve as a novel, safer and efficacious therapeutic approach to resisting ROS in POI and thereby may improve female reproductive health.

Funding Source: National Natural Science Foundation of China (81801515, 81801494), Suzhou science and technology for people's livelihood (SYS2018081), Suzhou introduce expert team of clinical medicine (SZYJTD201708).

T-2003

THE THERAPEUTIC EFFECTS OF ENHANCED HUMAN PLACENTA-DERIVED MESENCHYMAL STEM CELLS IN ACUTE OPTIC NERVE INJURY

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Cell death due to acute optic nerve damage occurs from a number of factors. Human placenta derived stem cells (hPMSCs) tissue-derived cells with self-renewing ability and can differentiate into various cell lineages. A number of studies supported that it has therapeutic potential. We also have been reported that hPMSCs can restore the optic nerve injury in previously study. We have recently demonstrated that hPMSCs has recovery abilities from hypoxic damage. We established enhanced hPMSCs (EhPMSCs) by exposure to hypoxic environment. Human PMSCs were exposed in short-term hypoxic conditions at 2.2 % O2 and 5.5 % CO2 concentration. After exposing, we found cell viability of EhPMSCs was more increased and neurogenic markers such as glial fibrillary acidic protein (GFAP), Thy-1, Neuroflament and Vimentin, were up-regulated under hypoxic conditions. Using EhPMSCs, we investigated recovery effects of EhPMSCs on optic nerve compression animal model. We injected naïve hPMSCs (2x106/30ul) or EhPMSCs (2x106/30ul) to rat disease model. After 1, 2, or 4 weeks, we analysed regeneration markers expression in rat retina and optic nerve. After 4 weeks hPMSC and EhPMSCs injection, the reduced Gap43 expression by injury was increased 30 %. In addition, we found Tuji-1 and GFAP expressions also were significantly increased in retina. We also investigated improved ability of EhPMSCs in R28 cells exposed to hypoxic condition. We examined cell viability of hypoxic damaged R28 cell with EhPMSCs was more increased and improved recovery function of EhPMSCs than naïve hPMSCs. We demonstrated recovery effect of hPMSCs on optic nerve injury models. Based on our finding, EhPMSCs would be expected to provide a foundation for the application of stem cells as a stable and effective cell therapy.

Funding Source: This research was supported by the Ministry of Health and Welfare, Republic of Korea (Grant/Award Number: HI16C1559).

T-2005

HUMAN AMNIOTIC MESENCHYMAL STEM CELLS DERIVED EXOSOMES IMPROVE PREMATURE OVARIAN INSUFFICIENCY THROUGH PI3K/AKT/ PTEN SIGNAL PATHWAY

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Although many reports show that various kinds of stem cells have the ability to recover the function of premature ovarian insufficiency (POI) few studies are associated with the mechanism of stem cell treatment of POI. We designed this experimental study to investigate whether human amniotic mesenchymal stem cell derived exosomes (hAMSC-Exos) retain the ability to restore ovarian function and how hAMSC-Exos work in this process.A POI mouse model and human ovarian granule cells (hGCs) collected from individuals with POI patients were prepared to assess the therapeutic effects and illuminate the mechanism of hAMSC-Exos in curing POI. Hematoxylin and eosin (HE) assay (HE) method was employed to assess the number of follicles. Enzyme-linked immunosorbent assay was used to detect the serum levels of sex hormones. The proliferation rate and marker expression level of hGCs were measured by flow cytometry (FACS). Real-time PCR and western blot assays were used to determine the mRNA and protein expression levels of PI3K, AKT and PTEN.After the hAMSC-Exos were transplanted into the POI mice model, the hAMSC-Exos exerted better therapeutic activity on mouse ovarian function, improving the folliclenumbers during four stages. ELISA assay results showed that hAMSC-Exos elevated the hormone level to the normal level. In addition, after hAMSC-Exos co-cultured with POI hGCs, our results showed that hAMSC-Exos significantly promoted the proliferation rate and inhibited the apoptosis rate. Furthermore, hAMSC-Exos also increased the marker expression of hGCs more higher than control group. Besides,mRNA and protein assays demonstrated that hAMSC-Exos the suppressed expression of PI3K and AKT, up-regulated the expression of PTEN in vivo and in vitro study.These findings demonstrate for the first time the molecular cascade and related cell biology events involved in the mechanism by which exosomes derived from hAMSCs improved ovarian function of POI disease via regulation of PI3K/AKT/PTEN signaling pathway.

Funding Source: Suzhou introduce expert team of clinical medicine (SZYJTD201708); Fund of State Key Laboratory of Reproductive Medicine,Nanjing Medical University (JX116GSP20171411); Suzhou Key Medicine Center (SZZX201505).

ADIPOSE AND CONNECTIVE TISSUE

T-2007

A NEW BALL FOR AN OLD TRICK: MAGNETIC ACTIVATED CELL SORTING OF HUMAN MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE

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Mesenchymal stem cells (MSCs) show great promise in therapeutic regeneration of cells found in solid tissues. Bone marrow remains the most common source of therapeutic MSCs. However, MSCs also reside in adipose tissue, and per unit volume, adipose tissue yields a considerably greater quantity of MSCs than bone marrow. Adipose tissue-derived mesenchymal stem cells (ASCs) may be isolated in clinically useful quantities without in vitro expansion, as many therapies using bone marrow-derived stem cells require. Thus, the therapeutic use of ASCs may intend comparative safety, economic, and logistic benefits. The purpose of this study is to validate a novel method, a method based on an august technique for the enrichment of therapeutic hematopoietic stem cells, for the enrichment of primary ASCs using paramagnetic beads. Primary rabbit anti-mouse antibodies (Thermo Fisher Scientific) were bound to Dynabeads® (Thermo Fisher Scientific). Secondary mouse anti-human antibodies (BD Biosciences), selective for ASCs, were then bound to the primary antibodies to construct so-called paramagnetic immunobeads (PIBs). PIBs were then added to fresh human lipoaspirate to create ASC-PIB conjugates (aPIBS) over 10 minutes. A hand-held magnet (Omega Magnets) was then placed adjacent to the lipoaspirate-aPIBs mixture, and over the next 10 minutes, the aPIBs were precipitated. No attempt was made to unconjugate the ASCs from the PIBs. Post-enrichment cellular analysis included morphology by scanning electron microscopy (SEM), quantification and immunophenotyping by flow cytometry, and function by tri-lineage differentiation. Live cell count per mL of lipoaspirate was 9.6 x 104. SEM revealed precipitates morphologically consistent with aPIBs. Flow cytometry identified cell-bound markers for CD90 and CD105 while culture confirmed differentiation to adipocytes, chondrocytes, and osteoblasts, all attributes diagnostic of ASCs. This study validates that functional ASCs may be isolated from lipoaspirate by magnetic enrichment in 20 minutes. As both the harvest of adipose tissue by liposuction and this ASC enrichment technique do not require electricity, fresh primary therapeutic ASCs may now be isolated in any point-of-care setting, even in developing countries where access to electricity is difficult if not impossible.

T-2009

HUMAN MESENCHYMAL STEM CELLS-INDUCED MACROPHAGES EXERT IMMUNOMODULATORY AND ANTI-INFLAMMATORY EFFECTS

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Kim, Hyun Ok - *Cell Therapy Center, Severance Hospital, Seoul, Korea*

Accumulative evidence has demonstrated that mesenchymal stem cells (MSCs)-derived paracrine factors are capable of regulating the immune system via interaction with various immune cells. In this study, adipose-derived MSCs and monocytes from human peripheral blood were isolated and cultured to investigate the effects of MSCs-induced macrophages (iMΦ) on anti-inflammatory and immune modulation. TNF-α, CD163 and arginase 1 (Arg1) of macrophages markers were tested by real-time PCR. Furthermore, we used phytohaemagglutinin-stimulated T cells to examine functional activity of iMΦ in vitro. Indirect co-culture with MSCs increased the expression of Arg1 and mannose receptor (CD206), markers of activated M2 macrophages in human peripheral blood mononuclear cells, demonstrating that MSCs-secreted factors promoted M2-MΦ polarization. iMΦ exhibited a similar inhibition effect on activated T cell growth compared with other group (MSCs only, MSCs plus iMΦ), implying iMΦ can play a sufficient functional role. Interestingly, the population of FoxP3 Treg cells co-cultured with iMΦ was significantly expanded, suggesting that iMΦ have an immunomodulatory effect by modulating FoxP3 expression. Notably, iMΦ expressed high levels of immunosuppressive and anti-inflammatory cytokines, IL-10 and TSG-6, as determined by quantitative PCR. Conclusively, our results suggest that iMΦ play a significant role in immune and inflammatory-mediated responses. Further, these iMΦ may be a novel type of stem cell-based cell therapies for immune-mediated inflammatory disorders.

MUSCULOSKELETAL TISSUE

T-2013

HUMAN MESENCHYMAL STROMAL CELL INJECTION IN NUDE RAT TONGUES FOR TREATMENT OF FIBROTIC SWALLOWING DYSFUNCTION

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Difficulty swallowing threatens the health and quality of life of patients with numerous disorders, including head and neck cancer. Surgical removal of cancer from the tongue and chemoradiation therapy pose significant risk of dysphagia due to tongue fibrosis. Malnutrition, aspiration, and reduced pleasure in eating commonly result. Many patients require gastrostomy tube for nutrition. Current treatment is limited to swallowing exercises and behavioral strategies which do not affect the dysfunctional tongue structure. A new treatment to restore tongue mobility is needed. This work investigates injection of human bone marrow-derived mesenchymal stromal cells (B-MSC) in a novel nude rat model of post-surgical tongue fibrosis. Previous work in this model demonstrated reduced fibrosis and inflammation after B-MSC injection during the wound maturation phase. Experiments presented here assess the engraftment of the injected cells. Membrane dye labeling and quantitative PCR for HLA-ABC demonstrate complete clearance of the B-MSC between 2 and 4 weeks after injection. TUNEL staining was strongly positive in the membrane-labeled cells, indicating apoptosis as the mechanism of cell death. Despite the cell elimination, smooth muscle actin content was reduced in injected tongues relative to injured controls. These findings support the potential of B-MSC injection in the tongue to reduce fibrotic wound healing which impairs swallowing, without long-term cell persistence.

T-2015

JUVENILE RADIOTHERAPY DAMAGES MOUSE MUSCLE STEM CELLS, IMPAIRING MUSCLE MATURATION AND REGENERATIVE CAPACITY

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Juvenile skeletal muscle growth is a dynamic period of maturation and development. During this time, muscle stem cells (satellite cells, SCs) contribute individual myonuclei to developing myofibers- essential for their ability to increase in size. Because juvenile SCs are active, they may be particularly susceptible to toxicities and damage. Using a small animal research radiation platform (SARRP), we have performed experiments investigating the effects of targeted radiation

on the juvenile SC pool. 4-week old mice were irradiated with a fractionated dose to the lower right limb. This regimen profoundly affected SC number and function immediately after the last dose. SC number was significantly reduced in both fast-contracting extensor digitorum longus (EDL) and slow-contracting soleus (SOL) muscles. Remaining SCs had severe deficiencies in proliferative ability in culture. Consistent with proliferative deficiency, gene expression analysis of irradiated SCs revealed the cell-cycle inhibitor p21 (Cdkn1a) to be significantly induced. Consistent with these findings, irradiated muscles had little to no regenerative capacity in response to experimental degenerative injury. Using a mouse model to track SC fate (Pax7CreERT2/+;Rosa26nTnG/+), irradiated EDL and SOL muscles demonstrated substantial reductions in SC-derived contributions to juvenile muscle growth. This coincided with significant reductions in myonuclear content and muscle fiber atrophy, one month post-juvenile irradiation. Collectively, these data demonstrate the susceptibility of juvenile SCs to radiation exposure, which leads to deficits in skeletal muscle maturation and regenerative capacity.

T-2017

A NEW PERSPECTIVE ON CARTILAGE REGENERATION: TEMPOROSPATIAL BIOCHEMICAL CONTROL OF THE SKELETAL STEM CELL NICHE

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By 2040 nearly 80 million Americans will have arthritis. Osteoarthritis (OA), the most common form of arthritis, is a major global health burden. Little is known about the exact mechanism of OA from a stem cell viewpoint. While there are a plethora of clinical pilot studies utilising a heterogenous population of Mesenchymal Stromal Cells the outcomes of these studies are often functional (symptomatic) or imaging-based. Our group are the first to identify the mouse and human Skeletal Stem Cell (SSC). In mouse and human we have successfully shown that; 1-with maturity there is a reduction on the number of resident SSC, 2-following acute injury there is a local increase in resident SSC and an increase in proliferation of those resident SSC, 3-SSC intrinsically change in their in vivo differentiation capacity and gene expression following injury, 4- the fate of those “activated” SSC can be controlled biochemically utilizing BMP2 and VEGFr1 to regenerate hyaline-like cartilage. For the first time, we have validated a surgical technique from a stem cell perspective and have added a controlled temporo-spatial biochemical niche that provides for effective and robust stable hyaline cartilage regeneration. The next step is clinical application of these FDA-approved components in a RCT.

Funding Source: The National Endowment for Plastic Surgery, The Plastic Surgery Foundation (PSF) of America. Transplant and Tissue Engineering Center of Excellence Leadership Group.

T-2019

DEFINING THE ROLE OF SOX9+ SKELETAL PROGENITOR CELLS IN LARGE-SCALE REGENERATION OF MURINE BONES

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The human skeleton has a limited ability to regenerate large bone defects beyond simple fractures. Current treatment of persistent skeletal injuries with autologous bone grafts or synthetic substitutes carry significant co-morbidities and high costs. For this reason, there is a strong need for alternative treatments to enhance skeletal healing by stimulation of endogenous repair mechanisms. Inspired by peculiar case-reports of large-scale rib regeneration in humans, our lab recently made the surprising discovery that murine ribs are capable of regenerating extraordinarily large skeletal segments. Using this novel model, we have identified the periosteum as a key source of highly-potent skeletal progenitor cells that generate the callus needed to mediate large-scale regeneration. In addition, with our collaborators at USC, our lab has recently discovered a rare Sox9+ skeletal progenitor population resident within the periosteum of the rib. Preliminary experiments have revealed that approximately 20% of the progenitor cells that migrate into the injury site and generate the reparative callus are derived from these Sox9+ populations. Interestingly, we observe that despite only representing a minority of repair cells, ablation of Sox9-lineage cells completely prevents regeneration. Furthermore,

genetic disruption of the Hedgehog signaling pathway in Sox9-lineage cells also drastically impedes healing. These results demonstrate that Sox9-lineage cells are required for large-scale rib regeneration and that their regenerative capacity requires activation of the Hedgehog signaling pathway. My future work will aim to further elucidate the mechanisms by which Sox9-lineage cells orchestrate large-scale bone repair, as well as evaluate the possibility of exploiting this knowledge to enhance skeletal regeneration in situations where natural healing fails.

Funding Source: NIH NICHD T32 Support to MS and SK NIH NIAMS RO1 Support to FM

T-2021

THE UBIQUITIN-PROTEASOME SYSTEM IS INDISPENSABLE FOR THE MAINTENANCE OF MUSCLE STEM CELLS

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Adult muscle stem cells (satellite cells) are required for adult skeletal muscle regeneration. A proper balance between quiescence, proliferation, and differentiation is essential for the maintenance of the satellite cell pool and their regenerative function. Although the ubiquitin-proteasome is required for most protein degradation in mammalian cells, how its dysfunction affects tissue stem cells remains unclear. Here, we investigated the function of the proteasome in satellite cells using mice lacking the crucial proteasomal component, Rpt3. Ablation of Rpt3 decreased proteasome activity in satellite cells. Proteasome dysfunction in Rpt3-deficient satellite cells impaired their ability to proliferate, survive and differentiate, resulting in defective muscle regeneration. We found that inactivation of proteasomal activity induced proliferation defects and apoptosis in satellite cells. Mechanistically, insufficient proteasomal activity upregulated the p53 pathway, which caused cell-cycle arrest. Our results show that the ubiquitin-proteasome system is indispensable for the maintenance of muscle stem cells in adult muscle.

T-2023

TARGETING INCREASED CDC42 ACTIVITY TO REJUVENATE AGED MUSCLE STEM CELLS

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Skeletal muscle, a multinucleated contractile tissue plays an integral role in locomotion and maintenance of homeostasis. Muscle stem cells (MuSCs) or Satellite cells are indispensable to preserve tissue homeostasis over time and for muscle regeneration. With aging, a progressive loss in total MuSC pool size with a decline in both MuSC and skeletal muscle function is observed. Although a deficit in their function has not been fully corroborated to age-related Sarcopenia (muscle wasting), several studies indicate a direct correlation of MuSC dysfunction to impaired muscle regeneration and decreased response to normal physiological stimuli. Interestingly, recent studies have given insights into the possibility of reversing MuSC aging. Gain of function of the small RhoGTPase Cdc42 exhibits premature aging-like syndrome, including muscle atrophy and sarcopenia. This evidence implies a potential role of high Cdc42 activity in impairing MuSC function and hence, efficient muscle regeneration with aging. In our study, we aim to better understand the role of Cdc42 activity and its implications in aging of skeletal muscles. Hence, we postulate that the increased Cdc42 activity in physiologically aged mice might drive (extrinsically or intrinsically) the impairment of MuSC regenerative capacity. We also propose that by pharmacologically targeting Cdc42 activity, the regenerative potential of MuSCs might be improved. Our data show that the systemic administration of CASIN (a specific small molecule inhibitor of Cdc42 activity) reduced Cdc42 active levels in aged muscles with significant increase in the proliferative potential of aged MuSCs in in vitro myogenic assays. Furthermore, in comparison to untreated aged mice, CASIN treated aged mice showed improvement in the overall regeneration of the injured tissue as well as significantly increased Pax7+, MyoD+, Pax7+/MyoD+ cell number after Notexin injury, indicating enhanced regenerative potential. As well, the aged CASIN treated mice performed better on Rotarod than the untreated littermates. Altogether, our data suggest that the increased Cdc42 activity in aged MuSCs might be a likely cause of functional impairment of aged MuSCs. Consequently, CASIN treatment might represent a possible therapeutic approach to rejuvenate aged MuSC function in vitro and in vivo.

CARDIAC TISSUE AND DISEASE

T-2025

AN IN VITRO 3D MODEL OF DESMOPLAKIN-LINKED CARDIOMYOPATHY USING HUMAN PATIENT-DERIVED IPS-CARDIOMYOCYTES

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Ventricular dilation is a common response to increased workload on the heart due to volume or pressure overload, but progression can vary widely across patients. In fact, little is known about the frequency and magnitude of loading, in combination with genetic predisposition, that drives this disease process. Desmoplakin mutations provide a unique opportunity to study this by providing a distinct defect in cell-cell adhesion molecules that lead directly to loss of cell-cell coupling. Clinically patient hearts with desmoplakin mutations are associated with changes in myocardial structure and occurrence of arrhythmias, but is challenging to study in detail. To address this, we developed an in vitro 3D model of desmoplakin-linked cardiomyopathy using patient-derived induced pluripotential stem (iPS) cells differentiated into cardiomyocytes. We engineered cardiac tissues with the unique ability to control both (i) the amount of mechanical loading on the tissue throughout the culture process and (ii) enable physiologic levels of muscle shortening (10-20%) to better mimic in vivo function. Briefly, iPS cells with desmoplakin mutations and controls were reprogrammed from dermal fibroblasts using established methods. These iPS cells were expanded, differentiated into cardiomyocytes using monolayer-based methods and lactate purified. Engineered cardiac tissues were formed by casting cardiomyocytes and cardiac fibroblasts in a collagen gel around the ends of a curved, horse-shoe shaped PDMS strip that applied a constant load. The engineered cardiac tissues were formed using PDMS strips with various thicknesses to tune the mechanical loading and were cultured for up to 4 weeks. The desmoplakin mutation tissues had reduced desmoplakin expression and clear loss of cell-cell adhesion, compared to controls. Functionally the desmoplakin mutation resulted in clear phenotypic difference with a 25% increase in diastolic tissue length and a 70% reduction in peak systolic stress compared to controls. This establishes that our 3D in vitro model can replicate aspects of the loading-induced disease progression, including associated chamber thinning and reduced cardiac output observed in desmoplakin-associated cardiomyopathies, and provide an in vitro model of volume-overload cardiomyopathy.

T-2027

STANDARDIZED GENERATION OF HUMAN PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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The use of human pluripotent stem cell (PSC) derived cardiomyocytes (CMs) is of high interest for drug testing, heart disease modeling and regenerative therapy. For these applications standardized protocols for the efficient generation of cardiomyocytes are needed. Even though several protocols for cardiac differentiation have been published, the majority of them have to be adjusted for each stem cell clone e.g. by titration of small molecule and cytokine concentrations, in order to obtain the optimal differentiation efficiency and cell yield. Moreover, lot to lot variations of media components modulate the outcome of the differentiation. These protocol optimizations are costly and time consuming. In order to circumvent these limitations, we have developed a cardiac differentiation medium, the StemMACS CardioDiff kit, enabling robust and standardized cardiac differentiation without the need for media adjustments. Contracting CMs can be generated within 8 days of differentiation with differentiation efficiencies of up to 90%. The cardiac differentiation protocol is robust, highly efficient, transferrable to different stem cell clones and scalable to various plating formats enabling standardized large scale manufacturing of PSC-derived cardiomyocytes. Generated CMs express the cardiomyocyte specific markers alpha actinin and Troponin T. Analysis of cardiomyocyte subtype marker expression (Irx4 and MYL2) revealed a ventricular-like subtype. However, an atrial-like cardiomyocyte phenotype can be induced by addition of

signaling pathway modulators at specific time points during differentiation. Translation of the described differentiation procedure to the integrated cell processing platform CliniMACS Prodigy® will pave the way for standardized, large scale manufacturing of the PSC-derived CMs.

T-2029

FROM STEM CELL TO DISEASE: HUMAN IPSCELL DERIVED CARDIOMYOCYTES MIMIC DIFFERENT FORMS OF INHERITED CARDIOMYOPATHIES

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Recent advances in genetic technology unraveled novel disease genes for inherited cardiomyopathies (CMPs). The goal of the project is to generate patient specific model systems by using human induced pluripotent stem cell (hiPSCs) derived cardiomyocytes (CMs). First dermal fibroblasts, obtained from patients carrying recently discovered homozygous mutations in genes causing DCM with ataxia syndrome (DNAJC19, IVS3-1G>C, DCMA), DCM with juvenile cataract (LEMD2, c.38T>G, p.L13R) and control cell lines, were reprogrammed into iPSCs. To compare the effects of the specific mutations within the remaining patient-specific genetic background, isogenic controls are currently generated by using CRISPR/Cas9 technology. Genome editing is used to reconstruct DCMA via DNAJC19 truncation in healthy iPSCs. Furthermore, two desmosomal proteins: plakophilin-2 (PKP2) and desmoglein-2 (DSG2) were knocked-out in healthy iPSCs for mimicking arrhythmogenic cardiomyopathy (ACM). Moreover the LEMD2 isogenic control iPSCell line is currently produced via homology directed repair. After successful differentiation of patient derived iPSCs and controls into CMs, challenges, as purity and immature state, are addressed by lactate based metabolic enrichment, MACS sorting and hormone controlled maturation experiments. Age-dependent volume increase (c-TnT [µm3]) and EM analysis reveal adult CM-like properties supporting a mature-like state of in vitro generated CMs. Phenotypic studies of ACM reveal in PKP2-KO CMs molecular changes in expression and localization of the corresponding desmosomal proteins. PKP2-KO CMs indicate contraction abnormalities due to a decrease in stimulus-dependent transients suggesting evidence for prolonged refractory phases due to abnormal Ca2+ homeostasis. Initial investigations of DCMA present in DNAJC19 IVS3-1G>C carrier CMs a fragmentation of mitochondrial structure and first functional changes measured by radioactive tracer uptakes (18F-FDG, 99mTc-MIBI). Further characterization of patient-specific CMs and their isogenic controls for structural, electrophysiological and molecular changes according to the individual disease are ongoing to display clinical aspects of the disease and to determine its suitability for drug compound testing.

T-2031

REGULATION OF CARDIOMYOCYTE MATURATION BY AN RNA SPLICING REGULATOR RBFOX1

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The maturation of stem cell derived cardiomyocytes (iCMs) is incomplete relative to the fully matured adult myocytes. Lack of maturation represents a major limitation to the applications of iCMs as heart disease models or heart failure therapies. Current attempts to promote iCMs maturation, such as prolonged culture, mechanical stretching and electronic pacing, are often based on empirical methods with poor reproducibility or little mechanistic basis. In order to better understand the molecular mechanisms driving cardiomyocyte maturation, we performed extensive transcriptome analyses in neonatal vs. adult hearts. In addition to metabolic and cell cycle regulatory pathways, Gene Ontology analysis revealed RNA splicing regulation is significantly enriched in the transcriptome reprogramming during postnatal maturation in heart. Specifically, we find a cardiomyocyte enriched RNA splicing factor Rbfox1 is dramatically induced in the perinatal maturating mouse hearts.

Ectopic expression of Rbfox1 in neonatal cardiomyocytes markedly promotes the cellular and molecular features of adult cardiomyocyte, including contractility, calcium handling, sarcomere organization, morphology, electrophysiology and gene expression. Most remarkably, expression of RBFox1 in human iPSC derived cardiomyocytes promotes similar maturation process as observed in the neonatal rat myocytes. At mechanistic level, RBFox1 expression in the iCMs enhances transcriptome maturation as indicated by targeted RNA splicing in genes involved in muscle contraction, gene expression, RNA processing and sarcomere organization. In summary, we have uncovered a novel molecular path towards neonatal myocyte maturation in perinatal murine hearts by targeted modulation of cardiomyocyte transcriptome via RNA-splicing. This approach has potential to be employed as a molecular approach to promote human iCMs maturation.

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T-2033

PROTEIN AND GENETIC INTERACTION ANALYSES IN HUMAN IPS-DERIVED CARDIOMYOCYTES TO STUDY PROTEIN QUALITY CONTROL DISEASE IN THE HEART

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Cardiomyocytes must maintain constant contractile function of the heart throughout a human lifetime. To achieve this, a complex network of chaperones and other proteins maintains the homeostasis of the system, facilitating the assembly and disposal of proteins as required by the cell. Protein quality control pathways are increasingly recognized for their importance in both inherited and sporadic cardiac disease, and as potential therapeutic targets. The co-chaperone BAG3 is particularly interesting, because genetic evidence suggests that variants of BAG3 can be both pathologic and protective. However, understanding the molecular mechanism underlying most of these disease-genetic variant associations remains a major challenge. Unbiased analysis of protein and gene interaction holds the potential to provide critical information to fill in this knowledge gap. Using genome engineering tools, we have generated a series of isogenic iPS cell lines bearing different variants on the BAG3 gene, along with an epitope tag fusion. Using affinity purification followed by mass spectrometry (APMS) on iPS-derived cardiomyocytes (iPS-CM), we have been able to identify interacting partners for the BAG3 protein expressed at endogenous levels in a disease relevant cell type. Our results show a surprisingly high number of cardiac-specific interactions. In addition, some BAG3 protein variants present distinct gain or loss of specific interactions, allowing us to narrow down the list of candidate hits. Using microscopy image analysis and deep neural networks, we performed a targeted knockdown screening on BAG3 interaction partners to dissect pathways of BAG3 interactors that are involved in the development disease phenotype. We hope the information obtained from this study will improve our understanding of the heart proteostasis network, enable the identification of potential therapeutic targets, and provide clues towards a broader understanding of the role of genetic variation in complex disease.

Funding Source: JAPB is a recipient of an American Heart Association predoctoral fellowship.

T-2035

BIOMECHANICAL REGULATION OF HUMAN ADIPOSE-DERIVED STEM CELL DIFFERENTIATION FOR CARDIAC MUSCLE REGENERATION

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Adipose-derived stem cells (ADSCs) have been identified as potential candidates for cell-based therapies for myocardial infarction due to their multipotency and immunomodulatory properties. Previous studies have shown that ADSCs can differentiate into cardiomyocyte-like cells in vitro and in vivo with the potential to promote cardiac tissue regeneration in myocardial infarct animal models. Various factors have been identified that are able to induce or enhance ADSC cardiomyocyte differentiation, such as biochemical cues and biomechanical forces, and physical properties of cell adhesion substrate such as topography and stiffness. Much is still unknown concerning the synergistic interactions that modulate stem cell differentiation, as well as what culture conditions are most conducive to generating mature MSC-derived cardiomyocytes. We used a novel high throughput biaxial oscillatory stretch system (HT-BOSS) to apply complex, time-varying strain waveforms to ADSCs cultured on flexible substrates coated with either collagen or fibronectin and with or without micropattern grooves. Stem cells were mechanically

stretched at 10% maximal strain at 1 Hz with either a sine or cardiac waveform mimicking physiological loading in the left ventricle, and simultaneously exposed to various biomolecules. Our findings showed that the cardiac waveform enhanced expression of proteins related to cardiomyocytes compared to the traditional sinusoidal waveform. Furthermore, mechanical loading in combination with chemical treatment synergistically increased expression of cardiac-related markers in ADSCs. Next generation sequencing of the cells demonstrated that there was increased gene expression for the cardiac fibroblast phenotype in ADSCs loaded with cardiac waveform. In contrast, ADSCs treated with the sine waveform of mechanical load expressed a cardiac muscle gene pattern. Overall, we were able to identify specific combinations of biochemical factors, pharmacological inhibitors, and biomechanical forces that potentially drive ADSCs toward a cardiomyocyte-like phenotype.

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T-2037

MODELLING POPULATION VARIABILITY IN LONG QT SYNDROME TYPE 2

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Congenital long QT syndrome type 2 (LQTS2) is caused by mutations in the KCNH2 gene that encodes the Kv11.1 potassium channel, one of the major contributors to repolarisation of the heart. LQTS2 is characterised by prolongation of the QT interval on the ECG and an increased risk of cardiac arrhythmias and sudden cardiac death. A major problem in risk stratification of LQTS2 patients is that there is significant variability in clinical phenotype seen across the population, even for patients with the same primary disease gene. In this study, we aimed to quantify how differences in the expression of rhythmonome genes that define the electrical environment of the cardiac cell contribute to this variable presentation. Preliminary transcriptomic analysis from a panel of 12 human iPSC-cardiomyocytes (hiPSC-CMs) from 'normal' patients showed differential expression of cardiac ion channel genes of up to 22-fold between lines. Phenotypic characterisation using high-throughput optical reporting of cellular calcium and membrane electrophysiology revealed significant differences in calcium transient and action potential parameters between lines. Subsequently, KCNH2 mutations that cause either Kv11.1 gating defects (R56Q) or heterozygous loss of function will be introduced into each background using CRISPR/Cas9 to assess the effect of genetic background on disease presentation. Our data show that phenotypic analysis of genetically diverse hiPSC lines allows modelling of population variability in vitro. Combination of this population modelling approach with gene-editing will allow us to explore how genetic background contributes to disease presentation in LQTS2 in order to develop better approaches to risk stratification for these patients.

T-2039

THICK HUMAN CARDIAC TISSUE CONSTRUCTS CONTAINING PATTERNED, PERFUSABLE HUMAN MICROVESSELS FROM PLURIPOTENT STEM CELLS

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Engineered cardiac tissues are a promising approach for both cardiac regeneration and disease modeling, however, vascularization and efficient perfusion are long-standing challenges to generating thick cardiac constructs. Previous work towards vascularization relies on the self-assembly of endothelial cells into connected tubes, resulting in improved tissue function but limited perfusion and slow vascular host integration upon implantation. Here, we engineer perfusable, patterned microvessels using human embryonic stem cell-derived endothelial cells (hESC-ECs) into collagen-based cardiac tissues. We first generated hESC-EC only constructs and demonstrate that hESC-ECs are highly angiogenic and form perfusable sprouts from the patterned microvessel under flow. Furthermore, perfusable patterning of hESC-ECs causes differential gene expression towards increased vascular development when compared to non-perfusable, self-assembled hESC-EC constructs. When implanted onto infarcted rat hearts, the perfusable microvessel grafts integrate with coronary vasculature to a greater degree than non-perfusable self-assembled constructs at 5 days post-implantation. We next integrated hESC-derived cardiomyocytes (hESC-CM) and human stromal cells into the surrounding collagen matrix of both perfusable microvessel and non-perfusable, self-assembled constructs. Implantation of vascularized cardiac grafts reveal

higher cardiomyocyte and vascular density for perfusable cardiac grafts, suggesting that perfusable microvessels enhance hESC-CM remodeling and could lead to enhanced hESC-CM engraftment long term. We will further demonstrate the ability to generate thicker cardiac tissues with higher vascular density by connecting multiple layers of patterned, perfusable vasculature. We hypothesize that integration of a multi-layer perfusable network of hESC-ECs will enable long term benefits to cardiomyocyte function within the tissues. Altogether, these findings demonstrate the beneficial role of perfusable vascular patterning to improve engineered cardiac tissues and support cardiac grafts after implantation. This work addresses the critical challenge of vascularization in engineered cardiac tissues and will facilitate the next generation of cardiac tissue design.

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T-2041

INTEGRATED ANALYSIS OF HUMAN IPSC-DERIVED CARDIOMYOCYTES IN DIVERSITY AND DISEASE MODELING

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Human cell types differentiated from induced pluripotent stem cells (hiPSC) offer an attractive human cellular platform for safety and efficacy testing. Here, we present data demonstrating the utility of hiPSC-derived cardiomyocytes (hiPSC-CMs) in safety assessment and cardiac disease modeling. Clinically-defined Type I cancer therapeutics-related cardiac dysfunction (CTRCD) may be associated with cellular death, structural changes, and permanent damage while Type II CTRCD may be associated with cellular dysfunction, no structural changes, and reversible damage. In this study, we include a comprehensive assessment of CTRCD compounds doxorubicin (type I) and sunitinib (type II) across a panel of hiPSC-CMs derived from 6 apparently healthy donors (DIV 14) at three concentrations [0.1, 1.0, and 10 ÂµM]. From these data, we were able to identify both type I and type II CTRCD by using a selected in-vitro cohort of hiPSC-CMs. These data further provide additional insight into sensitivities to cardio-oncology liabilities across different donors. Subsequently, we examined basic functional characterization data from several hiPSC-CM disease models, including hypertrophic cardiomyopathy MYH7 (R403Q), LMNA-related dilated cardiomyopathy LMNA (L35P), and Brugada syndrome type 3 CACNA1C (G490R) each with its respective isogenic control at DIV 14. We further identify the functional consequences of each mutation and demonstrate that each model recapitulates classical hallmarks of the disease phenotype. These data illustrate how hiPSC-CMs provide an excellent model system for assessing compound effects across multiple donors and disease models. Taken together, these examples help to create new avenues for safety assessment and efficacy studies, as well as serve as a template for future opportunities in cardiac disease modeling with hiPSC-CMs.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

T-2043

HIGHLY EFFICIENT ENDOTHELIAL AND SMOOTH MUSCLE DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS AND THEIR APPLICATION IN THE TREATMENT OF ISCHEMIA DISEASES

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Human pluripotent stem cell derived endothelial cells (ECs) and smooth muscle cells (SMCs) can be used to model human vascular diseases and for cell transplantation therapy. It is challenging to obtain large quantities of ECs and SMCs in a cost-effective manner. Here we described a very simple, xeno-free and chemically defined basal medium (named AATS) for vascular lineage differentiation. AATS culture system generates nearly pure ECs and SMCs in 5-8 days, through metabolic restriction and adhesive selection. Transcriptome profiling showed that hPSC-derived ECs and SMCs closely resembled their in vivo counterparts. Accessible chromatin analysis revealed that in AATS condition, cells acquired epigenetic feature bias towards vascular lineage differentiation. ECs and SMCs generated in AATS medium also lowly expressed immunogenicity genes. Combining with 3D microniche culture, AATS differentiation protocol permitted cost saving and large scale bioproduction of ECs derived from hPSCs. HPSC-derived ECs using AATS system exhibited strong revascularization

potential in the treatment of mice model of hindlimb ischemia and middle cerebral artery obstruction (MCAO). Our study provided a cost-effective and highly efficient platform to manufacture GMP compatible, off-the-shelf ECs and SMCs to model human diseases and for vascular repair.

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T-2047

AN IPSC DERIVED MICROFLUIDIC BLOOD BRAIN BARRIER MODEL PROVIDES LEAKTIGHT ENDOTHELIAL CELL TUBES FOR BARRIER PERMABILITY ASSESMENT

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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 development, maintenance, and disease of the BBB are difficult and time-consuming to study in vivo. 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 and have been hampered by the limited cell availability and low model fidelity. Human induced pluripotent stem cells (hiPSCs) are a promising source that enables large-scale production of specialized cells of human origin, for example brain endothelial cells, with high similarity to their in vivo counterparts. Thus, hiPSCs have a great potential to serve as an excellent infinite human cell source for in vitro BBB models. To further recapitulate the physiological conditions of brain vasculature micro physiological systems can be used where flow through tubes of endothelial cells can be added. Consequently, recreating the shear forces that endothelial cells in vivo experience, which have been proven to have important implications for development of many of the specialized features in brain endothelial cells. In this study hiPSC derived brain endothelial cells have been adapted to grow in micro physiological chips and form tube structures. These vascular tubes are perfused creating a more physiologically relevant model of the BBB. We show that hiPSC derived endothelial cells in this model create a leak tight barrier down to 4.4 kDa, and that it is possible to detect functional activity of important efflux transporters in the system. Comparing the micro physiological model to a static transwell model reveals that the dynamic model has improved mRNA expression of several important junction associated proteins and brain specific transporters. This model also has the advantage of suitability for higher throughput screenings since there are 20 micro fluidic units in one 384 well plate. Consequently, this microfluidic model of the BBB provides a promising starting point for using hiPSC derived micro physiological systems for predicting brain permeability of novel therapeutics.

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HEMATOPOIESIS/IMMUNOLOGY

T-2049

RED BLOOD CELL GENERATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Donor-derived red blood cells (RBCs) are the most common form of cellular therapy, coupled with donor-dependency, alloimmunization and blood borne disease risks. In vitro derivation of RBCs from an immortal source, as iPSCs provide an alternative solution to these issues. In addition, in vitro iPSC-RBCs allow genome editing for possible gene therapies, while large scale production would be beneficial in RBC drug delivery. The current limitation of iPSC-RBCs is their development immaturity, leading to low enucleation potential, which requires improvement prior clinical application. To further develop the process, we have established a GMP-compatible iPSC differentiation protocol that upon hematopoietic specification yielded erythroid cells (ERYs). This method resulted in 2-6*10^5 ERYs/iPSC with 100% CD71+/CD235+ (ERY-specific markers). Further maturation yielded orthochromatic normoblasts containing a mix of primitive and definitive erythroid waves, leading to poor enucleation potential and/or reticulocyte stability. Bulk RNAseq analysis comparing iPSC- to in vitro

derived definitive-ERYs revealed significant differences and identified genes that may be used to further augment iPSC-ERY terminal diff. (e.g. lack of erythroid regulators: MYB and KLF1 target genes SOX6, BCL11A). Single-cell RNAseq of early iPSC diff. was performed, aiming to recognize distinct waves of hematopoiesis. 5 cell clusters were identified: 1 was HSPC-like, 3 lineage committed and 1 of unknown origin. This data coupled with index sorting, defined the HSPCs as CD71+/CD235- and the iPSC-EBLs as CD71+/CD235+ expressing embryonic and fetal Hbs. In conclusion, we have found differentially expressed markers between the iPSC-ERYs and definitive-ERYs that may be used to better define specific erythroid waves during iPSC diff. and/or development. We have showed that CD71/CD235 is useful marker combination in early hematopoietic specification to distinguish between lineage-committed (developmentally immature) and HSPC-like cells. We hypothesize, that the identified HSPCs potentially give rise to developmentally more mature cells, which we aim to expand. Currently we are pursuing different approaches to support early iPSC-HSPCs giving rise to more mature definitive RBCs including proper enucleation potential.

T-2051

A DYNAMIC EXPRESSION OF MESENCHYMAL ASSOCIATED GENES DRIVES THE SEQUENTIAL FATE SWITCHES DURING HEMATOPOIETIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) provide an invaluable model for dissecting human hematopoietic development and an unlimited source for generating various kinds of functional blood cells. However, the mechanism underlying human hematopoiesis remains largely unknown. In this study, we identified a dynamic expression pattern of mesenchymal associated genes by transcriptome analysis with APLNR+, CD34+ and CD43+ subpopulation of cells during hPSCs hematopoietic differentiation. Interestingly, we found this dynamic expression pattern is also conserved in vivo. Functionally, the WNT/SNAIL1 axis activates the expression of mesenchymal associated genes during APLNR+ cells generation from hPSCs. In contrast, suppression of TGF-beta signaling or abolish the expression of MSX2, a master transcription factor of mesenchymal development, facilitates the CD43+ hematopoietic progenitor cells derivation from CD31+ endothelial cells. Our findings provide new insights for the understanding of human hematopoiesis and may facilitate the large-scale generation of functional blood cells for potential clinical applications.

T-2053

HOXC4 PLAY A KEY ROLE DURING HUMAN HEMATOPOIESIS AND HAS STRONG POTENTIAL TO PROMOTE HEMATOPOIESIS, ESPECIALLY ERYTHROGENESIS

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The HOX genes are initially characterized in Drosophila as a family of homeodomain-containing transcription factors, which function as master regulators of embryogenesis. In mammals the original HOX gene cluster was duplicated to give rise to 39 genes that were separated into A, B, C and D clusters. HOXC4 gene belongs to C clusters. It has been rarely researched and its function on hematopoiesis is still unclear. In our research HOXC4 coding region was inserted to PB-Tet-on-OE to construct piggyBac-based inducible overexpression vector, and then transfect human embryonic stem cells (hESCs) to establish transgenic cell line HOXC4/hESC, which has normal pluripotency and potential of hematopoiesis. HOXC4/hESCs co-cultured with AGM-S3 were induced overexpression of HOXC4 by doxycycline (DOX) from D0, D2, D4, D6, D8, D10, or D12 and done the FACS analysis at D14. The induction of HOXC4 from D10 could strongly promote the production of CD34-CD43+, GPA+CD71-, GPA+CD71+, CD34-CD45+, and CD34+CD45+ population. If from D10 the inhibitor of NF-KB signaling was added together with DOX, the effects will disappear, which indicated that such effects might be close related with NF-KB signaling. Because that HOXC4 was co-expression with GFP the GFP+ cells of co-culture induced from D10 were isolated by sorting at D14 so as to perform analysis of deep sequencing. It indicated that some important hematopoiesis-related signaling pathway, including NF-KB signaling, were up-regulated after HOXC4 was induced. The most obviously promoted population, such as CD34-CD43+ and GPA+CD71- in GFP+ cells, were sorted by FACS at D14 and detected by MGG staining, which show that they were main erythroid progenitors. Cell cycle status has no obvious difference between GFP+ cells of co-culture induced by DOX and the co-culture without DOX-treatment, which indicated that promotion of hematopoiesis is not related to the status change of cell cycle. Conclusion: the overexpression of HOXC4 during the late stage of hematopoiesis in AGM-S3 co-culture system could obviously promote the production of erythroid progenitors and hematopoietic stem/progenitor cells, especially the former. It might be through NF-KB signaling but not related to cell cycle, which revealed that HOXC4 play a key role in hematopoiesis.

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T-2055

HUMAN PLACENTAL MESENCHYMAL STROMAL CELLS DIFFERENTIALLY MODULATE RESIDENT ALVEOLUS VS RECRUITED BONE MARROW MACROPHAGE RESPONSES IN BACTERIAL PNEUMONIA TO IMPROVE SURVIVAL

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Tissue injury such as acute respiratory distress syndrome (ARDS) is one of the lethal complications of pathogen infections, and macrophages (MΦs) are the critical immune cells involved in post-infection tissue repair processes. Increasingly, tissue-resident MΦs appear to be distinct from classical bone marrow-recruited (BM) MΦs, with the former more important in tissue repair and the latter in pathogen clearance. While multilineage human mesenchymal stromal cells (MSCs) are known to have clinically relevant immunoregulation towards T cell-mediated diseases, little is known of its interactions with MΦ subpopulations. We therefore set up a mouse model of Klebsiella pneumoniae (KP)-mediated severe pneumonia with a clinical isolate of KP to assess MSC efficacy towards MΦ pathogen clearance versus tissue repair. t-Distributed Stochastic Neighbor Embedding (t-SNE) analysis showed that compared to KP-infected mice, intravenous injection of human placenta-derived MSCs (PMSCs) shifted lung MΦ subpopulations towards alveolus-resident MΦs and away from recruited BM MΦs. Intriguingly, antibacterial functions of phagocytosis and respiratory burst activity were enhanced in alveolar MΦs with PMSC injection, but conversely suppressed in BM-MΦs. Moreover, compared to KP-infected mice, injection of PMSCs significantly reduced the influx of inflammatory granulocytes in the lung tissue as well as systemic IL-6 levels, which led to significantly improved local and systemic bacterial clearance with decreases in alveolar and bronchial injury. Most importantly, PMSC injection significantly increased KP-infected mice survival rates. Taken together, our results demonstrate that PMSCs differentially enhance resident-alveolus MΦ function while suppressing recruited BM MΦ inflammatory function which overall led to improved survival of KP-infected mice. Our data strongly implicate a therapeutic role for PMSCs towards severe bacterial infections and its subsequent tissue injury.

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T-2057

LSD1/GFI1B CONTROLS HEMATOPOIETIC DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS

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Differentiation of induced pluripotent stem cells to hematopoietic lineages hold great promise to produce novel or replace existing advanced therapeutic and medicinal products (ATMPs). During embryogenesis, hemogenic endothelium cells (HE) give rise to hematopoietic stem and progenitor cells (HS(P)C) in the AGM region through a process called endothelium to hematopoietic transition (EHT), which involves the upregulation the hematopoietic and repression of the endothelial program. EHT has been observed during iPSC differentiation, however, bone marrow repopulating HSCs have been difficult to generate. We investigated the function of the chromatin demethylase LSD1/KDM1a and its specific partner, transcription factor GFI1B, during this process. Thereto, we used BDPLT17 patient-derived iPSC lines in which a dominant negative GFI1BQ287* is expressed. Flow cytometry revealed delayed and severely hampered hematopoiesis in BDPLT17 iPSC and LSD1 inhibited conditions, respectively. Although the formation of HE (CD144+/CD309+/CD31+) was not affected, hematopoietic committed cells (CD144+/CD309+/CD31+/CD43+/CD41+) were severely reduced upon LSD1 availability. Besides this, DLL4 expression, a hallmark of HE with definitive hematopoietic potential, was strongly reduced (GFI1BQ287*) or absent (LSD1). Mass spectrometry on CD144+/CD309+ from GFI1BQ287* and control revealed 39 deregulated proteins. Complementary ATAC-seq analysis showed minor changes of chromatin status at the transcription start site of these 39 genes. Interestingly, GATA4, a known mediator of endothelial differentiation, was upregulated in GFI1BQ287*, which may potentially begin to explain the reduced and lack of DLL4 positive HE in GFI1BQ287* and upon LSD1 inhibition. Hematopoietic outgrowth and specification toward the megakaryoid and erythroid lineages were severely delayed (GFI1BQ287*) or absent (LSD1), showing the need for sufficient LSD1 in in-vitro hematopoiesis. Based on the results we suggest that the endothelial program during EHT is partly controlled by LSD1/GFI1B and not efficiently down-regulated upon interfering with the function of LSD1 or upon expression of the dominant negative GFI1BQ287*. We expect these findings will help to unravel the molecular events that drive in-vitro hematopoiesis from iPSC.

T-2059

VCAM-1+ MACROPHAGES GUIDE THE HOMING OF HSPCS TO A VASCULAR NICHE

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Haematopoietic stem and progenitor cells (HSPCs) give rise to all blood lineages that support the entire lifespan of vertebrates. After HSPCs emerge from endothelial cells within the developing dorsal aorta, homing allows the nascent cells to anchor in their niches for further expansion and differentiation. Unique niche microenvironments, composed of various blood vessels as units of microcirculation and other niche components such as stromal cells, regulate this process. However, the detailed architecture of the microenvironment and the mechanism for the regulation of HSPC homing remain unclear. Here, using advanced live imaging and a cell-labelling system, we perform high-resolution analyses of the HSPC homing in caudal haematopoietic tissue of zebrafish (equivalent to the fetal liver in mammals), and reveal the role of the vascular architecture in the regulation of HSPC retention. We identify a VCAM-1+ macrophage-like niche cell population that patrols the inner surface of the venous plexus, interacts with HSPCs in an ITGA4-dependent manner, and directs HSPC retention. These cells, named ‘usher cells’, together with caudal venous capillaries and plexus, define retention hotspots within the homing microenvironment. Thus, the study provides insights into the mechanism of HSPC homing and reveals the essential role of a VCAM-1+ macrophage population with patrolling behaviour in HSPC retention.

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T-2061

TISSUE ORIGIN DICTATES MURINE MESENCHYMAL STROMAL CELL CHEMOKINE AND IMMUNOREGULATORY MOLECULE REPERTOIRE AND PREDICTS IN VITRO CHEMOTACTIC ACTIVITY

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Due to their anti-inflammatory and immunomodulatory properties, mesenchymal stromal cells (MSCs) are in the spotlight as potential cellular therapies in many disease settings. Most of the literature surrounding MSC phenotype and function is derived from studies focusing on bone marrow (BM) MSCs but MSCs can be isolated from most tissues. However, the ability of MSCs from different tissues to proliferate, differentiate and to modulate inflammation is highly variable. For this reason, the aim of this study is to objectively compare the phenotype and potential in vivo function of mouse MSCs isolated from the BM, adipose tissue (Ad) and Islet of Langerhans (Is) in a stringent, standardised manner to assess if MSCs isolated from specific tissues could be optimal for use in treating specific diseases. MSCs must interact with residing or migratory immune and non – immune cells, often within an inflammatory environment, to produce their beneficial therapeutic effect. Therefore, this study focuses on how MSCs could dampen inflammation in vivo by assessing and comparing the expression of MSC chemokine ligand and receptors, toll – like receptors, the complement system and cytokine and cytokine - regulated molecules at rest and under inflammatory stimulation. Moreover, this study focuses on assessing and comparing the secretion of the above-mentioned molecules and their immune cell attraction profile in vitro and in vivo. This study found that chemokine receptor expression by MSCs isolated from BM, Ad and Is was very low except for CCR1, CCR11 and ACKR4, while CCL2, CCL5, CCL7, CCL20, CXCL1, CXCL5 and CXCL10 were highly expressed and were upregulated under inflammatory conditions. TLR2 was upregulated while TLR4 was downregulated under inflammatory conditions in the MSCs isolated from the three tissues. In the complement system, CD59, CFH, C1Qc and C5AR1 were downregulated under inflammatory conditions in all the MSCs. IL-6, INOS and GMCSF were upregulated while TSG6, HGF, VEGF and CD142 were downregulated under inflammatory conditions in BM, Ad and Is derived MSCs. This study highlighted that the tissue origin of MSCs could affect MSC in vivo migratory capacity and their ability to chemoattract surrounding immune cells, thereby potentially influencing their clinical performance.

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T-2063

GENERATION OF T AND NK CELLS FROM PLURIPOTENT STEM CELL-DERIVED HEMATOPOIETIC PROGENITORS IN A STROMA-FREE, SERUM-FREE CULTURE SYSTEM

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Human pluripotent stem cells (hPSCs) could offer an unlimited source of T and NK cells for immunotherapy of malignancies and other disorders. Differentiation of hPSCs to lymphocytes has been difficult because cell cultures often fail to recapitulate the in vivo differentiation processes that are orchestrated temporally and spatially during ontogeny. We developed a culture system that promotes generation of T and NK cells from multiple PSC lines in the absence of stromal cells and serum. Human PSCs maintained in the mTeSR1 culture system were aggregated in AggreWell plates and cultured for ten days to induce mesoderm specification and hematoendothelial differentiation. The average frequency of CD34+ cells after this period was 65% (range: 24 - 95%, n = 30 experiments with WLS-1C, H9 and STiPS-F016 cell lines). Cell aggregates were then dissociated into a single cell suspension and CD34+ cells were isolated and maintained for two weeks in a stroma-free, serum-free T cell differentiation culture containing an Expansion Supplement on plates coated with Notch ligand to promote the generation of CD7+CD5+ lymphoid progenitors. The average frequency and yield of CD7+CD5+ cells was 27% (range: 2 - 59%, n = 27) and 3.7x10^5 (range: 4.4x10^3 - 1.5x10^6) per 5x10^4 CD34+ cells. To promote differentiation into CD4+CD8+ double-positive (DP) T cells, lymphoid progenitors were replated into Notch-ligand coated wells and cultured for two more weeks with a Maturation Supplement containing Flt3L and IL-7. DP cells arose at an average frequency of 13% (range: 1 - 42%, n = 11) and yield of 2.6x10^5 (range: 1.2x10^3 - 9.0x10^5) per 5x10^4 CD34+ cells. Some DP cells expressed CD3 and TCRαβ, suggesting that they may be able to mature further into functional T cells. To promote differentiation into NK cells the lymphoid progenitors were cultured for two weeks in medium containing IL-15. These cultures produced CD56+ NK cells with an average frequency of 81% (range: 59 - 97%, n = 11, from WLS-1C and H9 cells) and yield of 4.6x10^6 (range: 6.7x10^4 - 3.8x10^7) per 5x10^4 CD34+ cells. NK cells expressed other characteristic markers including NKp46 and CD16. These results show that hPSCs can be differentiated under stroma- and serum-free conditions into lymphoid progenitors that can generate large numbers of T and NK cells for basic and translational research.

PANCREAS, LIVER, KIDNEY

T-2065

SOMATIC MUTATIONS INCREASE HEPATIC CLONAL FITNESS AND REGENERATION IN CHRONIC LIVER DISEASE

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Normal tissues accumulate genetic changes, but it is unknown if mutations promote clonal expansion of normal cells in response to chronic injury. Whole exome sequencing of diseased liver samples from 83 patients revealed a complex mutational landscape in cirrhosis. Additional ultra-deep sequencing identified recurrent somatic mutations in PKD1, PPARGC1B, KMT2D, and ARID1A. The number and size of mutant clones increased as a function of fibrosis stage and extent of tissue damage. Also, chromosome 1 and 8 gains were observed in multiple liver samples, suggesting the existence of aneuploid clones in non-malignant tissues. To interrogate the functional impact of mutated genes, a pooled in vivo CRISPR screening approach was established. In agreement with sequencing results, examination of 147 genes again revealed that loss of Pkd1, Kmt2d, and Arid1a promoted clonal expansion. Furthermore, conditional heterozygous deletion of these genes in mice was also hepatoprotective in multiple liver injury assays. Although pre-malignant somatic alterations are often viewed through the lens of cancer, we show that mutations can promote regeneration, likely independent of carcinogenesis.

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T-2067

A GLIS3-CD133-WNT SIGNALING AXIS REGULATING THE SELF-RENEWAL OF ADULT MURINE PANCREATIC PROGENITOR-LIKE CELLS IN COLONIES/ORGANOIDS

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The existence and regenerative potential of resident stem and progenitor cells in the adult pancreas is a controversial topic. A question that has been minimally addressed in the field is a progenitor cell's self-renewal capacity; one key attribute that defines a stem cell. In prior studies, our laboratory has identified putative stem and progenitor cells from the adult murine pancreas and demonstrated the self-renewal and multilineage differentiation potentials of these stem/progenitor-like cells in a unique ex vivo colony/organoid culture system. We have named these cells pancreatic colony-forming units (PCFUs) due to their ability to give rise to colonies in a 3-dimentional space. However, the molecular mechanisms by which PCFUs self-renew has remained largely unknown. Here, we tested the hypothesis that self-renewal of PCFUs required Glis3, a zinc-finger transcription factor important in pancreas development. Pancreata from 2-4 month-old mice were dissociated, sorted for CD133highCD71low ductal cells, which are known to enrich for PCFUs, and virally-transduced with shRNAs to knock down Glis3 and other molecules. Subsequently, cells were plated into our colony assays and the resulting colonies analyzed for protein and gene expression. Here we report a previously unknown Glis3-to-CD133-to-Wnt signaling axis regulating the self-renewal of PCFUs. Additionally, we find that CD133, but not Glis3 or Wnt, is required for PI3K/AKT-mediated PCFU survival. Collectively, our results identify a novel molecular pathway in maintaining the self-renewal ability of adult murine PCFUs.

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T-2069

NGN3 ESTABLISHES HUMAN PANCREATIC PROGENITOR CELL COMPETENCE

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Here we describe evidence for a novel role for Neurogenin3 in establishing the competence of human pancreatic progenitor cells. Pancreatic progenitor cells are multipotent precursors to all cell types of the pancreas, and NGN3 expression in mouse pancreatic progenitor cells directs specification into the endocrine lineage. We have identified a patient with a NEUROGENIN3 (NGN3) loss-of-function mutation and generated induced patient-specific pluripotent stem cells

(PS-SCs) to investigate the functional requirement of NGN3 in the differentiation of human pancreatic cell types. Using established differentiation protocols, we found that PS-SCs were unable to differentiate into insulin-producing beta-like cells. Interestingly, the PS-SCs displayed significantly lower pancreatic progenitor cell differentiation with only 9.62% of the double positive PDX1+/NKX6.1+ cell population present, whereas the control hESCs produced 30.34% double positive cells. This decrease in pancreatic progenitor cells was observed upstream of canonical NGN3 transcriptional activity. Correcting the patient's NGN3 mutation using CRISPR cas9 restored the PDX1+/NKX6.1+ cell population at the multipotent pancreatic progenitor stage (25.3%) and enabled the cells to differentiate into insulin-producing beta-like cells. The decrease in PDX1+/NKX6.1+ multipotent progenitor population led us to investigate the capacity of PS-SCs to differentiate into mature exocrine cell types. When subjected to an exocrine differentiation protocol, PS-SC organoids were smaller in diameter and had decreased expression in exocrine markers. These in vitro conclusions are supported by a previously undiagnosed clinical finding of pancreatic insufficiency in the patient. Taken together, our data presents novel evidence for an undescribed role for NGN3 in establishing the competence of human pancreatic progenitor cells and their capacity to differentiate into both endocrine and exocrine lineages.

T-2071

IMMORTALIZED HEPATOCYTE-LIKE CELLS (IMHCS) DERIVED FROM HUMAN MESENCHYMAL STEM CELL SUPPORT IN VITRO LIVER STAGE DEVELOPMENT OF THE HUMAN MALARIAL PARASITE

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Plasmodium vivax represents as a great challenge to the global of malaria eradication because the parasites maintained their viability in dormant liver-stage form, named as hypnozoite. In this dormant stage, hypnozoite causes disease relapse in patients. The hepatocyte culture which supports hypnozoite formation has been hindered by the lack of robust and reliable model for in vitro culture of liver stage parasites. To overcome this limitation, immortalized hepatocyte-like cell (imHC) derived from hMSC was utilized as Plasmodium sp. infection model. The sporozoites of Plasmodium vivax and Plasmodium falciparum were collected prior to infect imHC. The imHCs maintained major hepatic functions and expressed essential host factors such as CD81, SR-BI and EpHA2, requiring for parasite entry and development. The imHCs could maintain in monolayer for long term without overgrowth, represents as a robust model for supporting growth of P. vivax liver-stages, including hypnozoites. Moreover, imHCs also host and support development of P. falciparum in liver-stages. The imHCs expressed major CYP450s activity which converted primaquine to active metabolite for treated liver-stage parasites. This observation paves the way to apply imHCs model for antimalarial drug screening. The imHCs, which maintained mature hepatocyte phenotypes and CYP450s expression, established an alternative host for in vitro Plasmodium liver-stage studies, essentially those focusing on the biology of P. vivax hypnozoite. This potential offers the possibility to achieve complete eradicate P. vivax hypnozoite from the patients.

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T-2073

CELL CYCLE ARREST PROMOTES THE DIFFERENTIATION OF BETA CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Several studies have shown that adult human beta cells proliferate with extremely low rate, and that the expression of anti-proliferative genes in beta cells improves function. We hypothesize that the exit from the cell cycle can promote differentiation and maturation of beta cells and reduce teratoma formation. We performed a targeted screen to induced cell cycle arrest at selected points of stem cell differentiation, using compounds interfering with different components of the cell cycle machinery. Remarkably, interference with DNA polymerase alpha resulted in a significant increase in the population of insulin expressing beta-like cells. These C-peptide positive cells expressed insulin and markers of beta cell identity at levels higher than untreated controls. In contrast to cycling stem cell derived beta cells that lost insulin expression and beta cell identity over time, cell cycle arrested beta cells were stable for at least one month of in vitro culture. Upon transplantation, serum human C-peptide was detected within a week and was secreted in response to physiological changes and glucose stimulation. G1 arrested cells could protect mice from diabetes without teratoma formation after long-term engraftment of over one year. Therefore, cell cycle progression is antagonistic to

terminal differentiation and adoption of a stable beta cell identity. The induction of G1 arrest is an efficient way to produce stem cell derived beta cells with stable beta cell identity and stable graft size after transplantation.

T-2075

ORGANOIDS DERIVED FROM HUMAN HEPATOCYTES FOR MODELING LIVER DISEASES

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Liver tissue, containing diverse cells, both vasculature and biliary system, function as the important synthesis and metabolism organ. Defects of liver cells would induce liver diseases, including liver cancers. Liver diseases are often diagnosed at late stages with poor prognosis. Preventive therapies at early stages would increase the survival, and the development of which requires improved understanding of liver diseases. However, proper models for studying human liver diseases are largely missing. Here, using human hepatocytes (hiHeps), we established organoids possessing liver architectures and functions, and genetically engineered them to model initial alterations in human liver cancers. By introducing the HCC oncogene c-MYC, hiHep organoids developed into bona fide HCCs in vitro and in vivo. Furthermore, through screening a panel of human ICC-enriched mutations, we demonstrated that hepatocytes could function as the cell of origin for human ICCs. Besides modeling liver cancer, we are currently trying to use human hepatocytes to construct a complex liver organoids with typical structures of the liver lobule. These liver tissues might be used for mimicking hepatobiliary diseases and liver regeneration. Together, human liver organoids represent a genetically manipulable and tractable system for modelling structural and molecular changes during liver diseases and identifying potential preventive therapies.

T-2077

DEVELOPMENT OF HUMAN IPSC-DERIVED PANCREATIC ACINAR CELLS FOR DISEASE MODELING

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The pancreas is mainly composed of two compartments: endocrine and exocrine. The endocrine islets secrete hormones such as insulin and glucagon to regulate blood glucose levels, while the exocrine produces mainly digestive enzymes. Although some groups have reported methods for the differentiation of human pluripotent stem cells (hPSCs) into pancreatic endocrine cells, there is a distinct lack of methods to turn human induced PSCs (hiPSCs) into exocrine cells. Consequently, in vitro models of exocrine diseases such as pancreatitis and pancreatic cancer are lacking. Pancreatitis affects more than 200,000 people in the U.S. every year and is the result of an early activation of the digestive enzymes within the acinar cells, which causes inflammation and auto digestion of the pancreas, while pancreatic cancer is a rare type of cancer that can sometimes arise as result from chronic pancreatitis. Limited treatments exist for this cancer and only 15-20% of patients are eligible for surgery. Thus, the development of in vitro patient-specific disease models will be beneficial to study these diseases. While there were some early attempts to generate acinar cells from hiPSCs, the efficiency was low, and assessment of functionality was not described. Here, we describe a method for the robust differentiation of blood-derived hiPSCs into pancreatic acinar cells for modeling pancreatic diseases by comparing them to isolated human primary acini. Our protocol robustly generated PDX1/SOX9 co-positive multipotent pancreatic progenitors (PP). We then determined the timing necessary for further acinar differentiation from PPs through analysis of gene expression levels of acinar specific genes such as PTF1A, MIST1, RBPJ, and GATA4. Our results suggest that the temporal monitoring and determining highest expression levels of these genes are critical prior to inducing late acinar differentiation. Here, we show

the successful differentiation of acinar cells from hiPSCs. Further improvements are still necessary to increase the efficiency of the protocol to generate a pure population of patient-specific pancreatic acinar cells. Our next step is to differentiate hiPSCs from patients with pancreatitis, such as those with the PRSS1 mutation to model pathogenesis.

EPITHELIAL TISSUES

T-2081

A NOVEL PEPTIDE WKYMVM HAS IMMUNOMODULATORY EFFECT VIA IRFS/STAT1 PATHWAY AGAINST ENDOTOXIN-INDUCED ACUTE LUNG INJURY

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There is no obvious treatment for acute respiratory distress syndrome (ARDS), although ARDS is a severe, acute inflammatory response within the lung. Recently, many studies have suggested therapeutic strategy through maintenance of microenvironments by modulating immune response in acute lung injury (ALI). Among them, WKYMVm hexapeptide (Trp-Lys-Met-Val-D-Met) as a strong FPR2 agonist has been thought to be responsible for wound healing process, re-endothelialization and promoting homing of stem cells. However, it is clearly unknown the therapeutic effect of WKYMVm in ALI. In this study, we investigate whether the WKYMVm has an immunomodulatory properties by regulating homeostasis of neutrophils functions. The peptide was intraperitoneally injected daily for 4 days, followed by injected with lipopolysaccharides (LPS) from E.coli O26:B6. After 24 hr, infiltrated inflammatory cells in bronchoalveolar fluid (BALF) was validated using Wright-Giemsa and H&E staining. The expression of pro-inflammatory cytokines, myeloperoxidase (MPO) activity expressed by neutrophil and nitric oxide (NO) level were measured with ELISA, MPO assay and Griess assay, respectively in BALF or serum. The protein levels of IRFs/STAT were determined by Western blot analysis in mouse lung tissues. Results showed that the inflamed lung with LPS instillation was mildly blocked by WKYMVm treatment in mice. Moreover, WKYMVm inhibited release of pro-inflammatory cytokine and encouraged antimicrobial function such as MPO activity and NO release in BALF of LPS-induced mice and in differentiated HL-60 toward neutrophil-like cells through FPR2-dependent IRFs/STAT1 pathway. In alveolar progenitor cell surviving parenchyma and neutrophils, the effect of WKYMVm was mediated IRF7/STAT1 at Tyr701- and IRF1/STAT1 at Ser727 residue respectively in cell-specific manner. Taken together, we have concluded that WKYMVm peptide has immune-stimulating effects with antimicrobial activity of neutrophils and anti-inflammatory effect of alveolar progenitors via IRFs/STAT1 axis in ALI, and it is able to a critical signaling for endotoxin-induced ARDS mice.

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T-2083

CMIT/MIT INDUCE APOPTOSIS AND CELL CYCLE ARREST THROUGH ERK/JNK/P38 AXIS IN ALVEOLAR TYPE II PROGENITOR CELLS

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The 5-chloro-2-methyl-2h-isothiazolin-3-one and 2-methyl-2h-isothiazol-3-one (CMIT/MIT) is a chemical mixture that commonly found in many water-soluble consumer products including dentifrice, germicide and shampoo etc. Although it is emerging a certain risk factor threatening public health, it is unknown to be associated with pathological cellular- and molecular-mechanisms. Alveolar epithelial cells include type I and type II cells. When type I cells is damaged, type II progenitor cells differentiate into type I cell for repair. Therefore, in this study, we investigated the pathophysiological role of CMIT/MIT using mouse epithelial type II progenitor cell, MLE-12. The cells were treated with CMIT/MIT (0-50 μM) for 24 hours. In MTT assay, cellular proliferation was significantly decreased in response to CMIT/MIT treatment. In western blot analysis, protein levels of BAX/Bcl-2 and cleaved caspase-3 were significantly increased. Moreover, p21 and p53, cell cycle arrest markers, expression were also increased. In ELISA, CMIT/MIT increased the release of pro-inflammatory cytokine, TNF-α and IL-1β. Furthermore, CMIT/MIT increased the phosphorylated- ERK1/2, -p38, and -JNK1/2 protein levels in MLE-12 cells. Therefore, these findings suggest that CMIT/MIT exposure interrupt alveolar progenitor cells via apoptosis/cell cycle arrest following inflammation and activation of MAPK pathway.

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T-2085

WNT/BETA-CATENIN SIGNALING IS REQUIRED FOR MAINTENANCE OF ADULT MERKEL CELLS

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Merkel cells are involved in gentle touch responses and localize to epithelial touch dome structures. Merkel cells display similarities to Merkel cell carcinoma, an aggressive cancer that predominantly occurs in elderly and sun-exposed skin, and Merkel cell progenitors are a possible cell of origin for this devastating disease. In adult mouse skin, a tractable model for studying Merkel cell maintenance, Merkel cell numbers fluctuate during the hair follicle growth cycle. Merkel cells are thought to be repopulated from KRT17+ touch dome cells; however, the mechanisms controlling this process are unclear. We found nuclear beta-catenin and Wnt reporter expression localize to KRT17+ touch dome cells and a subset of Merkel cells in adult mouse skin. To determine whether Wnt/beta-catenin signaling marks self-renewing stem/progenitor cells in the touch dome, lineage tracing analysis was performed with mice carrying Axin2-CreERT2 knockin allele and fluorescent Cre reporters that permit lineage tracing of Wnt responsive cells. These experiments identified self-renewing Wnt-active touch dome progenitors that were maintained over natural regeneration and gave rise to Merkel cells. To determine whether Wnt/beta-catenin signaling is required for Merkel cell renewal, we induced expression of the Wnt/beta-catenin inhibitor Dkk1 or deletion of epithelial beta-catenin in adult mouse skin epithelia. Either of these manipulations caused gradual depletion of Merkel cells without affecting touch dome maintenance. We further identified Wnt10a as the key Wnt ligand for maintenance of Merkel cells in adult mouse skin. Merkel cells and touch dome cells were present in the newborn Wnt10a null pups, however, with age, Merkel cells but not touch dome cells, were gradually lost from Wnt10a mutant skin. To determine whether Wnt/beta-catenin signaling is specifically required within Merkel cells for their repopulation between hair growth cycles, we used Sox2-CreERT2 or Atoh1-CreERT2 to induce deletion of epithelial beta-catenin in adult Merkel cells. These manipulations resulted in a dramatic reduction in Merkel cell numbers. Taken together, our data identify Wnt-active self-renewing stem cells in touch domes and demonstrate that WNT/beta-catenin signaling is required in Merkel cells for their renewal in adult life.

T-2087

LINEAGE CHOICES OF RESIDENT STEM CELLS DURING DEVELOPMENT, HOMEOSTASIS AND REGENERATION OF MOUSE SALIVARY GLAND

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The epithelial tissue in salivary glands (SG) is organized into three main compartments, including terminal secretory units or acini, a ductal network for transporting and modifying saliva, and a contractile myoepithelial tissue to aid in expulsion of saliva from acini through ducts. Salivary dysfunction, which severely affects oral and overall health, is mainly due to the loss of acinar cells. Previous studies in the adult mouse submandibular gland have shown that despite the existence of a ductal stem cell population in proximity of acini, acinar cell renewal during homeostasis or following mild injuries is maintained by self-duplication of acinar cells. However, it is not clear how acini are regenerated after severe injuries when almost all acinar cells are lost and the self-duplication of acinar cells is not an option. Here, by combining genetic lineage tracing of several distinct epithelial and mesenchymal cell populations with a model of severe but reversible glandular injury, we find that regeneration of acini involves transdifferentiation of multiple epithelial cell populations. During homeostasis, ductal stem cells marked by cytokeratin K14 are the only cell population displaying robust proliferative and differentiation capacity in organoid cultures. However, in response to the wound environment, not only K14+ ductal stem cells, but also c-Kit+ ductal cells and myoepithelial cells acquire remarkable lineage plasticity and contribute to regeneration of acini. Our data indicate that both ductal and myoepithelial cells have the potential to replenish acinar cells, providing new insights into the mechanism of salivary gland repair and regeneration.

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T-2089

SIRT1 SUPPRESSES MIR-1185, A NOVEL TUMOR-SUPPRESSOR MICRO-RNA, TO INCREASE HUMAN COLON CANCER STEM CELL PROPERTIES

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The cancer stem cell (CSC) properties such as self-renewal, drug resistance, and metastasis have been indicated to be responsible for poor prognosis of patients with cancers. Based on previous studies, epigenetic regulator SIRT1 as a regulatory hub positively influences cancer-related pathways such as proliferation and stress-resistance. It mediates deacetylation of histone H3 lysine 9 (H3K9), which results in repressive chromatin structure and low transcriptional activity. However, the precise mechanisms between CSC properties and abnormal histone

modification by SIRT1 are still unknown. Here, we found that SIRT1 signaling pathway was highly associated with colon CSC properties through the suppression of the novel miRNA miR-1185, a tumor-suppressor miRNA, via histone deacetylation. The miR-1185-1 suppressed CD24, a colon CSC marker gene, through targeting the 3â€™UTR of CD24 mRNA. Besides, inhibition of SIRT1 by RNA interference leading to elevated H3K9 acetylation in the promoter region of miR-1185 and increased the expression of miR-1185 expression, which further represses CD24 translation and colon cancer stem cell properties. In the xenograft model, the tumor size of miR-1185-1 overexpressed tumor cells was smaller. Overall, these findings suggest SIRT1-miR1185-CD24 axis plays an important role in colon cancer stem cell properties and tumorigenesis.

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EYE AND RETINA

T-2091

GENERATION OF RETINAL NEURONS FROM HUMAN PLURIPOTENT STEM CELLS USING A SCALABLE 3D SPHERE CULTURE SYSTEM

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An essential requirement for the development of cell-based therapies is the establishment of robust manufacture process that allow the derivation of large quantities of highly pure transplantable cells from renewable sources, which recapitulate the characteristics of the endogenous cell types intended to replace. Retinal neurons including photoreceptors precursor cells (PRPCs) generated in vitro from human pluripotent stem cells (hPSCs) are potential cell source for regenerative therapies, drug discovery and disease modeling. However, numerous approaches to differentiate hPSCs into retinal neurons and PRPCs for the purpose of cell replacement therapy produced undesirable results in terms of efficiency, purity, homogeneity and scalability. This study describes a robust, defined and scalable 3-dimension (3D) sphere culture system for the generation of highly enriched retinal neurons at different developmental stages from hPSCs, including early and late committed retinal neuron progenitors (CRNP), PRPCs as well as photoreceptor-like cells by synchronizing the differentiation process, which can be easily adapted to current general manufacture practice (cGMP) protocol. This novel protocol starts with hPSCs as 3D spheres, which are directly induced to differentiate into early CRNPs, late CRNPs, PRPCs and photoreceptor-like cells by a combination of small molecules with continuous sphere dissociation/reaggregation and sphere reformation approach in bioreactors under matrix-free conditions. This well controlled 3D sphere system overcomes numerous limitations, especially the scalability, facing conventional adherent 2D culture and traditional embryoid body as well as organoid systems. Our novel approach routinely generates 3-4.5 x 10^8 PRPCs starting with 3 x 10^6 hiPSCs with a purity of approximately 95%. Multiple levels of analyses, including immunofluorescence staining, flow cytometry, and quantitative gene expression by RT-qPCR confirmed the identities of early and late CRNPs, PRPCs and photoreceptor-like cells. This novel 3D sphere platform is amenable to the development of an in vitro GMP-compliant retinal cell manufacturing protocol from multiple renewable hPSC sources for future preclinical studies and human cell replacement therapies.

T-2093

REGENERATION OF RETINAL GANGLION CELLS BY REPROGRAMMING FULLY DIFFERENTIATED INTERNEURONS IN VIVO

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Glaucoma is a leading cause of blindness worldwide. It is characterized with a progressive loss of retinal ganglion cells, the only output neurons of the retina that convey visual information to the brain. Current therapies for glaucoma are aimed at preserving ganglion cells by lowering intraocular pressure, therefore can only slow the progression of the disease; they do not improve or restore vision that already has been lost. Regeneration of retinal ganglion cells has been viewed as an ideal therapeutic strategy for vision restoration in glaucoma patients. Here we explore the feasibility of regenerating retinal ganglion cells by activating endogenous regenerative sources in animal models. We demonstrate that fully differentiated retinal interneurons still possess regenerative potential in adult mice. By extopic expression of transcription factors essential for ganglion cell fate determination, retinal amacrine interneurons can be efficiently converted into ganglion cells. New generated ganglion cells extend axonal projections into the brain and form synaptic connections with other neurons of the visual circuitry. The regeneration of ganglion cells in adult mammals points to a new therapeutic strategy for vision restoration in glaucoma patients.

T-2095

METFORMIN AMELIORATES CTRP5 MUTATION-ASSOCIATED ALTERATIONS IN AMPK ACTIVITY UNDERLYING LATE-ONSET RETINAL DEGENERATION AND IS ASSOCIATED WITH DELAYED ONSET OF AMD PATHOGENESIS

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Dysregulation of lipid metabolism is thought to play a pivotal role in the etiology of age-related macular degeneration (AMD). We generated induced pluripotent stem cell derived retinal pigment epithelium (RPE) from patients with late-onset retinal degeneration - a rare mendelian disorder with AMD-like clinical manifestations that stem from a dominant missense mutation in gene CTRP5. CTRP5 was preferentially secreted across the apical membrane of RPE cells. Mutant CTRP5 showed reduced affinity for adiponectin receptor R1 (ADIPOR1) resulting in constitutively elevated pAMPK levels that were insensitive to the cellular energy status. Uncoupling of intracellular energy sensory pathway led to susceptibility to epithelial-mesenchymal transition and diminished lipid metabolism. Metformin, an anti-diabetic drug, re-sensitized AMPK, alleviated lipid deposition, and counteracted EMT phenotype in patient-RPE. Consistently, our retrospective clinical study revealed metformin significantly delayed the onset of AMD signifying it as an effective therapy for AMD.

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T-2097

ROLE OF MICRORNA IN GENE REGULATION DURING HUMAN RETINAL FORMATION

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The use of human pluripotent stem cells is an invaluable tool used to study human diseases in vitro. This, in combination with innovative 3D retinal organoid technology, gives us the ability to explore exciting new therapeutic interventions. However, this system is still evolving and there is much to learn about the molecular mechanisms that are involved during the transition from undifferentiated stem cells to fully developed retinal organoid. A deeper understanding of these mechanisms will enable more reliable disease model systems and offer new insights into human-specific retinogenesis. With the goal of understanding the deeper processes that evolve within our cells, transcriptomic analysis was conducted in order to identify changes in microRNA expression levels during various time points of differentiation in 3D retinal organoids, specifically as uncommitted stem cells progress to retinal progenitor cells. MicroRNAs (miRNAs) are noncoding RNA molecules which function as regulators that silence mRNA post transcriptionally in order to regulate overall gene expression. MiRNAs play an important role in the biological function of our cell systems and in the regulation of the majority of our genes. To pinpoint their specific role in retinal development, SIX6-GFP and VSX2-tdTomato stem cell lines were created using CRISPR-Cas9 technology. SIX6 was chosen as a marker for early eye field development while VSX2 is distinguished more specifically in retinal progenitors. Stem cell lines were then differentiated and cells were collected at specific time-points based on their expression of GFP and tdTomato, and thus SIX6 and VSX2, respectively in order to conduct FACS enrichment. Bioinformatic analysis identified specific miRNAs targeting SIX6, VSX2, and other retinal genes, suggesting miRNA involvement in retinal development. More interestingly, different miRNAs were identified during the earlier eye field versus more retina-defined time points. Through the knockout of these specific individual miRNA targets using the CRISPR-Cas9 system, stem cell lines were created in order to observe their effect of different stages of retina formation in vitro. Since miRNA are involved in many cellular processes, the further analyses of their roles may be the key to a deeper understanding of human-specific retinogenesis.

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T-2099

FUNCTIONAL INVESTIGATION OF DANONS DISEASED RETINAL PIGMENTED EPITHELIUM

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Danons disease (DD) is a severe, genetic, x-linked disorder that is caused by mutations in the lysosome associated membrane 2 (LAMP2) gene leading to near complete loss of LAMP2 and buildup of autophagic vacuoles. The disease is currently diagnosed by a myriad of cardio-skeletal myopathies, phenotypically experienced through severe or mild symptoms, the former almost exclusively associated with males. Current treatment plans are limited to complete heart transplants. Recent evidence has shown that DD patients also suffer from ocular issues including: blurred vision, impaired visual acuity and loss of retinal pigmented epithelium (RPE) pigmentation. RPE cells are responsible for engulfing millions of shed photoreceptor outer segments (POS) daily. Monitoring RPE phagocytosis in situ has uncovered some of the key mechanisms and their respective molecular counterparts. Several studies have identified the important players responsible for binding and engulfing POS, including: RPE integrin αVβ5/MFG-E8 and RPE MerTK/Gas6, however; much of the digestive mechanism is poorly characterized during POS breakdown. Hence the molecular basis of LAMP2 mediated retinal impairment in DD patients is currently unknown and may provide insight to the pathology. In order to elucidate the molecular mechanism of this retinal pathology, we investigated RPE cells derived from patient-derived induced pluripotent stem cells (iPSC). DD-iPSC were differentiated into RPE and DD-RPE identity was characterized using: qRTPCR, flow cytometry, western blot, ELISA and ICC. Mature DD-RPE expressed key markers including: RPE65, PMEL and BEST1, and PEDF was secreted apically and VEGF basally. Furthermore, DD-RPE lack LAMP2 mRNA/proteins, consistent with clinical diagnosis. To assess phagocytosis, mature RPE monolayers were incubated with fluorescein isothiocyanate (FITC) conjugated Bovine POS and relative FITC signal was collected using a microplate reader. Degradation of FITC-POS was assessed over a 24hr chase period. We found that DD-RPE were defective in the degradation of POS compared to wildtype ($p<0.0001$). These data suggest that lack of LAMP2 results in improper degradation of autophagosomes. Future work will use this in vitro functional platform for dissection of molecular pathways and high-throughput drug screening.

T-2101

GENERATION OF FUNCTIONAL CORNEAL ENDOTHELIAL-LIKE CELLS FROM PLURIPOTENT STEM CELLS FOR REGENERATIVE THERAPY OF THE HUMAN CORNEA

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The cornea is the clear window that lets light into the eye. The inner surface of the cornea is lined by corneal endothelial cells (CECs) that function as an active metabolic pump to maintain the transparency of this avascular tissue. Corneal disease is one of the leading causes of blindness and visual disability, affecting over 15 million people worldwide. In developed countries, corneal endothelial disease is responsible for most corneal opacities. State of the art therapy involves selective replacement of the corneal endothelium with that of a donor. However, only one donor cornea is available for every seventy patients in need. Stem cell differentiation into corneal endothelial cells could open the possibility to obtain large amounts of cornea endothelium in a rapid and reproducible way to use in personalized regenerative medicine. To date, scientists have succeeded in differentiating pluripotent stem cells into a corneal endothelial phenotype; nevertheless, protocols lack a functional test of the differentiated cells and do not demonstrate the active transport of the corneal endothelium. We set out to design an integrated device to culture and test the functionality of the stem cell-derived endothelial cells. Both human embryonic (hESC) and induced pluripotent stem cells (iPSC) were successfully differentiated into corneal endothelial-like cells according to previously published protocols. Then the differentiated cells were cultured on a collagen membrane in the device for 5 days until cell confluency was confirmed by microscopic observation. At this point, the functionality of the differentiated cells was assessed measuring the K+/Na+ and L-lactate transport across the membrane containing the cells. With this, we are able to validate that the stem cell-derived endothelial cells show similar functionality to native corneal endothelial cells.

T-2103

SINGLE CELL RNA SEQUENCING OF HESC-DERIVED 3D RETINAL ORGANOIDS REVEALS NOVEL GENES REGULATING PROGENITOR COMMITMENT IN EARLY HUMAN RETINOGENESIS

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The development of the mammalian retina, aka retinogenesis, is a complicated process involving generating distinct types of neurons from retinal progenitor cells (RPCs) in a spatiotemporal-specific manner. The progression of RPCs during retinogenesis includes RPC proliferation, commitment, and neurogenesis. In this study, by performing single-cell RNA-sequencing (scRNA-seq) on cells isolated from neuroretina of human embryonic stem cell (hESC)-derived 3D retinal organoids, we successfully deconstructed the RPC progression during early human retinogenesis. We identified two distinctive subtypes of RPCs with unique molecular profiles, namely multipotent RPCs and neurogenic RPCs. We found genes related to the Notch and Wnt signaling pathway, as well as chromatin remodeling were dynamically regulated during RPC commitment. Interestingly, our analysis identified CCND1, a G1-phase cell cycle regulator, was co-expressed with ASCL1 in a cell-cycle independent manner. Temporally-controlled overexpression of CCND1 in retinal organoid suggested a role for CCND1 in promoting early retinal neurogenesis. Together, our results revealed critical pathways and novel genes in early retinogenesis of humans.

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STEM CELL NICHES

T-2105

HUMAN ADULT MYOCARDIUM-RESIDENT MESENCHYMAL STEM-LIKE CELLS WITH A PERICYTE PHENOTYPE EXHIBIT COLONY FORMING ABILITY AND POTENCY INTO MULTIPLE CARDIOVASCULAR LINEAGE CELLS

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Accumulating evidence demonstrates the various types of endogenous cardiac cells with stem cell properties, both self-renewal capacity and multipotency into cardiovascular lineage cells, in the adult mammalian hearts. It is critical to identify the type and origin of cells capable of regenerating the human myocardium for developing regenerative strategies for intractable heart disease. Here, we identified a unique myocardium-resident stem cell population isolated by a niche-preserving organ culture method. The engineered provisional matrix-supported organ culture of the human adult myocardium enabled the cell renewal of myocardial pericytes and outgrowth of in vitro renewed cells into matrix. Most in vitro renewed cells were found the perivascular spaces of myocardial Interstitium and expressed pericyte-related markers. The outgrown cells uniformly expressed both mesenchymal stem cell- and pericyte-specific genes and markers. Intriguingly, these cells expressed cardiomyocyte-specific transcription factors, such as Nkx-2.5 and GATA-4. The matrix-specific proteolysis allowed efficient recovery of outgrown cells from matrix which retained a robust clonogenic and growth potential and multipotency into cardiovascular and mesodermal lineage cells in vitro. Transplanted cells showed the protective and regenerative effects on the infarcted myocardium. Taken together, these human adult myocardial-resident stem cells with a pericyte origin might be tissue-specific cells participating myocardial regeneration.

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T-2107

SAFETY EVALUATION OF THE INTRA-ARTICULAR INJECTION OF MESENCHYMAL STEM CELLS FOR OSTEOARTHRITIS TREATMENT

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Osteoarthritis (OA) is a major degenerative joint disease without effective therapy. Recently, intra-articular injection of mesenchymal stem cells (MSCs) has been shown to be a promising tool to treat OA. Here we examined the biodistribution of MSCs to evaluate the safety of intra-articular injection of MSCs for OA treatment. MSCs were labeled with the far-red fluorescent membrane dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide (DiR) and 3 x 106 labeled MSCs were intra-articular injected into the right knees of 10 SD rats. In vivo fluorescence images were acquired using an IVS spectrum CT (PerkinElmer, Waltham, MA) 1, 3, 8 and 21 days after cell transplantation, respectively. Immunohistochemistry and qPCR were conducted to assess the tissue distribution of human MSCs. All rats behaved normally after injection. In vivo fluorescence signal was only observed in the right knees where the labeled MSCs were injected while no fluorescent signal was detected in the other parts of the body. The fluorescent signal lasted 21 days after cell transplantation. Ex vivo revealed that there was no detectable fluorescence signal in major other organs. We then conducted immunohistochemistry staining to assess the distribution of transplanted human MSCs in the major organs with a specific anti-human mitochondria antibody. As expected, no positive staining cells was found in all the major organ tissue sections. To further determine whether the MSCs migrated out of the joints, a qPCR method specifically detecting human DNA was adopted. The qPCR results showed human genomic DNAs were only able to be amplified in the rat genomic DNA samples from the right knees of rats. There was no amplify signal be detected in rat genomic DNA samples from other major organ tissues. The qPCR results were in consistence with the immunohistochemistry results. Labeling of MSCs with DiR is an efficient tracking method for analysis MSC biodistribution. Intra-articular administration of a single MSC injection was well tolerated and the biodistribution of MSC was restricted in the injected knee. Our data support the idea that clinical application of human MSC for osteoarthritis cell therapy is safe.

T-2109

EFFECTS OF LONG-TERM NEUROTROPHIN SIGNALING ON HUMAN EMBRYONIC STEM CELL-DERIVED SPIRAL GANGLION-LIKE NEURONS

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The neuroprotective effects of neurotrophic factors (i.e. brain-derived neurotrophic factor) on spiral ganglion neurons (SGNs) has been widely studied in the setting of sensorineural deafness. Many have shown that SGNS continue to survive post-neurotrophic factor treatment cessation. However, the verdict is unclear as others have established that the eventual depletion of neurotrophic factors (NTFs) leads to an accelerated decline in neuronal survival. Additionally, these studies do not assess the response of stem-cell derived SGNs to neurotrophic factor treatment. In this study, we set out to investigate the effects of various sustainable BDNF sources on stem-cell derived SGNs. The hESC-derived SGN-like cells were derived from human embryonic stem cells (hESCs) (H1, H7, and H9) through a protocol developed by our laboratory. The sustainable BDNF sources studied were PODS (Cell Guidance Systems, UK), BDNF-mimetic amphiphile gels, and, lentivirus-infected Schwann cells. PODS (POlyhedrin Delivery System) consist of the Bombyx mori polyhedrin microcrystal structure co-expressed with BDNF. The PODS degrade via protease activity and release the growth factor at a sustained rate. Three-dimensional diffusion was modeled graphically prior to experimentation to optimize initial PODS concentrations. Immunocytochemistry was used to stain and visualize the SGN-like cells after experimentation. Preliminary result demonstrated that neurites were growing towards the BDNF PODs crystals. It appeared that the length of neurite was proportional to the number of PODS crystals. We will compare the length of neurites from hESC-derived SGN-like cells across PODS, a BDNF-mimetic amphiphile gel and, primary Schwann cells. Furthermore, long-term three-dimensional BDNF diffusion may help differentiate hESC-derived ONPs into a SGN lineage. The application of this technology needs to be tested in an animal model as a next step.

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T-2111

TRANSPOSONS-ACTIVATED IMMUNE SIGNALING IN THE AGED NICHE DROPS DROSOPHILA GERMLINE STEM CELLS

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Transposons participate in tissue aging, but the impacts on stem cells remain unclear. Here, we report that in the Drosophila ovarian germline stem cell (GSC) niche, aging reduces expression of Piwi (a transposon silencer) to derepress retrotransposons. This derepression activates Glycogen synthase kinase 3 (GSK3) to impair beta-catenin-E-cadherin-mediated GSC anchorage, leading to GSC loss. Supplementation of Piwi in the aged niche delays age-dependent GSC loss, while elimination of Piwi in the young niche accelerates this loss. In the piwi-knockdown niche, suppressing GSK3-dependent beta-catenin degradation or inhibiting retrotransposon duplication restores GSC anchorage. We also report that the gypsy retrotransposon generates endogenous virus to activate GSK3 via Toll-mediated immune signaling. Suppression of virus generated by retrotransposons or disruption of Toll signaling in the piwi-knockdown niche decreases GSK3 activity and prevents GSC loss. Our results document that during aging, retrotransposon-mediated GSK3 activation impairs stem cell maintenance, a finding that may have relevance to aging-related processes in many tissues.

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T-2113

A METHOD TO ISOLATE AND TRANSPLANT MOUSE HEMATOPOIETIC STEM CELLS ALONG WITH THEIR NICHE ASSOCIATED WITH IMPROVED FUNCTIONAL HEMATOPIETIC ENGRAFTMENT WITHOUT MYELOABLATION

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Hematopoietic stem cell (HSC) transplants provide the best chance for cure for a range of malignant and non-malignant diseases, and for facilitating immune tolerance in organ and facial transplantation. Methods for increasing the efficiency of HSC homing and engraftment are important due to the rarity of HSCs in donor tissue and the toxicity of myeloablative regiments necessary to enable HSC engraftment. Attempts at expanding HSCs ex vivo prior to clinical transplantation have generated mixed results and culturing may not maintain the functional hematopoietic repopulating cells. We investigated the survival and engraftment potential of HSC from Luciferase-GFP transgenic mice isolated with their normal niche microenvironments as intact units. We devised a new micro-fluidics based method to purify intact niches containing phenotypic CD150+CD48-Sca+cKit+Lin- HSCs surrounded by associated Thy+, 6C3+, VECadherin+ stromal cells, then tested the ability of HSC-Niche units to support HSC survival in vitro and HSC engraftment into non-myeoablated immunodeficient mice. For the in vitro experiments, HSCs were cultured (1 x 105/10cm3 well) for 2 weeks either: a) within their cellular niches, or b) alone. Cultured HSCs were then dispersed from niches or culture by mechanical dissociation and transplanted into myeloablated mice by intravenous injection to assay HSC hematopoietic reconstitution. Resulted showed HSCs-Niche cultured for up to 2 weeks were able to maintain long-term multi-lineage reconstitution after transplant. For the in vivo niche transplantation experiments, HSC-Niches were isolated and transplanted directly into the right femoral cavity of C57BL mice. Two weeks after intra-femoral transplant mice underwent whole-body lethal irradiation, with shielding of the transplanted region with 1cm2 of lead. Re-colonization of irradiated bone marrow was assessed by tail bleeding recipient mice at 2, 4, and 6 days post irradiation, and looking for evidence of engraftment in irradiated regions of the left femur by IVIS in vivo imaging. Results indicated that isolating and transplanting HSC-

Niches facilitates ex vivo survival and non-myeloabaltive HSC engraftment. Our approach also suggests a new paradigm for stem cell therapy by isolating and transplanting stem cells with their niches as intact units.

T-2115

HYDROGEL NANOPOROUS MICROCAPSULES SUPPLEMENTED WITH TRANSITIONAL MATRIX PROVIDE A PORTABLE STEM CELL NICHE FOR TRANSPLANTATION OF STEM AND PROGENITOR CELLS

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Endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) have shown promise for the treatment of cardiac and vascular disease, but therapies are limited by poor cell persistence and lack of engraftment. We hypothesized that microencapsulation of late-outgrowth (L)-EPCs or MSCs will increase transplanted cell survival and retention in the lungs and result in greater engraftment by promoting cell egress and tissue penetration. Rat bone marrow MSCs and L-EPCs were encapsulated in 3.5% agarose microgels supplemented with fibrinogen and fibronectin by vortex-emulsion, producing cell-loaded microgels of 39±12 um in diameter, most containing 1 or 2 cells. Microgel encapsulation enhanced viability of L-EPC cultured in serum free conditions compared to 'naked' cells (71% vs. 27% by WST1 assay). Microgel composition was further optimized by addition of gelatin to enhance degradation and improve cell egress. Gelatin-agarose microgels (1:2% gel/ag) maintained MSC viability at 24h (89% by WST1 assay) however increased cell egress over 48h quantified by time-lapse microscopy compared to 3.5% agarose microgels (47% vs. 27%, respect.). Luciferase-transduced L-EPCs were administered by jugular vein injection in a rat monocrotaline (MCT) model of pulmonary hypertension 3 days post MCT, and L-EPC fate was tracked by bioluminescence imaging (BLI) for up to 3 weeks. Immediately following injection, non-encapsulated and encapsulated L-EPCs were retained in the lungs and had equivalent baseline signal. However, at 4 and 24 hours post-injection only encapsulated L-EPCs could be detected by BLI (28±12% and 12±8% of baseline). Three weeks post cell injection, microencapsulation of L-EPCs led to significant improvements in right ventricular systolic pressure compared to MCT alone (56±24 vs. 80±7 mmHg), whereas no improvement in pulmonary hemodynamics was observed with delivery of non-encapsulated L-EPCs (79±14 mmHg). However, empty 3.5% agarose microgels were seen to persist for up 3 weeks in the lungs, highlighting the importance of fine-tuning microgel composition to promote efficient degradation after cell egress. Therefore, these data suggest that portable stem cell niches can be engineered to maintain cell viability, increase in vivo retention, and enhance therapeutic efficacy of stem cell therapies.

CANCERS

T-2117

BREAST CANCER DERIVED EXOSOMES ENHANCE THE DIFFERENTIATION AND ANTI-INFLAMMATORY EFFECT OF THE HUMAN MESENCHYMAL STEM CELLS

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Exosomes are small extracellular vesicles, enriched for bioactive materials including lipids, proteins, and different forms of RNA and DNA. Exosomes affect cellular functions by means of transfer of these bioactive material via the extracellular fluids. Tumor cells were shown to secrete increased amounts of exosomes compared to normal proliferating cells. Tumor-derived exosomes circulate in the body fluids and recruit the targeted cells, which could contribute to tumor growth. In our study, we tested the hypothesis that tumor derived exosomes promote tumor progress and vasculogenesis via their effect on mesenchymal stromal cells. Exosomes were purified from culture-supernatant of breast cancer cell line MCF7 by differential ultracentrifugation. MCF7 derived exosomes were cultured with human adipose tissue-derived mesenchymal stromal cells (ASCs) at a concentration of 10 µg/ml for 48 hours. Our data showed that breast cancer-derived exosomes cultured with ASCs resulted in downregulation of the pluripotency markers Oct4, Sox2 and Nanog. ASCs showed increased expression of myofibroblast differentiation marker αSMA, and concomitant increase in angiogenesis markers vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF). Treatment of ASCs with breast cancer exosomes enhanced secretion of tumor necrosis factor-inducible gene 6 protein (TSG-6) anti-inflammatory cytokine along with significant decrease in

expression of CD44 marker. The cells also showed decreased expression of mesenchymal markers, N-cadherin and Slug and increased expression of epithelial marker, E-cadherin. Our results show that coculture of breast cancer exosomes with ASCs promoted their differentiation into vascular-like cells, as evidenced by decreased pluripotency markers, increased angiogenic markers and enhanced mesenchymal to epithelial transition. The contribution of cancer exosomes to the inflammatory milieu of the cancer microenvironment requires further investigation.

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T-2119

OVERCOMING WNT-DEPENDENT ANTI-CANCER THERAPY RESISTANCE IN CANCER STEM CELLS

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Cancer therapeutic resistance remains a critical, unsolved problem. Residual leukemia stem cells (LSCs) underlie resistance but targeting them remains elusive. The Wnt/β-catenin and PI3K/Akt pathways cooperatively promote tumorigenesis, stem cell survival and proliferation, and resistance to anti-cancer therapies. Here, we used a mouse model with activation of both pathways to study therapeutic resistance. Unlike bulk leukemic blast cells, LSCs driven by activation of both pathways are not only chemoresistant but expand in response to chemotherapy. Since Akt can activate β-catenin by C-terminal phosphorylation, inhibiting this interaction might target therapy-resistant LSCs. Unexpectedly, high-throughput screening (HTS) identified doxorubicin (DXR) as an inhibitor of Akt:β-catenin interaction at low doses. We repurposed DXR as a targeted inhibitor rather than a traditional, broadly cytotoxic chemotherapy. Targeted use of DXR reduced Akt-activated β-catenin levels in chemoresistant LSCs, prevented LSC expansion in response to chemotherapy, reduced LSC tumorigenic activity, and substantially increased survival. Mechanistically, β-catenin binds multiple immune checkpoint gene loci, and targeted DXR treatment inhibited expression of multiple immune checkpoints on LSCs, including PD-L1, TIM3, and CD24. However, clinical doses induced oncogenic resistance mechanisms, reversing this inhibition of immune checkpoints. Using patient samples, low-dose DXR treatment also inhibits leukemia-initiating activity of samples exhibiting chemoresistant LSCs expressing Akt activated β-catenin, and similar treatment of relapsed or refractory patients reduced LSCs expressing Akt activated β-catenin. Overcoming anti-cancer therapy resistance and immune escape are expected to reduce relapse. Our findings indicate a more efficacious remedy for overcoming cancer therapy resistance and immune escape.

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T-2121

DISSECTING HUMAN GLIOBLASTOMA RESPONSES TO STEM CELL NICHE FACTORS AT SINGLE-CELL RESOLUTION USING MASS CYTOMETRY

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Glioblastoma (GBM) is the most common primary malignant adult brain tumor. Our lab has shown that GBM patient outcome can be stratified based on tumor radiographic contact with the neural stem cell niche of the ventricular-subventricular zone (V-SVZ), with V-SVZ contact predicting worse patient outcomes. Available demographic, genetic, and transcriptomic data have been unable to explain this observation. My hypothesis is that V-SVZ-enriched soluble and membrane-bound factors provide tumor cells with an advantage after therapy by promoting patterns of intracellular signaling associated with survival and growth. Many such factors, particularly growth factors and cytokines, have been shown to enhance stem cell maintenance in the non-neoplastic niche. I have developed a novel approach to quantitatively measure per-cell signaling responses in primary

GBM patient samples exposed to V-SVZ enriched factors via phospho-specific flow cytometry. Using a workflow developed by my laboratory and collaborators, I have collected single-cell data on 28 human GBM samples using mass cytometry to measure cell surface, intracellular, and signaling proteins. Analysis of these data using computational tools including clustering algorithms, marker enrichment modeling, and earth mover's distance calculations indicates that basal signaling and protein expression do not differ between V-SVZ-contacting and non-contacting tumors, suggesting strongly that per-cell response to acute stimulation is likely critical for understanding the differences between these tumor types. I am now analyzing differences in response to stimuli between contacting and non-contacting tumor specimens ex vivo, and connecting these findings to subpopulations of cells associated with patient outcomes. This work will help determine the biological differences between V-SVZ contacting and non-contacting tumors as well as the functional importance of niche-enriched factors on tumor cell outcomes. More broadly, the approach developed here – snapshot proteomic analyses of signaling events in primary human tissue – creates a novel technical resource for the study of normal and neoplastic stem/progenitor cells in the brain, and the identification of post-translational events which may be pharmacologically targeted in these cells.

T-2123

OVERCOMING THE EPIGENETIC RIGIDITY OF THE CANCER DILEMMA IN REPROGRAMMING AND DIFFERENTIATION PROCESSES

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The tumor recurrence and tumor heterogeneity represent an ongoing challenge in the field of cancer therapy. The chemotherapy and radiation treatment can kill some cells in the tumor, but tumor recurrence quickly, it is mentioned there is a population cancer subsets resist chemotherapy and radiation, and have the ability to differentiate into cancer cells and regrow the tumor. Tumor possesses a characteristic of the stem-like state, but not normal functional stemness. The most common IDH1 mutations in glioma result in a single allele of an amino acid substitution at arginine 132 (R132). The mutations do not result in a simple loss of function but a gain-of-function called a neomorphic enzymatic function. The affinity of the mutant enzymes is reduced compared with the wild type enzymes for isocitrate, their affinity for α-ketoglutarate (α-KG) and NADPH is increased, allowing the generating of the new production R(2)-2-hydroxyglutarate (2HG) to affect cellular metabolism. GASC1 (Lysine-specific demethylase 4C) can demethylates H3K9me3 and H3K9me2 in vitro through a hydroxylation reaction requiring iron and α-KG, producing formaldehyde and H3K9me1. The less α-KG production causes GASC1 mediated hypermethylation in IDH1 mutation cells. We construct a wild type IDH1 or IDH1 R132H (arginine 132 is mutated to histidine) neural progenitor cells (NPC) for reprogramming to ipsc by overexpress four transcription factors: Oct4, Sox2, Klf4, and c-Myc (OSKM). And we construct a wild type IDH1 or IDH1 R132H human embryonic stem cell (hESC) differentiation to induced neural cells (iN) by overexpress Ngn2 (a neuronal marker). Compare the change of reprogramming efficiency and differentiation efficiency caused by IDH1 mutation. The one single point mutation of IDH1 in cells causes the difficulty in reprogramming and differentiation. IDH1 mutation cells blocked in the middle of reprogramming and differentiation. We try to modify the epigenetic state by induce the expression of histone demethylase GASC1 to open the chromatin, it maybe activates the anti-cancer genes and repress the oncogenes to drive the mutation cells into a terminal differentiation stage to lose its stemness, then maybe overcome the rigidity of cancer dilemma in reprogramming and differentiation processes.

T-2125

MULTIANALYTE ASSESSMENT OF CAR T-CELLS USING BULK POPULATION AND SINGLE CELL ANALYSIS METHODS

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Cancer is the leading cause of mortality worldwide and is the largest barrier to extending life in developed countries. Traditional cancer therapies are non-specific, have numerous side effects, and cannot eliminate drug resistant tumors. Immunotherapy has emerged as a promising answer to the downfalls of traditional cancer therapy by harnessing the specificity and potency of physiological immune reactions. Cellular immunotherapy involves the isolation, activation, alteration, expansion, and re-infusion of patient derived immune cells. Chimeric antigen receptor (CAR) T-cells have proven an effective cellular immunotherapy and are approved for the treatment of relapse/refractory hematological malignancies. Current methods to determine CAR T product potency rely on a hazardous and laborious cytotoxicity assay (Chromium 51 release) and/or a single analyte (IFN-γ) secretion analysis that is not consistent between lots and does not correlate with patient response. As the field of cellular immunotherapeutics expands, there exists an unmet need to develop simplistic and more predictive potency assays. In this study, we identify optimal methods to characterize immune cell potency by correlating cytotoxic activity to single and multi-analyte secretion on a bulk population and single cell level. T-cells were isolated from different donors and transduced with lentivirus containing an anti-CD19 second generation (CD3ζ and 4-1BB) CAR. The CAR T-cells were expanded in vitro and extensively phenotyped for differentiation and exhaustion markers. The potency of these

CAR T-cells was characterized through correlating cytotoxic activity to the measurement of 32-plex analyte panel comprised of effector, stimulatory, regulator, and inflammatory analytes measured on a bulk population and single cell level. Results from this study facilitates a simplistic and integrated workflow that is relevant for functional characterization of both autologous and allogeneic CAR T products and can guide pre-clinical development of novel immune cellular therapies.

T-2127

THE COMBINED EFFICACY OF OTS964 AND TEMOZOLOMIDE FOR REDUCING THE SIZE OF POWER-LAW CODED HETEROGENEOUS GLIOMA STEM CELL POPULATIONS

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Glioblastoma resists chemotherapy then recurs as a fatal space-occupying lesion. To improve the prognosis, the issues of chemoresistance and tumor size should be addressed. Glioma stem cell (GSC) populations, a heterogeneous power-law coded population in glioblastoma, are believed to be responsible for the recurrence and progressive expansion of tumors. Thus, we propose a therapeutic strategy of reducing the initial size and controlling the regrowth of GSC populations which directly facilitates initial and long-term control of glioblastoma recurrence. In this study, we administered an anti-glioma/GSC drug temozolomide (TMZ) and OTS964, an inhibitor for T-Lak cell originated protein kinase, in combination (T&O), investigating whether together they efficiently and substantially shrink the initial size of power-law coded GSC populations and slow the long-term re-growth of drug-resistant GSC populations. We employed a detailed quantitative approach using clonal glioma sphere (GS) cultures, measuring sphere survivability and changes to growth during the self-renewal. T&O eliminated self-renewing GS clones and suppressed their growth. We also addressed whether T&O reduced the size of self-renewed GS populations. T&O quickly reduced the size of GS populations via efficient elimination of GS clones. The growth of the surviving T&O-resistant GS populations was continuously disturbed, leading to substantial long-term shrinkage of the self-renewed GS populations. Thus, T&O reduced the initial size of GS populations and suppressed their later regrowth. A combination therapy of TMZ and OTS964 would represent a novel therapeutic paradigm with the potential for long-term control of glioblastoma recurrence via immediate and sustained shrinkage of power-law coded heterogeneous GSC populations.

T-2129

DIET-INDUCED METABOLIC LIABILITIES IN MURINE INTESTINAL STEM CELLS AND CANCER INITIATION

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Our understanding of the relationship between diet, stem cells, and cancer is critical to the prevention and treatment of this disease. We previously demonstrated that a pro-obesity high fat diet (HFD) increases intestinal stem cell (ISC) number and niche-independent growth. Furthermore, the HFD promotes stem cell self-renewal and tumorigenesis through the activation a robust peroxisome proliferator-activated receptor delta (PPAR-dd)) signature in intestinal stem and progenitor populations and that pharmacologic activation of PPAR-dd recapitulates the effects that a HFD has on these cells. PPAR-dd is a lipid sensor that transcriptionally activates a metabolic program to utilize fatty acids (FA). An implication of this work is that reliance on a PPAR program generates a dependency on fatty acid oxidation (FAO). We find that in the HFD state, the PPAR lipid sensors instruct an FAO program necessary to increase intestinal stemness whereby loss of mitochondrial FA import through Cpt1a reduces stem cell number and functional clonogenic capacity. We further demonstrate that tumor initiation in HFD ISCs is abrogated in absence of functional Cpt1a suggesting that adaptation to FAO renders stem cells vulnerable to FAO inhibition. These findings highlight that a PPAR/FAO axis may be targeted or exploited for therapeutic benefit.

NEURAL DEVELOPMENT AND REGENERATION

T-3001

IMPROVED DERIVATION AND SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF HUMAN CHOROID PLEXUS EPITHELIAL CELLS DERIVED FROM EMBRYONIC STEM CELLS

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The Choroid Plexus (ChP) is a vital tissue within the central nervous system (CNS), which maintains cerebral homeostasis by producing cerebrospinal fluid (CSF), removing toxins from the CNS, and forming the blood-CSF barrier. The main functional units of the ChP are its epithelial cells (CPECs), which have been implicated in several neurological diseases including Alzheimer's disease (AD). Despite their importance, CPECs are highly understudied, especially human CPECs, for which useful sources have not been available. To address this issue, we previously described a CPEC derivation protocol from mouse and human embryonic stem cells (ESCs), but this protocol was relatively inefficient and cumbersome. Here we describe a simpler and more efficient CPEC derivation protocol using human ESCs, which incorporates several optimizations, and have begun to characterize the human derived CPECs (hdCPECs) using immunocytochemistry, single cell transcriptomics, and new functional assays. Immunocytochemistry assays for CPEC identity, polarization, multiple primary cilia, and barrier function demonstrated the progressive differentiation of hdCPECs and their acquisition of a more three-dimensional morphology reminiscent of the developing ChP in vivo. Single cell RNA sequencing of the hdCPECs revealed high degrees of similarity to published mouse and human bulk sequencing data and preliminary evidence of distinct hdCPEC lineages using a novel algorithm called SoptSC. ELISA and Westerns of hdCPEC conditioned media indicated high and increasing levels of TRANSTHYRETIN (TTR), the most abundant protein in the CPEC secretome. Lastly, based on prior evidence of beta amyloid uptake by CPECs, we show the ability of hdCPECs to rapidly take up beta amyloid 42, the specific peptide associated with Alzheimer's disease. These immunocytochemical, transcriptomic, and functional data begin to validate the hdCPECs derived using our new protocol and to provide insights into an important, yet understudied cell type.

Funding Source: This work is supported by NIH-IMSD GM055246 (HM) and NIH R21 MH109036 (ESM).

T-3003

ELUCIDATING THE ROLE OF DACH1 IN EARLY HUMAN CEREBELLAR CORTICOGENESIS USING HUMAN ESC-DERIVED NEURAL CULTURES AND ORGANOIDS

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With their ability to undergo self-renewal and pluripotent differentiation in vitro and in vivo, human pluripotent stem cells (hPSCs), provide an opportunity to study the mechanisms underlying cellular differentiation during early development. These stem cells are the only human biological sources with which to study early human development in the absence of complex ethical issues. Several laboratories have demonstrated that hPSCs have an intrinsic tendency to form three-dimensional polarized cerebral cortex tissues and cerebral organoids that resemble the embryonic cortex. The ability to grow these organoids offers an excellent opportunity to further investigate the detailed molecular mechanisms of human corticogenesis. In this study, we demonstrated that LHX2, a LIM-HD transcription factor, has a critical role in regulating forebrain neural transcription factors, such as PAX6, and a WNT signaling component, CER1, to modulate the formation of early forebrain lineages. Furthermore, our results indicate that several transcription factors, which are highly expressed in early hESC-derived neural progenitors and co-expressed with LHX2 and/or PAX6, affect early cortical gene expression. Among these transcription factors, DACH1, a nuclear factor that is expressed during mouse neural development, is involved in regulating the patterning of hESC-derived cortical structures. In this context, we propose that the LHX2-PAX6-DACH1 axis and its associated genetic factors, including non-coding RNAs are likely to be important for modulating cortical development in human. Consequently, the interplay between the genetic factors and the machinery controlling the orientation and mode of cell division may also play a role in cortical cell fate determination and cortex formation.

T-3005

INTRACEREBRAL TRANSPLANTATION OF DOPAMINE NEURONS DIFFERENTIATED FROM HUMAN EMBRYONIC STEM CELLS TO PARKINSONIAN NON-HUMAN PRIMATES PROMOTES FUNCTIONAL RECOVERY

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Differentiation of human embryonic stem cells (ESCs), constitutively expressing GFP, to dopamine neurons yields neural progenitors expressing Nestin, Lmx1a and Foxa2, that progress to dopaminergic cells positive to TH and GIRK2. RNA-seq profiles analyzed at pluripotent, neural precursor and differentiated neurons stages, were consistent with appropriate dopamine differentiation. In vitro produced dopamine neurons display electrophysiological maturation over time, and released dopamine in depolarizing conditions. After approval of the Institutional Animal Care and Use Committee, non-human primates (Chlorocebus pygerythrus) were intoxicated with MPTP, which caused them motor alterations in displacement, reaching and food ingestion, produced by the acute degeneration of dopamine neurons. Suspensions of dissociated ESCs-derived neuronal cultures containing 8 million cells were grafted in the putamen, with stereotaxic coordinates, and immunosuppression was administered throughout the protocol. Transplantation was associated to recovery of the motor alterations, assessed in the hallway test, while no spontaneous recovery was found after sham surgery. In agreement, dopamine release in the brain was augmented in grafted animals, when compared to the sham condition. MRI anisotropy analysis revealed changes in neural tracts after transplantation. PET imaging suggests that active dopamine sites are present in the grafted animals. Post-mortem histology after 11 months identified over 500 000 GFP-positive dopamine neurons in the putamen of grafted animals. These results show that grafting of dopamine neurons provide functional and biochemical improvement with surviving dopamine cells in this pre-clinical model of Parkinson disease.

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T-3007

A PIGLET MODEL OF CORTICAL DEVELOPMENT SHOWS POSTNATAL MIGRATION INTO THE FRONTAL AND TEMPORAL LOBES

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Recent studies have shown ongoing inhibitory neuron (interneuron) migration into the frontal lobe of the human infant brain, supportive of a protracted and region-specific developmental timeline for cortical development. We use a gyrencephalic model, the domestic pig (Sus scrofa domesticus), to identify cortical regions that receive neurons during the perinatal period. We mapped doublecortin (DCX) positive cells in embryonic day 89 (E89), postnatal day 0 (P0), and P2 piglet brains and found persistent streams of DCX+ young migratory neurons that target the cingulate gyrus and superior frontal gyrus, as seen in the neonatal human brain, but also in areas not previously analyzed such as the middle frontal gyrus and the insular gyrus. The largest population of DCX+ cells in the piglet brain was in the anterior body of the lateral ventricle, the area characterized as the Arc in the human brain. DCX+ cells at E89 and P0 were Gad67+ interneurons and expressed the regional-specific transcription factors Nkx2.1, LHX6, CoupTFII, or Sp8. This suggests that these cells arise from both the medial and caudal ganglionic eminences (MGE and CGE respectively), the ventral progenitor zones that give rise to interneurons in the fetal brain. The proportion of DCX+ cells that are MGE- or CGE-derived varies along the AP axis; however, Sp8+ cells made up the greatest subpopulation of DCX+ cells. Lastly, we found high Reelin expression in the areas of the postnatal brain that contained the highest concentration of DCX+ cells. Many of these DCX+ cells were positive for the Reelin receptor VLDLR, which suggests a role for Reelin signaling in late migrating interneuron populations. This work shows that late migrating interneurons have heterogeneous origins and target multiple cortical regions that are implicated in neurodevelopmental disorders such as autism and epilepsy.

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T-3009

JDP2-DEFICIENT MOUSE CEREBELLAR GRANULE IPS CELLS ARE RESISTANT TO ROS-MEDIATED NEURAL CELL DEATH

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Cerebellar development is controlled by a set of transcription factors, however, the role of Jun dimerization protein 2 (JDP2) in this process remains to be elucidated. Here we found that JDP2 was predominantly expressed in granule cells of the cerebellum, demonstrated by Jdp2-promoter-Cre transgenic mice. Jdp2-knockout (KO) mice exhibited impaired development of the tubular structure of the cerebellum. We have generated induced pluripotent stem cells (iPSCs) from mouse embryonic fibroblasts (MEFs). The cerebellar granule cells (CGCs) from Jdp2-KO mice were less proliferative but were more resistant to ROS-dependent apoptosis compared with CGCs from wild-type (WT) mice. In Jdp2-KO CGCs, we found an elevation of reduced glutathione, and lower levels of reactive oxygen species (ROS) and antioxidant response element (ARE)-driven luciferase. Overexpression of nuclear factor-E2-related factor 2 (Nrf2) and musculoaponeurotic fibrosarcoma-K (MafK) did not rescue ARE-luciferase promoter activity, indicating an essential role of JDP2 in inducing ARE activity. Moreover, both the expression of cyclin-dependent kinase inhibitor 1 (p21Cip1) and the interaction between p21Cip1 and Nrf2 were increased in Jdp2-KO CGCs. Knockdown of p21Cip1 induced higher levels of ROS and apoptosis in CGCs from Jdp2-KO CGCs than in those from WT mice, demonstrating the pivotal role of p21Cip1 in controlling oxidative stress and apoptosis of CGCs in the absence of JDP2. These results suggest that the interplay between JDP2, Nrf2, and p21Cip1 may regulate the proliferation and apoptosis of CGCs, which is critical for normal development of the cerebellum.

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T-3011

SMALL-MOLECULE COCKTAILS FOR RAPID MATURATION OF MULTIPLE 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, different 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 96 or 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, dozens of compounds were identified that accelerate SYP expression specifically for human spinal motor neurons (MN) and cortical glutamatergic neurons (Glut). After further optimizing the combination and concentrations of these compounds, we have developed BrainFast MN maturation supplement and BrainFast Glut maturation supplement. After treating with these supplements, the MNs or Glut 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. These rapid maturation cocktails will enhance the ability to model CNS disease processes and screen new drugs.

T-3013

EMERGENCE OF COLUMNAR SYNAPTIC CIRCUIT DURING EARLY HUMAN CORTICAL DEVELOPMENT

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The human brain is as complex as the universe and the best “animal model” is the human brain itself. Mental diseases create enormous burdens on society, particularly neurodevelopmental diseases that have impacts throughout the lifespan. Early brain circuit activity is extremely important for normal development and is sensitive to environmental changes and disruptions of early circuit elements that are found in multiple neurodevelopmental diseases. We thus focus on applying modern techniques to human tissue to explore the principles underlying human early circuit components by applying tissue culture, viral tools including genetically modified rabies, time-lapse imaging, calcium imaging, electrophysiological recordings, and multiplex

fluorescence imaging. We find that cells with synaptic contacts are arranged into columnar structures. Most of the synaptic units consist of immature excitatory neurons. The synaptically coupled columnar structure includes both stationary and migrating neurons. Functionally, cells with synaptic contact tend to have synchronous activity. Understanding the basic principles of human early circuit elements will provide important hints of the causes and potential treatments of human neurodevelopmental disorders.

T-3015

NOVEL NEURAL PROGENITOR MARKER IMPLICATED IN INTELLECTUAL DISABILITY

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The human neocortex is made up of a myriad of cells. While the major cell types are known, the molecular mechanisms and transition states giving rise to these cells are not well understood. In previous work, using single-cell RNA sequencing (Drop-seq), we have developed a catalog of cell types in developing human neocortex, including neural progenitor cells (NPCs) and their neuronal progeny. Analysis of the transcriptional profiles of these cells and their transitions allowed us to identify 1) transition states from NPCs to neurons, and 2) transcription factors enriched in specific cell types (including ZFHX4) which may act as drivers of those fates. Here, I have found that 5-13% of the S-phase cells in the progenitor laminae express markers of both radial glia and neurons, supporting a model where a neurogenic program is induced before cell division. I have also begun characterizing the transcription factor ZFHX4, validating its enrichment in NPCs using RNA-FISH. This transcription factor is within the 8q21.11 microdeletion, causing a syndrome exhibiting intellectual disability, hypotonia, and decreased balance. Future experiments will elucidate the function of ZFHX4 in NPCs. Together, these findings validate Drop-seq results and begin bringing insight into mechanisms of normal brain development and neuropsychiatric disease.

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NEURAL DISEASE AND DEGENERATION

T-3017

SPT4 GENE-EDITED STEM CELL THERAPY IN HUNTINGTON'S DISEASE: TRANSPLANTATION OF SPT4 KO HD IPSC-DERIVED NEURAL PRECURSOR CELLS RESCUES NEURONAL DYSFUNCTION IN THE YAC128 MOUSE MODEL OF HD

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Induced pluripotent stem cell (iPSC) technology has provided the possibility that patient-specific iPSCs can be generated and utilized for autologous cell therapy without the concern of immune rejection. However, when iPSCs are developed from patients carrying a genetic mutation(s), the resulting iPSCs will still carry such mutation(s). Huntington's disease (HD) one of the most typical genetic diseases, caused by abnormally expanded CAG repeats in the N-terminus of huntingtin gene. Spt4 is a translation elongation factor involved in the expansion of CAG repeats. In this study, we first knock-outed the Spt4 gene in the Q57 HD-iPSC-derived neural precursor cells (NPCs) using CRISPR/Cas9 technology, which showed 80~90% indel efficiency. We next transplanted this Spt4-knockout Q57 HD iPSC-NPC, together with unedited Q57 HD iPSC-NPC and normal iPSC-NPC, into the striatum of 6 month-old YAC128 transgenic mouse model of HD. Human-specific nuclei (hNu) antibody staining indicated that transplanted cells were detected in all three groups at 1 week post-transplantation, Interestingly, the unedited cells showed the expression of mutant huntingtin protein (EM48) in the transplant. However, no EM48-positive cells were detected in the transplanted Spt4-knockout cells, suggesting that the gene editing of Spt4 resulted in the removal of mutant huntingtin proteins in Q57 HD iPSC-NPCs. We also observed that Spt4 knockout group showed behavioral improvement. From two months after transplantation, Spt4 knockout group showed improvement of motor functions, judged by rotarod and grip strength tests. Improvement of emotional functions, judged

by elevated plus maze test, was observed from three months after transplantation. Although histological analysis has yet to be done, these results strongly suggest that ex vivo editing of Spt4 gene can remove EM48 expression efficiently and can also improve the behavioral deficits in YAC128 HD mice, providing a new approach of silencing the mutation of huntingtin gene, which will be useful to develop autologous cell therapy using HD patient's own iPSC. Given the nature of Spt4 gene, this approach can be generally applicable to any types of HD iPSC lines.

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T-3019

ASTROCYTE-MEDIATED NEUROPROTECTION IN THE YAC128 MOUSE MODEL OF HUNTINGTON'S DISEASE BY TRANSPLATED HUMAN IPSC-DERIVED NEURAL PRECUSOR CELLS

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Huntington's disease (HD) is an autosomal-dominant disease, in which medium spiny neurons present in the striatum is selectively degenerated by the neurotoxicity from the extended CAG repeat sequences in the N-terminus of huntingtin gene. Dysfunctional astrocytes have been implicated in the development of various pathological symptoms of HD. The purpose of this study is to investigate the potential of astrocyte differentiation and the neuroprotective effects of human iPSC-derived neural precursor cells (iPSC-NPCs) following transplantation into the YAC128 transgenic mouse model of HD. We detected hNu-positive transplanted cells at 1, 3, 5, and 20 weeks post-transplantation and found that hNu-positive cells co-localize with MAP2 (a neuronal marker), or hGFAP (a human-specific astrocyte marker). Using immunohistochemistry and western blot analyses, we observed the increase of EAAT, a marker for glutamate transporter, in the hGFAP-positive cells. These results strongly suggest that the transplanted cells can increase the glutamate reuptake in the hGFAP-positive cells, thereby reducing the glutamate toxicity in the host brain. We also found that the transplanted hGFAP-positive cells can give rise to anti-inflammatory effects via the release of BDNF, as well as to the BBB stabilization by the formation of astrocytic end-feet surrounding blood vessels. As a result, astrocyte differentiation from the transplanted iPSC-NPCs increased the striatal density through the recovery of the host astrocyte functions. Transplanted animals showed improvement of motor and cognitive functions. Taken together, these results strongly suggest that the transplanted iPSC-NPCs led to astrocyte-mediated neuroprotection by the modulation of glutamate excitotoxicity or release of growth factors, providing a new possibility that astrocytes may play a major role in the cell therapy of HD.

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T-3021

POPULATION-SCALE PHARMACOGENOMICS IN-A-DISH: MAPPING ALLELES TO STRATIFY PATIENT RESPONSE TO THERAPEUTIC INTERVENTION USING IPSC-DERIVED MASSIVELY MOSAIC EXPERIMENTAL SYSTEMS

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A fundamental need in biomedical research is to understand how human allelic variation shapes cellular phenotypes. Our goal is to be able to genetically dissect any phenotype of interest to measure its heritability and discover how genetic variation converges to affect biological processes, vulnerability to illness, and response to therapeutics. We developed population-in-a-dish experimental systems in which cells from hundreds of donors can be simultaneously phenotyped and subjected to genetic analyses. Co-culturing cell lines in this way provides orders-of-magnitude increases in scalability allowing statistically meaningful genotype-phenotype correlations, and minimizes aspects of variability that would otherwise plague comparisons of individual cell lines. As a model phenotype, we focused on discerning the effects of common variation on expression of Survival Motor Neuron (SMN), encoded by paralogues SMN1 and SMN2. We show that the power and scalability of our system affords the opportunity to rapidly and inexpensively correlate SMN phenotypic variation (as measured by flow cytometry) with underlying SMN copy number (CN), markedly surpassing genome-wide significance ($p = 8.72 \times 10\text{-}22$). We then asked whether our approach could illuminate the genetic foundation for patient variation in drug response and map the basis of this pharmacogenetic effect. We used flow cytometry

to detect changes in SMN expression following treatment with the splicing enhancer LMI070. We show that LMI070 response correlates strongly with SMN2 CN (but not SMN1, p = 6.55 x 10-10) confirming the hypothesis that LMI070 increases SMN levels by specifically modulating splicing of SMN2. We questioned why some donors with the same SMN2 CN showed variation in response to LMI070 and performed an in-depth re-analysis of the genomic architecture of the SMN locus. We discovered a novel drug-nonresponsive allele of SMN that lacks exons 7 and 8 (including drug binding site), rendering these donors unable to enhance SMN protein production in response to LMI070 treatment. These results provide a therapeutic guideline for genomics-informed stratification of patients with Spinal Muscle Atrophy, and illuminate the power of population-in-a-dish systems for connecting human genetic variation with disease-relevant biological processes.

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T-3023

THE NINDS CELL AND HUMAN DATA REPOSITORY - A STEM CELL RESOURCE FOR THE GLOBAL COMMUNITY

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Since its inception in 1998, RUCDR Infinite Biologics (RUCDR, www.rucdr.org) has provided the global scientific community with the highest quality biomaterials, technical consultation, and logistical support. In 2011 with the rising interest in utilizing induced pluripotent stem cells (iPSC) to model human development and disease progression, for drug screening and for toxicology testing RUCDR began its stem cell lab offering a wide range of stem cell services, including source cell and iPSC banking, iPSC generation, iPSC gene editing and source cell and iPSC distribution. In 2015 RUCDR became the home of the NINDS Cell and Human Data Repository (NHCDR), which houses fibroblasts, iPSC and clinical data from over 300 subjects. These cells are available to academic and for profit researchers worldwide and can be viewed on the NHCDR website (https://bioq.nindsgenetics.org/). After completing an MTA (also available online) and a simple statement of research intent, cell lines can be ordered via a cart based check out system. The NHCDR functions via a cost recovery model and iPSC are available to not-for-profit researchers for $500 a vial and to for-profit researchers for $1500 per vial. Not-for-profit researchers can obtain fibroblasts (FCL) for $350 per vial and $500 per vial for for-profit researchers. Currently we have distributed more than 1500 vials of cells from the NHCDR and other collaborative projects such as the NIH Regenerative Medicine Program (NIH RMP), Target ALS, and the Myotonic Dystrophy Foundation. The NHCDR is also distributing 9 isogenic pairs of iPSC developed through CRISPR gene editing and a GMP grade iPSC as well as a non-GMP grade cell line from the same subject for pre-clinical research. All cell lines are distributed with a certificate of assurance, guidelines for culturing the cells and any technical support required to help a client meet their research goals.

T-3025

USING HUMAN PLURIPOTENT STEM CELLS TO INVESTIGATE THE ROLE OF GLIA IN PSYCHIATRIC DISORDERS

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Many types of psychiatric disorders, such as autism and schizophrenia, are complex and heterogeneous neurodevelopmental disorders affecting the brain at both the cell and structural levels. Due to inadequate animal models and limited capacity to isolate human brain tissue, modeling these disorders and understanding the underlying mechanisms can be challenging. Here, we use human induced pluripotent stem cells (hiPSCs) as a powerful tool to dissect the molecular and cellular pathways implicated in these psychiatric disorders during the early stages of neurodevelopment with the overarching aim of identifying novel therapeutics. Given the heterogeneity of these disorders, we focus here on subtypes of autism or schizophrenia associated with brain enlargement or undergrowth. Studies have shown that subjects with autism who have enlarged brains have more severe cognitive impairments and a poorer prognosis compared to autistic patients with normal brain sizes. Here, we first develop a new protocol for deriving brain-derived neuroglial cells from human iPSCs. We then differentiate the patient-specific iPSCs into neuroglial cells to investigate changes at the molecular and cellular levels. In particular, we investigate changes in cell size, proliferative capacity, cell survival, and signaling pathways that may be underlying the development of autism or schizophrenia. This work demonstrates the utility of a new neuroglial directed differentiation protocol and shows that psychiatric disorders can be modeled using human iPSCs. Furthermore, this work begins to shed light on signaling pathways regulating brain size that may suggest new therapies for the treatment of autism or schizophrenia.

T-3027

ALS-IMPLICATED PROTEIN TDP-43 SUSTAINS LEVELS OF STMN2, A MEDIATOR OF MOTOR NEURON GROWTH AND REGENERATION

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The findings that amyotrophic lateral sclerosis (ALS) patients almost universally display pathological mislocalization of the RNA-binding protein TDP-43 and that mutations in its gene cause familial ALS have nominated altered RNA metabolism as a disease mechanism. The RNAs regulated by TDP-43 in human motor neurons and their connection to neuropathy, however, remain to be identified. Using pluripotent stem cell technologies and RNA sequencing, we identified transcripts whose abundances in motor neurons are sensitive to TDP-43 depletion. Notably, expression of STMN2, which encodes a microtubule regulator, declined after TDP-43 knockdown and TDP-43 mislocalization as well as in patient-specific motor neurons and postmortem patient spinal cord. STMN2 loss upon reduced TDP-43 function was due to altered splicing, which is functionally important, as we show STMN2 is necessary for normal axonal outgrowth and regeneration. Notably, post-translational stabilization of STMN2 rescued neurite outgrowth and axon regeneration deficits induced by TDP-43 depletion. These findings were enabled by human pluripotent stem cell disease modeling as this regulation of STMN2 by TDP-43 is not found in mice. We propose that restoring STMN2 expression warrants examination as a therapeutic strategy for ALS.

Funding Source: J.R.K. is the Project ALS Tom Kirchhoff Family Postdoctoral Fellow.

T-3029

EVS DERIVED FROM GLIOBLASTOMA PROMOTED THE TRANSFORMATION OF NEURAL PROGENITOR CELLS INTO GLIOBLASTOMA

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Grade IV gliomas is highly infiltrative growth and aggressive glioblastoma (median survival time are 14.5-16.6 months). Extracellular vesicles (EVs) are membrane-contained vesicles shed from cells. EVs contain proteins, lipids, and nucleotides, all of which play important roles in intercellular communication. In the progression of cancer, cancer-derived EVs can induce endothelial cells angiogenesis, adipose-derived stem cells (ASCs) differentiation, microglias, neutrophils, macrophages polarization, and stromal cell EMT (epithelial–mesenchymal transition). The human glioblastoma cell lines U87-derived EVs carrying miRNA-221, promote glioblastoma cell proliferation, migration and invasion. We posit that EVs derived from glioblastoma promoted the transformation of neural progenitor cells into glioblastoma. Release of EVs, which was confirmed by electron microscopy, nanoparticle tracking analysis (NTA), and Western Blot in glioblastoma cell line (U87). Furthermore, EVs derived from U87 increased levels of proliferation and migration of neural progenitor cells. Interestingly, U87-derived EVs activated PI3K/Akt/mTOR and Ras/Raf/ERK signal pathways, and the proteomics analysis of U87-derived EVs was consisted with our previous results. These findings suggest that the EVs derived from glioblastoma promoted the transformation of neural progenitor cells into glioblastoma. Release of EVs, which was confirmed by electron microscopy, nanoparticle tracking analysis (NTA), and Western Blot in glioblastoma cell line (U87). Furthermore, EVs derived from U87 increased levels of proliferation and migration of neural progenitor cells. Interestingly, U87-derived EVs activate PI3K/Akt/mTOR and Ras/Raf/ERK signal pathways, and the proteomics analysis of U87-derived EVs was consisted with our previous results. These findings suggest that the EVs derived from glioblastoma promoted the transformation of neural progenitor cells into glioblastoma.

Funding Source: Natural Science Foundation of China (81830037)

T-3031

PRELIMINARY RESULTS OF PHASE I CLINICAL TRIAL OF INTRACEREBRAL TRANSPLANTATION USING BONE MARROW STROMAL CELL (BMSC) AGAINST ACUTE ISCHEMIC STROKE IN HUMAN (RAINBOW PROJECT)

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Recent breakthrough in cell therapy is expected to reverse the neurological sequelae of stroke. Prior studies have demonstrated that bone marrow stromal cells (BMSCs) have therapeutic potential against stroke. In this study, we investigated the use of autologous BMSC transplantation for acute ischemic stroke through direct transplantation route with several new aspects as a next-generation cell therapy for treating stroke. This study is called the Research on Advanced Intervention using Novel Bone marrOW stem cell (RAINBOW, UNIN ID: UMIN000026130). RAINBOW is a phase 1, open-label, uncontrolled, dose-response study, with the primary aim to determine the safety of the autologous BMSC administered to the patients with acute ischemic stroke. Estimated enrollment is 6 patients suffering from moderate to severe neurological deficits. Approximately 50 mL of the bone marrow is extracted from the iliac bone of each patient 15 days or later from the onset, and BMSCs are cultured with allogeneic human platelet lysate (PL) as a substitute for fetal calf serum and are labeled with superparamagnetic iron oxide for cell tracking using magnetic resonance imaging (MRI). BMSCs are stereotactically administered around the area of infarction in the subacute phase. Each patient will be administered a dose of 20 or 50 million cells. Neurological scoring, MRI for cell tracking, 18F-fuorodeoxyglucose positron emission tomography, and 123I-Iomazenil singlephoton emission computed tomography will be performed throughout 1 year after the administration. This is a first-in-human trial to use labelled BMSC to the patients with acute ischemic stroke. We expect that intraparenchymal injection can be a more favorable method for cell delivery to the lesion and improvement of the motor function. Moreover, it is expected that the bio-imaging techniques can clarify the therapeutic mechanisms.

T-3033

CHARACTERIZATION OF APOE PROTEIN UPTAKE IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

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Alzheimer's Disease (AD) is the most prevalent form of dementia among the elderly. There is currently no viable cure for AD. While Amyloid Precursor Protein (APP), Presenilin 1 and 2 (PSEN1 and PSEN2) mutations are the major causative genetic factors to familial AD (accounts for ~5% of AD patients), the causal factors to the most common sporadic AD (~95% of AD patients) are still unclear. The Apolipoprotein E allele ε4 (APOE4) gene has been identified as the most significant risk factor associated with sporadic AD [AKA Late Onset Alzheimer's Disease (LOAD)], which occurs in people 65 years of age and older. In humans, APOE genes have three common alleles (ε2, ε3, and ε4). As a major component of lipoprotein complexes, APOE proteins play important roles on lipid homeostasis in the adult brain, where they are secreted and maturated by astrocytes and then internalized by adult neurons. APOE4 protein has been shown to promote neurodegenerative diseases, potentially through the altering of APP processing, interfering with Amyloid Beta clearance, and exacerbating tauopathy. However, whether APOE4 protein uptake and cellular trafficking by human neurons play a role in AD pathogenesis is still unknown. Thus, we hypothesize that among three isoforms, APOE4 protein is differentially internalized in human brain neurons to promote AD progression. To test our hypothesis, we investigated the APOE protein internalization in human induced pluripotent stem cell (iPSC) derived cortical neurons. We treated the iPSC-derived neurons with three APOE isoforms (APOE2, APOE3, and APOE4) and examined APOE neuronal uptake using immunostaining and fluorescent microscopy. Intracellular distribution of APOE protein is determined by the colocalization of MAP-2 and Tau staining (neuron markers). Early endosomal marker (EEA1) and vesicle recycling and sorting factors (Rab proteins) were used to monitor the potential APOE trafficking pathways. The immunostaining signal was quantified using ImageJ. To this end, we characterized APOE isoform internalization in human cortical neurons and confirmed that APOE4 protein is differentially internalized by neurons compared with APOE2 and APOE3 proteins. This higher APOE4 internalization by human neurons may consequently promote APOE4 mediated AD pathogenesis.

Funding Source: California Institute for Regenerative Medicine (CIRM)

T-3035

COMBINATIONAL TREATMENT OF DOCOSAHEXAENOIC ACID AND CAROTENOIDS DECREASES SECRETED AβS VIA β-SECRETASE INHIBITION IN HUMAN IPS CELL DELIVED NEURONS

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by impaired memory and cognition. One of major pathological hallmarks in the AD patient's brain is senile plaques, which are mainly composed of heterologous amyloid-β (Aβ) peptides. A lot of studies have indicated that accumulation of Aβ peptides in vulnerable brain regions plays a central role in AD pathogenesis: Aβ is a culprit of neurotoxic agent, leading to synaptic dysfunction and eventual neuronal loss. Aβ is generated through sequential cleavages of amyloid-β precursor protein (APP) by enzymes called β- and γ-secretases. Alternatively, APP can be subjected to a non-amyloidogenic processing by α-secretase within the Aβ sequence domain. In prior study, we have found that combinational administration of Docosahexaenoic acid (DHA, 22:6 n-3) and two Carotenoids (Lutein or Zeaxanthin, which are structural isomers of Lutein, and Capsanthin) reduces accumulation of Aβ and suppresses cognitive decline in the brain of AD mouse model (APPswe/PS1ΔE9). However, the effect of these compounds on humans and the detailed mechanism of their action remain to be determined. In this study, to investigate whether this combinational treatment of DHA and carotenoids containing Lutein and Capsanthin (named as DLC) attenuates Aβ production in human neuronal cells, we first developed in vitro culture system, in which we differentiated induced pluripotent stem (iPS) cells into human cortical neurons. Next, the levels of secreted Aβ species in this iPS-derived neurons were quantified following DLC administration. Here, we revealed that DLC significantly decreased secretion of Aβs through specific inhibition of β-secretase activity without altering BACE1 protein level. Furthermore, Carotenoids alone, but not DHA alone, have a sufficient effect on anti-Aβ phenotypesto a lesser extent than DLC combination, suggesting Carotenoids as a major efficient components. In sum, DLC is largely expected to become novel candidate compounds for therapeutic drug and/or disease prevention of AD.

T-3037

MUTATIONS IN CHCHD10 DISRUPT MITOCHONDRIAL STRUCTURE AND FUNCTION IN IPSC-DERIVED MOTOR NEURONS (MN)

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Mutations in coiled-coil-helix-coiled-coil-helix-domain containing 10 (CHCHD10), a nuclear-encoded mitochondrial protein, have recently been implicated in a number of neurodegenerative diseases including frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS). While the function of the protein remains unknown, conflicting evidence exists, which suggests that mutations in CHCHD10 and/or knockdown of expression may cause considerable deformation of mitochondrial cristae membranes in a variety of cell types including yeast, HeLa and fibroblasts. Furthermore, mutations appear to alter respiration and ATP synthesis in a mutation- and cell type-specific manner. Studies to date have explored the role of CHCHD10 in non-neural cell types, an important limitation due to these cells' inability to accurately replicate the cell biology of neurons, critical players in neurodegenerative disease. Due to the inaccessibility of motor neurons (MN) in patients, induced pluripotent stem cells (iPSCs) can serve as a limitless source of MN to aid in studying protein function and disease biology. Here, we investigate how mutations in CHCHD10 disrupt mitochondrial structure and function in iPSC-MN through the use of genome editing, 3D reconstruction of subcellular components using electron tomography and various molecular biology techniques. To explore the role of CHCHD10 in the central nervous system, we utilize human patient iPSC-derived MN harboring the R15L mutation as well as a CHCHD10 knockout (KO) cell line we've engineered via CRISPR/Cas9 gene editing. When compared to healthy control donor lines, both the R15L and KO cell lines exhibit significantly altered mitochondrial area and cristae abundance, respectively. Preliminary studies show that this altered ultrastructure is accompanied by diminished mitochondrial respiration and spare respiratory capacity. Transcriptomic analysis also reveals misregulated pathways related to oxidative phosphorylation and ECM-receptor interaction in both R15L and KO iPSC-MN. Identifying dysregulated elements of mitochondrial biology will expand our knowledge of disease onset and progression among several multi-etiologic neurodegenerative diseases, which share clinical, neuropathological and genetic features.

ORGANOIDS

T-3041

VASCULARIZED HUMAN CARDIAC SPHEROIDS AS AN IN VITRO MODEL TO STUDY CARDIAC DISEASE

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Stem cell derived cell- and tissue models with three-dimensional (3D) architecture cultured under perfusion conditions are on the rise as in vitro platforms with increased physiological relevance. Compared to conventional two-dimensional (2D) cell culture models, these microphysiological systems (MPS) intend to better mimic organ functions in order to provide new insights to in vivo processes including the release of disease-specific biomarkers, or to predict the impact of drug candidates on humans already during the pre-clinical trials. Two critical challenges for MPS and spheroid models are 1) to provide essential factors such as oxygen and nutrients throughout the dense 3D cell-construct and 2) to establish homotypic and heterotypic paracrine cell-cell communications between relevant cell types of the local tissue environment. We are therefore developing cardiac spheroids consisting of iPS-derived cardiomyocytes, cardiac fibroblasts, and cardiac endothelial cells where vascularization within the 3D spheroids can occur by self-assembly of the endothelial cells into capillary networks. The cardiac spheroids were cultured under perfusion of cell culture medium for physiologically relevant shear stress exposure, which previously have been shown to have positive effects on both cardiomyocytes and endothelial cells. Here, we present the effect of different levels of shear stress on the vascularization process. We further investigated the effects of the vascularization and the shear stress on cardiomyocyte maturation. In upcoming experiments, we intend to induce cardiac hypertrophy in the cardiomyocytes by exposing the spheroids to endothelin-I. Vascular spheroids like the ones presented here, can for example become valuable tools in the study of diseases such as cardiac hypertrophy or be part of more complex MPS platforms with multiple cell types mimicking the interactions between several organs.

Funding Source: The Swedish Knowledge Foundation

T-3043

IN VITRO RECONSTITUTION OF WOLFFIAN DUCT USING HUMAN PLURIPOTENT STEM CELLS

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The Wolffian duct (WD) is a pair of tubes that arises in the trunk of elongation stage embryos. The WD initially develops as the pronephric duct from the anterior intermediate mesoderm population, then elongates caudally and connects to the cloaca. By contacting with the metanephric mesenchyme (MM) which is derived from posterior intermediate mesoderm, the WD develops the ureteric bud (UB) on that contact surface. The UB then initiates branching and induces nephrogenesis of the MM by secreting various growth factors. Recently, it was revealed that the WD lineage and the MM lineage separate post primitive streak (PS) stage, which is regulated by the timing of cell migration from the primitive streak region to the trunk. Based on this knowledge, using human pluripotent stem cells (hPSCs), we demonstrated that anterior-posterior patterning of intermediate mesoderm could be controlled by duration of Wnt/β-catenin pathway activation, which mimics a period of PS stage. More recently, induction of WD cells and successive UB structure from hPSCs was reported from a couple of groups. However, the induction efficiency remains moderate, and these procedures require time-consuming process such as cell sorting and re-plating. Also, in vitro reconstitution of the WD tubule structure, which is necessary for generating kidney organoids with functional ureter, has not been achieved. Thus, our group aimed to establish an efficient and simple method of the directed induction of the WD lineage. Based on a strategy of our previous study, we have achieved induction of KIT+/CXCR4+ WD progenitor from hPSCs with ~80 % efficiency by a 3-step protocol on simple 2D culture system. We are also trying to reconstitute the WD tubulogenesis, the WD elongation, and eventually the kidney organogenesis in vitro. Here, we present current progress of the above research.

Funding Source: We thank RIKEN BDR-Otsuka Pharmaceutical Collaboration Center for their financial support to this project.

T-3045

NOVEL HYPER CROSS-LINKED CARBOHYDRATE POLYMER FOR THREE-DIMENSIONAL TUMOR ORGANOID DRUG SCREENING

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The medical industry shifts towards developing effective cancer treatments and improved screening platforms focusing on cancer initiating cells (CIC), which are hypothesized to be self-renewing, drug-resistant precursors to tumors. To better monitor drug behavior on cancer cells such as CIC, a reliable in vitro representation of their in vivo counterpart is needed. Historically, drug screens utilized monolayer culture or in vivo animal models. Although monolayer culture is amenable to low cost high-throughput drug screening, in vivo cancer malignancy and drug sensitivity are often not faithfully replicated. In vivo animal models provide a more complete context including stromal interplay; however, preliminary drug screening with animal models are limited due to cost and species-specific differences (e.g., human tumor in a rodent model). To bridge this gap, organoid culture has become increasingly popular. With an organoid culture system that can faithfully recapitulate the key physiological factors driving malignancy and drug response in the CIC niche, the use of animal models and associated time

and cost may be reduced. Previous studies showed great potential for cell culture on a novel synthetic hyper cross-linked carbohydrate polymer (HCCP). In this study, HCCP was evaluated to support a cancer organoid. To better mimic in vivo tumor development with the contribution of cancer-associated fibroblasts in cancer aggression, lung cancer cells were co-cultured with fibroblast cells, seeded onto HCCP, and cultured for one month. HCCP was then harvested and paraffin embedded for analysis. Immunohistochemistry analyzed for epithelial and mesenchymal markers showed high marker expression comparable to its in vivo counterpart, including presence of CIC, beyond typical findings with other organoid systems. Cancer organoids were successfully recovered from the HCCP backbone and investigated for downstream analyses and applications. Organoids generated in HCCP offer an enhanced in vitro system to study cancer and drug development at scale within vivo-like context where CIC and stromal interplay can be reviewed. Further studies are currently investigating the response of cancer organoids containing CIC to chemotherapeutic drugs in a high-throughput format, which is critical for early drug screening.

T-3047

CARDIAC ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS RESEMBLING KEY FEATURES OF EARLY HUMAN HEART DEVELOPMENT

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Organoids are three-dimensional (3D) cellular aggregates that better resemble features of native organs regarding functionality and morphology compared to conventional cell culture. They can be used as in vitro models for organ development and diseases, drug development, and potentially for future regenerative therapies. Although comprehensive organoids have already been published for a wide range of tissues including small intestine, kidney and brain, advances in the cardiovascular field are limited. Ideally, cardiac organoids should resemble heart morphology at early developmental stages. This should include proper formation of the three heart layers (epi-, myo-, and endocardium) and an organ-typical tissue composition, in particular cardiomyocytes, myofibroblasts and endothelial cells. However, to date cardiac organoids that mimic the human embryonic heart properly have not been published. This project aims to overcome these prior limitations. We have established a protocol, which leads to the highly reproducible generation of cardiac organoids from human pluripotent stem cells, which contain at least two heart layers in a structured 3D pattern. We also show that the organoids are composed of all cell types present in proper heart tissue and include the formation of endothelialized blood vessel-like networks. Moreover, we demonstrate the application of these organoids as a functional model for drug screening i.e. to monitor teratogenicity and for congenital heart defects. Together, we show that these novel organoids represent a superior in vitro model for early human heart development, thus opening new perspectives in pharmacological research and organ-specific lab-on-chip approaches.

T-3049

IN VIVO EVALUATION OF SCAFFOLDS COMPATIBLE FOR ORGANOID ENGRAFTMENTS ONTO INJURED MOUSE COLON EPITHELIUM

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Epithelial regeneration is one of the critical steps necessary for the healing of wounds at the surface of small intestine and colon. Colon organoid is a useful tool in exploiting the regenerative medicine potential of tissue resident stem cells for treating human digestive disorders. Transplanted colon organoids from Lgr5+ stem cells readily integrated into damaged mouse colon, which formed self-renewing functional crypts. Organoids suspended in Matrigel engrafted better than organoids in PBS, suggesting a role for the simultaneous supply of extracellular matrix to develop organoid based therapeutics. However, Matrigel, which is used as a transplantation scaffold for colon organoids, cannot be used clinical applications due to its undefined composition and tumorigenicity. This study identifies FDA-approved scaffolds that are effective for organoid transplantation in damaged intestinal mucosa. The colon crypt was isolated and cultured from CAG-EGFP mice into EGFP+ organoids and subsequently transplanted into the EDTA colitis mouse model using gelatin, collagen type Ia, or fibrin glue scaffolds. When organoids were transplanted with

gelatin, collagen, and fibrin glue into the EDTA colitis mouse model, all groups were found to be successfully engrafted after 1 week. However, the EGFP+ area increased 4 weeks after transplantation only in the group using organoid suspended in a collagen scaffold. The transplantation of organoids with collagen did not induce scaffold mediated mucosal inflammation and toxicity in the recipients' colon and were thus deemed safe when locally administrated. We provide evidences for the safety and utility of collagen to develop organoid-based therapeutics for treating de-epithelialized colon diseases. Furthermore, this work provides insight into tissue engineering underlying development of the organoid therapeutics and points to future opportunities for regeneration of the digestive tract.

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T-3051

NEURONAL NETWORK FUNCTION AND PLASTICITY IN BIOENGINEERED NEURONAL ORGANOIDS

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To study human neuronal network function, we developed a defined, Matrigel-free 3D cell culture system termed human bioengineered neuronal organoids (BENOs). Neural differentiation of pluripotent stem cells (iPSCs) embedded in a collagen matrix was directed under serum-free conditions. Calcium imaging revealed spontaneous tetradotoxin (1 µM)-sensitive signals by d25. Co-ordinated spontaneous activity of multiple neuronal clusters was abolished by GABAergic (picrotoxin, 58 µM; saclofen, 330 µM) inhibition and re-instated upon washout (n=2). On the other hand glutamatergic (CNQX 15 µM / MK-801 0.2 µM) inhibition strongly reduced the event frequency but did not affect the synchronicity. Stimulation (injected current: 20-100 µA)-evoked Ca2+ influx in remote regions (distance from electrode 0.5 to 1.5 mm) suggesting a neuronal network that extends throughout the organoid. Paired-pulse stimulation demonstrated a Ca2+ influx pattern similar to paired pulse depression (PPD), which could be alleviated by a GABA-A inhibition and restored upon washout (n=2). Multielectrode array analysis of BENO slices (n=6), showed high frequency-induced long- and short-term potentiation and depression) in different areas of the BENO providing strong evidence for neuronal plasticity. In conclusion, iPSC-derived BENOs contain electrically active neuronal networks and exhibit typical forms of plasticity observed in the human brain.

T-3053

EXPLORING THE POTENTIAL OF A NOVEL IN-HOUSE REPROGRAMMING APPROACH MARKED BY FGF2 SECRETION SIGNATURE TO BUILD INNER EAR ORGANOIDS FOR HEARING LOSS REGENERATION

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The success in establishment of faithful inner ear organoids (IEO) that would facilitate recreation of cochlear hair cells will provide for an opportunity for disease modeling, drug screening and cell-based transplantation therapies in sensorineural hearing loss (SNHL) patients. Developmental studies strongly implicate bFGF (basic fibroblast growth factor) signaling as a primordial pathway in induction of otic placodes. Precise spatial-temporal regulation of bFGF/FGF2 signaling with controlled exogenous supplementation of FGF2 is integral part of current inner ear induction protocols. We explored the potential of our recently established reprogramming approach- SPIR (serine protease induced reprogramming), which triggers de novo bFGF secretion in ARPE19 (adult retinal pigment epithelial) cells, to generate IEO. The following exciting possibilities in prolonged SPIR cultures of ARPE19 cells were explored- a) stand-alone approach- endogenous bFGF induction by SPIR (independent of supplementation with other signaling cues) directed a pre-otic fate; b) reliable re-routing approach- modifying standard protocols to include/exclude established signaling cues to above approach; c) secretome approach- employing ARPE19 secretome (containing bFGF) to CACO-2 (colon carcinoma) cell line, to harness the possibility of recreating cochlear stereocilia by hijacking and coaxing the structurally similar microvilli machinery of CACO cells into stereocilia. Our preliminary data suggests morphological features of IEO demonstrated by inner-ear like unique spiral organoids (several microns to 1 mm in size); b) immunofluorescence studies suggest expression of inner ear marker MYO7A and supporting cell marker SOX-2; c) pilot

proteomic studies using liquid chromatography tandem mass spectrometry (LC-MS) indicate upregulation of intracellular bFGF, neurotrophic growth factors and synaptic vesicle proteins in ARPE19 IEO. Myosin 14 (MYH14) is implicated in neuritogenesis, apical cell junction maintenance in cochlea, and mutations in MYH14 are associated with DFNA4-type hearing impairment. Active actin remodeling and upregulation of MYH14 in CACO-derived IEO cells offers a completely novel approach with potential scope and permissiveness towards stereocilia/ inner hair cell generation.

Funding Source: Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

T-3055

GENERATION OF HUMAN BRAIN ORGANOIDS FROM L-MYC IMMORTALIZED NEURAL STEM CELLS

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Neural organoids can recapitulate aspects of tissue formation and cell differentiation of developing brain in a readily manipulated format. Here we present the developmental progression of organoids formed using L-myc expressing human neural stem cells (hNSC; LM-NSC008). These organoids originate with LM-NSC008 cells embedded in Matrigel, are grown in defined stem cell medium under both normoxic and hypoxic conditions, and survive without signs of necrosis for well over three months. Over time cells organize into regions of circumferential and radial orientation, and become quite complex structures. By immunofluorescence and confocal microscopy, we observe emergence of spatially-patterned morphologically-distinct cells of multiple neural lineages (GFAP, tubulin III, FoxG1, Fzd9, MAP2, NeuN), temporal and spatial expression of marker genes and appearance of synaptic junctions (synapsin1, PSD95), along with indications of endothelial trans-differentiation (CD34, von Willebrand Factor) and tight junctions (ZO-1). At the same time, populations of neural progenitor cells (Sox2, nestin, Pax6, GLAST, phospho-vimentin) are maintained. We do not see signs of uncontrolled growth or possible tumor formation. Taken together, these observations suggest successful neural tissue development from LM-NSC008 cells, which may provide a physiologically-relevant model for preclinical in vitro studies, as well as a source of tissue for direct transplantation into injured brain for purposes of cell replacement or regeneration.

T-3057

ESTABLISHING AND FUNCTIONAL CHARACTERIZATION OF 3D NEURAL SPHERIOD MODELS FROM MONOLAYER EXPANDED NSCS: OPTIMIZATION AND COMPARISON TO 2D CULTURES

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The expanding application of 3D culture methods to generate stem cell derived models of neuronal development, maturation and disease enables the creation of more complex cellular models which more faithfully recapitulate in vivo neural architectures and physiology than traditional 2D cultures. Currently, many approaches to create 3D models rely on reagents and tools designed for 2D monolayer systems. Here we evaluated and compare the differentiation and functional maturity of NSCs (neural stem cells) generated and expanded in monolayer, and then transferred to conditions which promote 3D spheroid formation relative to those maintained in monolayer conditions. Multiple media combinations, culture conditions and reagents were assessed for optimal differentiation of the NSC to neurons and the influence they had on proliferation, gene expression and maturation. We demonstrate that our Sox2 /Nestin positive NSCs are capable of forming 3D spheroids that could be matured to generate electrically active neurons. Through modulation of culture conditions we showed reduced numbers of immature cell types in the spheroid subsequent observable changes in the morphology and shape of the spheroid. In order to facilitate analysis of specific properties of the spheroids we transitioned them from suspension culture to poly-D-Lysine/laminin coated surfaces, to which they readily attached, and maturing neurons were observed to migrate out of the spheroid across the surface of the well. Spheroids anchored on multi-well Multi Electrode Array (MEA) plates showed electrical activity as early as 4 weeks. Our results clearly demonstrate the feasibility of transitioning a 2D NSC culture system to 3D and that importance of optimizing several key culture system parameters in order to reproducibly generate neural spheroids. Clearly additional work to help define the relative benefits of modeling and analysis of neural biology in 2D vs 3D is an area of active investigation and several points of consideration are highlighted in our work.

TISSUE ENGINEERING

T-3059

3D BIOPRINTING AND ORGANOTYPIC CULTURE OF HUMAN SKIN UNIT

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3D bioprinting is a platform for automated fabricating complex structure, which offers many advantages over traditional methods for tissue engineering regarding high throughput, precision, flexibility, and reproducibility. 3D bioprinting has recently been used to engineer human organs and tissues, including the skin. To make the skin equivalent unit, keratinocytes and fibroblasts were used to constitute the epidermis and dermis, respectively. Collagen and other bio-degradable materials were used for the dermal matrix of the skin. We then made the dermal unit containing the collagen-based bio-ink and fibroblasts using the RegenHU Biofactory 3D plotter, followed by seeding the keratinocytes on the dermal unit. To mimic physiological relevant conditions of the skin, 3D organotypic units were cultured in submerged media and then lifted to the air-liquid interface for epidermis maturation and stratification. We modified the collagen-based bio-ink and culture media to achieve higher strength of the bio-ink to prevent matrix contraction. We additionally explored the 3D printing parameters to control gelation speed and structure integrity in the engineered dermal unit. Importantly, we optimized co-culture conditions of human keratinocytes and primary fibroblasts for the epidermal stratification. Using histological and immunostaining analyses, we determined the similarity between 3D printed skin equivalents and in vivo skin tissues.

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T-3061

RAT BONE MARROW MESENCHYMAL STEM CELLS ISOLATION, CULTURE AND CHARACTERIZATION IN BIODISPOSITIVES FOR REGENERATION OF INFARCTED MYOCARDIUM

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Currently tissue engineering strategies for myocardial regeneration after infarction are being explored, including scaffolds that offer mechanical support and cell delivery into the injury. Bone marrow mesenchymal stem cells (MSC) are important candidates for cell therapy due to its ability to differentiate into cells of cardiac tissue. However, the underlying mechanisms of MSC to promote tissue regeneration are not fully understood. The present study examines the undifferentiated and differentiated MSC's behavior on a biopolymer to assess cell viability and cell migration. The MSC were isolated from Wistar rats aged between 4 and 8 weeks. An improved isolation protocol was executed to optimize the performance of the cells in the scaffold. Group 1 (G1) of scaffolds (750 cells/µL) and group 2 (G2) (5000 cells/µL) were studied through trypan blue exclusion test to compare cell viability during 4 weeks. To assess cell migration group 3 (G3) were cell-seeded in a homogenous distribution and group 4 (G4) in a divided distribution, both at the same cell concentration of 2250 cells/µL. Cell migration was estimated through fluorescent microscopy. The isolation and cell culture protocol resulted in optimum confluence (>90%) in passage 4 to seed all the scaffolds. The cell viability assay determined G1 live cells had an average viability percentage of 98.23 ± 3.35 and for G2 an average of 98.38 ± 1.95. Distances measured in cell migration resulted highly similar (cv<1%). MSC showed optimal behavior during culture and differentiation and should be considered as good candidates for tissue regeneration. Their viability was significantly high, and it was not affected by the concentration of cells in the scaffold, the gelation method with ammonium hydroxide, the use of PETG in 3D printing, or the integration to the biopolymer. Closeness in the distances evaluated between cell reference points for cell migration showed that there was no significant cell migration. This suggests that cells did not generate sufficient tensile forces to create focal adhesions in the scaffold. Despite the favorable characteristics of MSC, it is important to extend the study by modifying the biopolymer and submitting cellular constructs to paracrine factors of the natural myocardial infarcted microenvironment.

T-3063

ENHANCING THE DIFFERENTIATION POTENTIAL OF AGED STEM CELLS FOR BONE RECONSTRUCTION

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More than half a million patients per year undergo surgeries to repair of large bony defects. The main options currently available for bone reconstruction are autologous bone harvested from the patient involving additional and complex surgeries, or cadaver bone grafts. Yet, both incur significant morbidity and risks. Therefore, bone engineering offers an attractive alternative approach for regenerative medicine. Multipotent stromal cells (MSCs) have promise for bioengineering bone grafts and were shown to generate bone cells in-vitro in three dimensional (3D) printed scaffolds. However, the therapeutic application of human MSCs is still limited by a physiologic aging-related decline in differentiation potential, compromising the feasibility and reproducibility of therapies. Moreover, bone grafts generated from MSCs are only millimeters in size limiting their use in a clinical setting. Telomere shortening with aging and cell division restricts cell proliferation and leads to a loss

of stem cell function. We have designed a highly innovative approach, to overcome this critical barrier, and developed novel methods to increase the differentiation capacity of aged MSCs cultured on 3D printed scaffolds. Our data show that induction of the ZSCAN4 mechanism enhances bone differentiation of aged MSCs and rescues telomere length. Our research goal is to generate novel protocols for rapid generation of patient-specific bone grafts from MSCs attained from minimally invasive procedures. Our research has impact for the treatment of numerous bone degenerative diseases and bone reconstructive procedures.

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T-3065

INJECTABLE SCAFFOLDS FOR TISSUE ENGINEERING

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Intervertebral disc diseases are a significant medical problem affecting many people around the world. In Poland, the statistics of the Social Insurance Institution (Medical Abuse in 2016) indicate that low back pains and other intervertebral disc diseases constitute 17% of the total number of days of sick leave. In connection with the above, current work describes design of a composite scaffold as a carrier in cell therapy, which will contribute to the regeneration of the intervertebral disc, including the increase of its height. Our composite scaffold include nanofibers that were prepared with the use of the electrospinning method. This method is a simple but powerful technique for fabricating desirable nano- and microfibers by using a high potential electric field. Human mesenchymal stem cells (MSCs) were cultured on the scaffold from poly(L-lactide). Proliferation kits and fluorescence microscopy were used to asses cells' viability and adherence to the nanofibers' surface. hMSCs were efficiently cultured on the nanofibrous scaffold and could be readily detected in porous structure of the scaffold after 7 and 14 days of culture. Viability and proliferation kits proved that the material is not toxic. Drug release from nanofibrous material of model growth factor was conducted with pharmacopeia protocols. Drug release of the 14 kDa growth factor was achieved for 14 days without burst release. Nanofibrous biomaterials prove their advances in many tissue engineering applications. Adjustable porosity of the scaffold and the biocompatibility of the biomaterial make it perfect candidate for cells' scaffold in many medical procedures and also as a drug release carrier. With the use of our single nanofibers, such biomaterials can also be readily used in minimally invasive procedures to regenerate IVD.

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T-3067

COMPARISON OF HUMAN MESENCHYMAL CELLS ADHERENCE AND VIABILITY ON ELECTROSPUN PLGA FIBROUS SCAFFOLDS AND THE COMMERCIALLY AVAILABLE COLLAGEN MATRIX NEVELIA

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The number of people in Brazil needing skin transplants has been constantly increasing over the last 10 years. There are some commercially available skin substitutes nowadays, most of them being acellular. Mesenchymal stem cells (MSCs) are frequently used in the field of regenerative medicine due to their plasticity, which is their capacity of giving rise to several types of cells. MSCs, when associated with a dermal substitute, could consistently contribute to tissue regeneration. In this study, comparison was done regarding adherence and morphology between the MSCs on the fibrilar scaffolds of 18% PLGA (poly lactic-co-glycolic acid) and the collagen matrix, Nevelia. Fibrous scaffolds were produced by the electrospinning method. The morphology of the fibers was evaluated by scanning electron microscopy and revealed two populations of fibers with diameters of 0.3±0.5µm (thinner) and 1.3±0.06 µm (thicker) with an average fiber diameter of 0.82±0.47 µm. The MSCs were isolated and prepared from human deciduous teeth pulp and characterized by flow cytometry and differentiation assays. The MSCs were cultivated either on the surface of the PLGA scaffolds or the Nevelia membrane, and their viability was analyzed by the WST8 assay at day 1 and 7 after seeding. The

WST8 assay showed that the MSCs cultivated on the culture dish presented a higher viability at day 7 (4.18 ±0.6) than the cells cultivated on the surface of the PLGA fibers (0.14 ±0.1) or the Nevelia (0.57±0.07). However, MEV analysis of the cells on both scaffolds showed good adherence. Further cell types, such as fibroblasts and keratinocytes, should be tested as possible cell components of the two analyzed scaffolds. In conclusion, the scaffolds developed in this study are potential biomaterials for use in the treatment of large skin loss, as with burn patients.

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T-3069

IKVAV, LRE AND GPQGIWGQ ALTER EXTRACELLULAR MATRIX DEGRADATION AND ENZYME EXPRESSION LEADING TO AXON EXTENSION IN ENCAPSULATED HUMAN IPSC DERIVED NEURAL STEM CELLS

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Human stem cells and neural progenitors are being widely used in experimental treatments to restore function after central nervous system trauma or degeneration. However, these cells often do not survive, fully mature, or integrate into the host tissue when transplanted. Inclusion of biomaterial supports with the cells enhance survival and integration. Recently, enzymatic remodeling of the extracellular matrix has been identified as a key driver of neural differentiation. This makes the development of matrices that can manipulate the expression of enzymes to further promote cellular maturation and integration key therapeutic targets to improve cell therapy efficacy in the central nervous system. Ile-Lys-Val-Ala-Val (IKVAV) and Leu-Arg-Glu (LRE), both originally derived from laminin, have been shown to modulate enzyme activity, while GPQG↓IWGQ is an established enzymatically degradable crosslinker. Using human induced pluripotent stem cell derived neural stem cells, a promising clinically relevant therapeutic cell type, this study examines the effects of peptide signaling and enzymatically degradable crosslinkers on axon extension and enzyme expression. Inclusion of peptides did not significantly alter the material or mechanical properties of the matrix. All matrices had a similar degradation rate in hyaluronidase, but inclusion of GPQG↓IWGQ increased degradation by collagenase. Inclusion of IKVAV, LRE and GPQG↓IWGQ was found to significantly increase axon extension 4 weeks after encapsulation. After 2 weeks of encapsulated culture increases in latent gelatinase and uronic acid, a byproduct of HA degradation, were observed in the IKVAV, LRE and GPQG↓IWGQ compared to other matrix groups. Protease expression was unaffected by the peptide inclusion. Collectively, this work implies that enzymatically degradable crosslinkers play a more active role in modulation cellular behavior through interaction with other signaling pathways than previously thought.

Funding Source: Our research was supported by the Bentson Stroke Center, Department of Neurosurgery and William Stamps Farish Fund.

T-3071

NONINVASIVE APPLICATION OF HESC-DERIVED MSC SPHERES ACCELERATES WOUND HEALING IN WILD-TYPE AND DIABETIC MICE

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It has been reported that mesenchymal stem cells (MSC) derived from adult tissues are effective in promoting wound healing. However, the cell quality varies and cell number is limited as both depend on donations. Moreover, dissociated MSC delivered to an inflammatory lesion are subject to challenges to their survival and functions. Here we demonstrate that dropping of spheres of MSC derived from human embryonic stem cells (EMSC) onto murine dermal wound had much higher survival and efficacy than topical application of dissociated EMSC. Similar efficacy was observed in both wild-type and diabetic mice. RNA sequencing on cells isolated from the wound highlights the CXCL12-CXCR4 signalling in the EMSC sphere-mediated efficacy, which was verified via CXCL12 knockdown in EMSC and CXCR4 inhibition in target cells such as vascular endothelial cells, epithelial keratinocytes, and macrophage. Finally, we enhanced the biosafety of EMSC spheres by engineering the cells with an inducible suicide gene. Together, we propose topical application of EMSC spheres as an unlimited, quality-assured, safety-enhanced, and noninvasive therapy for wound healing and the CXCL12-CXCR4 axis as a key player in the treatment.

Funding Source: University of Macau Research Committee and Macau Science and Technology Development Fund

T-3073

HEPATOBLAST ORGANOIDS HAVE BIPOTENTIAL FATE IN ENGINEERED LIVER TISSUE

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Recent advances in tissue engineering have led to development of artificial human liver tissue containing human hepatocytes, blood vessels, and stromal cells. These tissues can perform key liver functions after implantation in mice, but they lack an organized biliary network – an integral component of liver structure and function. Incorporating hepatocytes and cholangiocytes (the cells that line bile ducts) into engineered liver tissues remains a critical challenge. Towards this goal, we generated hepatoblast organoids from fetal liver tissue and encapsulated these organoids in fibrin hydrogels. We hypothesized that upon implantation in mice with liver injury, endogenous signaling would drive hepatoblasts to differentiate into both hepatocytes and cholangiocytes. After implantation, we found by histology and immunohistochemistry that numerous cell clusters resembled densely packed hepatocytes and stained positively for hepatocyte markers cytokeratin 18, arginase-1, alpha-1-antitrypsin, and albumin. Human albumin was identified in mouse blood serum, confirming successful engraftment and integration with host vasculature, as well as suggesting that organoid tissues are functionally active by synthesizing human protein. In addition to functional hepatocyte clusters, we also identified numerous cholangiocyte-like cells that had self-assembled into bile-like duct structures and stained positively for cholangiocyte marker cytokeratin 19. These results suggest that endogenous signaling upon engraftment can direct immature hepatoblasts within a tissue engineered construct towards both hepatocyte and cholangiocyte phenotypes. This work is an important step toward building clinically relevant human liver tissues and provides a new model to study human liver development. Future work will focus on directing maturation of differentiated cells to generate more complex and fully functional engineered liver tissues.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

T-3075

INCREASING AWARENESS OF THE ISSCR GUIDELINES FOR STEM CELL RESEARCH AND CLINICAL TRANSLATION

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The International Society for Stem Cell Research (ISSCR) published its most updated Guidelines for Stem Cell Research and Clinical Translation (ISSCR guidelines) in May 2016. This study investigated the communication and visibility of these guidelines to clinicians and researchers through the websites of the twenty-one stem cell research programs at Universities and Institutions listed by the National Institutes of Health (NIH), and the top 50 medical institutions for research. In our methods, we performed a search of the websites of the top 50 medical institutions, and the 21 stem cell programs listed by the NIH's, in order to identify if the ISSCR guidelines were cited or linked. Our results showed that twelve out of twenty-one (12/21) NIH-listed stem cell research programs and twenty-six out of the top fifty (26/50) medical schools for research , either directly, through hyperlinks, or internal searches within the respective websites, referred to the ISSCR guidelines. This study demonstrates that the ISSCR1 could improve the visibility of the ISSCR guidelines through the websites of medical and research institutions with stem cell research programs to benefit researchers.

T-3077

PUBLIC SURVEY IN JAPAN ON HUMAN GENOME EDITING FOR RESEARCH PURPOSES

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The world's first genome editing (CRISPR-Cas9) on human embryos for research purposes was conducted in March 2015 in China. This research has sparked heated ethical debate. In response, the International Society for Stem Cell Research (ISSCR) and the National Academy of Science and Medicine (NAS) in the United States have brought attention to the importance of including diverse stakeholders in policymaking on human genome editing. According to new guidelines that will be established soon in Japan, genome editing on human embryos will only be permitted for basic research, and even then, the embryos are restricted to surplus IVF embryos. However, whether these restrictions are in accord with the Japanese public has not been established. More efforts to gather the opinions of the general public on human genome editing for policymaking are required. At present, most surveys have focused on genome editing for clinical purposes, with few exceptions. One is a 2017 survey conducted by Musunuru and colleagues, but in this study the respondents were scientists and medical doctors in the US. Moreover, no survey on human genome editing for research purposes has been done in Japan. Thus, we will conduct an Internet-based questionnaire survey of about 4,000 members of the general public to grasp the acceptance of human genome editing on germ cells, embryos, and somatic cells for research purposes. The survey will present multiple research purposes for the use of each cell type including basic biology, the study of infertility treatments, and the study of treatments of intractable diseases. Through this survey, we will show how the Japanese public view human genome editing that has been approved or will soon be approved in different countries including Japan. We believe that our survey framework will provide valuable information to policymaking around the world.

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CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

T-3081

PARACRINE EFFECT OF SMOOTH MUSCLE PROGENITOR CELLS DERIVED FROM HUMAN-INDUCED PLURIPOTENT STEM CELLS

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Our previous study demonstrated the regenerative effect of smooth muscle progenitor cells (pSMCs) derived from human-induced pluripotent stem cells (hiPSCs) in a rodent model of urethral sphincter muscle injury. Injection of pSMCs into the chronically injured sphincter resulted in increased expression of elastin and collagen in the lower urinary tract. Hence, we hypothesize that pSMCs may improve urethral function through a paracrine effect on the extracellular matrix (ECM). In this study, we sought to examine the proteins secreted by pSMCs in vitro and their effect on cells from the urinary tract. Cells from a hiPSC line were differentiated into pSMCs using our xeno-free, chemically-defined differentiation protocol. The conditioned media was collected from pSMCs throughout the cell expansion process. Bladder smooth muscle cells (bSMCs) and vaginal fibroblasts were cultured from tissues from female donors. To test whether there is a paracrine effect, bSMCs and vaginal fibroblasts were treated with pSMC conditioned media, while cells in the control groups were treated with the baseline smooth muscle growth supplement (SMGS). RNA and protein of the cells were extracted after 24 and 48 hours of treatment. mRNA expression of extracellular matrix proteins (TIMP1, TIMP2, MMP2, Collagen I, Collagen III, Elastin) was examined by PCR. mRNA expression of all six ECM proteins was significantly increased in both cell types treated with the conditioned media compared to the control groups. Proteomic analysis of the conditioned media revealed several candidate proteins involved in ECM metabolism. In summary, these data suggests that pSMCs can mediate ECM metabolism through secretion of proteins involved in ECM deposition and remodeling. These findings support the longstanding hypothesis that urinary incontinence is associated with weakening of the peri-urethral connective tissue. Equally important is that they suggest that these paracrine factors could be used as therapy to restore urethral sphincter function, instead of cell therapies that may contain residual, tumorigenic pluripotent cells.

Funding Source: California Institute for Regenerative Medicine

T-3083

MELATONIN RESTORES THE LPS-INDUCED INHIBITION OF BMP2/4 ACTIVITY IN MOUSE CALVARIAL OSTEOBLASTS DURING OSTEOGENIC DIFFERENTIATION

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Bone morphogenetic protein (BMP) is widely used to treat bone defects but the efficiency decreases in the inflammatory condition. Melatonin has an anti-inflammatory function and enhances bone regeneration. We hypothesized that melatonin perform the synergistic effect with BMP in restoring osteogenic activity at inflammatory condition in bone healing. Osteoblasts were isolated from calvaria of newborn mice, and various inflammation-related signals, including lipopolysaccharide (LPS), pro-inflammatory cytokines, such as TNF-α and IL-1β, hypoxia inducer, such as Dmog, DFO and COCl2, and ROS inducer, such as H2O2, were introduced. The viability of osteoblasts was quantified using an MTT assay, and the osteogenic differentiation was measured by Alizarin Red S staining. Among inflammation-related signals, 24h pre-treatment of LPS significantly decreased the osteogenic differentiation potential of osteoblasts without affecting cellular viability. In non-inflammatory condition, BMP2/4 effectively enhanced the osteogenic differentiation of osteoblasts in a dose-dependent manner. Melatonin alone did not show a significant effect at low dose treatment, but the high dose treatment of melatonin (500 uM) significantly increased the osteogenic differentiation. Co-treatment of BMP2 and melatonin showed the additive effect in promoting osteogenic differentiation. Co-treatment of BMP4 and melatonin showed the biphasic response with the additive effect at the mid-dose of BMP4 and the negative effect at the high-dose of BMP4. Pre-treatment of LPS for 24 h on osteoblasts significantly inhibited the effect of BMP2/4 on osteogenic differentiation. However, co-treatment of high-dose melatonin (200 uM, 500 uM) meaningfully restored the activity of BMP2/4 in osteogenic differentiation of LPS-pre-treated cavarial

osteoblasts. These results suggest that LPS-induced inhibition of BMP2/4 activity can be restored by high-dose treatment of melatonin, in which melatonin shows the synergistic interaction with BMP2/4. The noble findings of the current study may lead to the development of new medical protocols in treating patients suffering from craniofacial bone loss with inflammatory condition.

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T-3085

NEURAL AND CHONDROITINASE ENCAPSULATED MICRORIBBONS FOR RESTORING SPINAL CORD INJURY CIRCUITRY

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Human neuronal and glial cells derived from human pluripotent stem cells have potential to restore damaged circuitry of the injured spinal cord. However, there is a need for new cell replacement strategies to improve cell survival and integration with the host tissue while guaranteeing safety, efficacy, and reproducibility. Our goal is to generate fully characterized cells for transplantation into the damaged spinal cord, and to optimize cell delivery methods taking into account the SCI microenvironment and need for removal of inhibitory signals. We use published induced pluripotent stem cells (hiPSCs) and isolate MUSE (multilineage differentiating stress enduring) cells that are recalcitrant to tumor formation. MUSE cell multipotency is validated by differentiation into multiple germ layers and by NSC generation and expression of Sox2, Nestin and MAP2 biomarkers. Spinal cord region identity NSCs (scNSCs) from our hiPSC or MUSE derived Sox2+/Bra+ neuromesodermal progenitors have characteristic hallmarks of multipotency and neurosphere formation. The spinal motor neurons (HB9+, Isl1/2+, ChAT+, NF-H+) generated from the scNSCs are capable in vitro of forming synaptic networks, innervating rodent myotubes, and exhibit active mitochondrial transport in neurites. We encapsulate scNSCs in alginate microribbons, or brachial spinal motor neurons and oligodendrocyte progenitor cells (A2B5+, Olig2+, O4+, Sox10+) that interact with motor neurons, with and without chondroitinase (chABC). The synthesized chABC is used to provide enzymatic attenuation of the inhibitory chondroitin sulfate proteoglycan injury (CSPG) microenvironment in vivo and we have quantified its extended release from microribbons in vitro by CSPG glial scar models. Encapsulated scNSCs retain robust viability and differentiation potential upon recovery as assessed by neurogenesis and maturation (synaptogenesis) even following long-term shipping at 37°C (From Albany, NY to Houston TX). Surgical implantation of microribbons is done by syringe injection. In summary we demonstrate that alginate microribbons offer a tunable and reproducible platform for delivery of therapeutic neural cells, retention of cell viability, injury site modification and capability for circuitry reformation.

Funding Source: NY State Spinal Cord Injury Review Board (NYSCIRB) funded project: “Healing the Contusion Injured Spinal Cord Microenvironment with Nanotechnology and Stem Cells”.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-3087

UNRAVELLING THE CELLULAR AND TRANSCRIPTIONAL MECHANISMS UNDERLYING THE ESTABLISHMENT OF EXPANDED POTENTIAL STEM CELLS

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Totipotency, the unbiased ability of a cell to differentiate into any cell type of the body, including extraembryonic cell types, is one of the fundamental properties characterizing cells of the pre-implantation embryo. However, totipotency is rapidly lost upon development, when cell fate decisions segregate the trophectoderm from the inner cell mass, then the epiblast from the primitive endoderm, ultimately preventing switching from one cell lineage to another. Recent studies have reported the derivation of expanded potential stem cells (EPSCs), which can give rise to both embryonic and extraembryonic lineages. Despite these advances, whether the totipotent state can be induced in vitro is still an open question. Here, we combine differentiation experiments and transcriptional profiling at a population level to investigate the ability of EPSCs to activate extraembryonic genes and the transcriptional dynamics accompanying the conversion of embryonic stem cells (ESCs) into EPSCs. We found that the transcriptional state of EPSCs is rapidly induced during the conversion of ESCs into EPSCs, and involves changes in the expression of developmental, cell adhesion and cell migration genes, among others. Evidence will be presented that EPSCs transcriptionally differ from totipotent cells of the pre-implantation embryo and maintain silencing of 4-16 cell stage embryo marker genes. Furthermore, when exposing EPSCs to trophectoderm differentiation conditions, silencing of trophectoderm genes is maintained, suggesting that EPSCs are resistant to differentiation toward the extraembryonic lineage. In addition, transcriptional analyses uncovered changes in the expression of genes involved in cell adhesion. These results raise the possibility that EPSC culture conditions could induce a switch in cell phenotype, enabling cells to localize to extraembryonic sites when aggregated in chimeric embryos. However, we found that such mislocalized cells do not express appropriate extraembryonic markers and likely do not functionally contribute to extra embryonic tissues. Understanding the cellular mechanisms regulating the establishment and maintenance of specific stem cell states and the epigenetic basis of reprogramming will have major implications for the development of regenerative medicine approaches.

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T-3089

DEVELOPMENT OF AN EXTENDED CULTURE SYSTEM THAT SUPPORTS SELF-RENEWAL OF HUMAN PRIMORDIAL GERM CELL-LIKE CELLS

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Primordial germ cells (PGCs) are the early progenitors of what will become mature mammalian gametes. Appropriate specification and differentiation of PGCs is critical for reproductive health, as germ cells are the only cells capable of passing on genetic and epigenetic information from one generation to the next. Given this critical role, a model system for differentiating and expanding hPGCs in vitro is required. Recent work has shown that human pluripotent stem cells can differentiate into human PGC-like cells (hPGCLCs) in vitro, and this has allowed for the investigation of mechanisms required for hPGC specification. However, an approach for promoting hPGCLC self-renewal without reversion to a pluripotent stem cell state has been elusive. Here, we developed a culture system using three different human pluripotent stem cell lines and the differentiation of hPGCLCs that captures the self-renewing properties of hPGCLCs for a minimum of three additional weeks in culture. During this time, we show that the cultured hPGCLCs undergo global epigenetic reprogramming without evidence of reversion to the pluripotent state. This culture system provides a critical new approach for expanding the number of hPGCLCs for downstream technologies, including transplantation and screening or possibly the differentiation of hPGCLCs into gametes by in vitro gametogenesis.

T-3091

UNIQUE EPIGENETIC PROGRAMMING DISTINGUISHES FUNCTIONAL SPERMATOGONIAL STEM CELLS IN THE IMMATURE MOUSE TESTIS

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In the mammalian testis, spermatogonial stem cells (SSCs) sustain steady-state spermatogenesis leading to the production of ~100 million sperm per day by an adult man. SSCs self-renew and also give rise to progenitors that enter the spermatogenic differentiation pathway. The extent to which SSCs and progenitors represent distinct spermatogonial subtypes, and whether, in addition to SSCs giving rise to progenitors, progenitors may revert back to SSCs, are unresolved questions. The Id4-egfp transgenic mouse model facilitates FACS-based isolation of SSC-enriched/progenitor-depleted and SSC-depleted/progenitor-enriched spermatogonial subpopulations,

functionally validated by the capacity to seed spermatogenesis following transplantation to a recipient testis. We examined genome-wide patterns of a) gene expression by RNA-seq, b) six histone modifications – H3K4me1-3, H3K9me1, H3K27me3 and H3K27ac – by ChIP-seq, c) chromatin accessibility by ATAC-seq, and d) DNA methylation by MeDIP-seq in SSC-enriched and progenitor-enriched subpopulations from the immature mouse testis. We found consistent differences in epigenetic landscapes associated with consistent differences in gene expression between the two spermatogonial subtypes. Differential enrichment of H3K27me3 or H3K27ac at promoters, and differential enrichment of H3K4me1 or H3K27ac at enhancers of differentially expressed genes, as well as sites of differential methylation (DMRs) in intergenic regions, appear to be the most consistent epigenomic distinctions associated with genes differentially expressed in SSCs and progenitors. Our results suggest that regulated changes in epigenetic landscapes promote the transition from SSCs to progenitors. Motif enrichment analysis of differentially programmed promoter and enhancer regions revealed binding sites for candidates for upstream regulators of spermatogonial subtype-specific epigenetic programming. Our own and previously reported ChIP experiments confirmed that FOXP1, DMRT1, and DMRTB1, are differentially bound at differentially programmed enhancers. Thus, SSCs and progenitors are distinguished by 1) differential developmental potential, 2) differential gene expression, 3) differential epigenetic programming, and 4) differential binding of master regulators.

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T-3093

SPATIO-TEMPORAL ANALYSIS OF HUMAN PREIMPLANTATION DEVELOPMENT REVEALS DYNAMICS OF EPIBLAST AND TROPHECTODERM

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Recent technological advances such as single-cell RNAseq1-3 and CRISPR-CAS9-mediated knock-out4 have allowed an unprecedented access into processes orchestrating human preimplantation development5. However, the sequence of events which occur during human preimplantation development are still unknown. In particular, timing of first human lineage specification, the process by which the morula cells acquire a specific fate, remains elusive. Here, we present a human preimplantation development model based on transcriptomic pseudotime modelling of scRNAseq biologically validated by spatial information and precise time-lapse staging. In contrast to mouse, we show that trophectoderm (TE) / inner cell mass (ICM) lineage specification in human is only detectable at the transcriptomic level at the blastocyst stage, just prior to expansion. We validated the expression profile of novel markers enabling precise staging of human preimplantation embryos, such as IFI16 which highlights establishment of epiblast (EPI) and NR2F2 which appears at the transition from specified to mature TE. Strikingly, mature TE cells arise from the polar side, just after specification, supporting a model of polar TE cells driving TE maturation. Altogether, our study unravels the first lineage specification event in the human embryo and provides a browsable resource for mapping spatio-temporal events underlying human lineage specification.

CHROMATIN AND EPIGENETICS

T-3095

PLURIPOTENT STEM CELLS MAINTAIN A REDUCED CENTROMERE AND KINETOCHORE SIZE: IMPLICATIONS FOR CHROMOSOME SEGREGATION FIDELITY

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Maintaining a stable karyotype is essential for the use of pluripotent stem cells (PSCs) in regenerative medicine and translational and basic research. Although around 10-30% of PSC lines present karyotypic abnormalities, the molecular mechanisms underlying this genomic instability are largely unknown. Centromeres, the chromosomal loci that drive chromosome segregation are central to mitotic fidelity. Maintenance of centromeres in somatic cells is tightly cell cycle coupled, as centromeric chromatin assembly is strictly dependent on transition into G1 phase. PSCs have an atypical cell cycle structure with a truncated G1 and proliferate at unusually rapid rates. How this affects mitotic fidelity in general, and centromere assembly in particular are essential questions in reprogramming biology. We now show that although the total pool of CENP-A is higher in PSCs, when compared to differentiated cells, this H3 histone variant is depleted at the centromere. This may indicate that regulation of CENP-A levels is altered in PSCs, possibly due to the shortened G1 phase. Moreover, our preliminary results suggest that loss of CENP-A at the centromere is an early event during the reprogramming of somatic cells to PSCs. Characterisation of key centromeric and kinetochore proteins in PSCs indicate that these cells have weaker centromeres and kinetochores than differentiated cells. We are currently attempting to strengthen both centromere and kinetochore function, by modulating the levels of key proteins in PSCs. This will also allow us to assess if the weakened centromere/kinetochore underlies the increased mis-segregation observed in these cells. This in depth characterisation of centromere and kinetochore structure in PSCs will contribute to determining the mechanisms regulating proper chromosome segregation in PSCs and during somatic cell reprogramming.

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T-3097

CHROMATIN CONFORMATION INDUCES DNA METHYLATION CHANGES DURING CULTURE EXPANSION OF MESENCHYMAL STROMAL CELLS

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Replicative senescence impacts on the functional characteristics of mesenchymal stromal cells (MSCs) and is important for quality control of therapeutic cell preparations. This process of cellular ageing is associated with highly reproducible DNA methylation (DNAm) changes at specific sites in the genome, albeit it is largely unclear how these epigenetic modifications are controlled. In this study, we identified CG dinucleotides (CpGs) that become continuously hyper- or hypo-methylated during primary culture expansion and provide a biomarker to estimate the number of passages. Upon reprogramming into induced pluripotent stem cells (iPSCs), senescence-associated DNAm is reversed with similar kinetics as pluripotency-associated DNAm changes, indicating that epigenetic rejuvenation might be directly associated with the pluripotent state. Bisulfite barcoded amplicon sequencing (BBA-seq) demonstrated that DNAm patterns of neighboring CpGs become more complex during culture expansion of MSCs without evidence of continuous pattern development. We used multicolor lentiviral barcode labeling to demonstrate oligoclonality at later passages, but this was apparently not related to clone-specific epigenetic patterns at the senescence-associated regions, which rather exhibited stochastic fluctuations of DNAm. BBA-seq with hairpin-linked DNA molecules demonstrated that many senescence-associated CpG dyads are only methylated on either the forward or the reverse strand. This hemimethylation was conserved over many passages, indicating that it was not due to insufficient maintenance of DNAm upon cell division. Circularized chromatin conformation capture (4C) of senescence-associated CpGs revealed highly reproducible interaction changes, enrichment in CTCF binding sites, and association with lamina-associated domains. Taken together, our results suggest a model where senescence-associated DNAm is not regulated in a targeted manner but is rather caused by higher order chromatin conformation states.

T-3099

CHD7 REGULATES THE CELLULAR IDENTITY OF HUMAN NEURAL PROGENITORS

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It is still elusive how the mutation of a single genetic factor leads to multiple congenital disorders. In this study, by using human neuroepithelial (NE) cells and CHARGE patient-derived cells as in vitro models, we identified a critical role of CHD7 in neuroepithelial-neural crest bifurcation, providing a missing link between central nervous system (CNS) and craniofacial anomalies observed in CHARGE syndrome. We found that CHD7 is required for the epigenetic activation of super-enhancers and CNS-specific enhancers, which underlies the maintenance NE and CNS lineage identities. We further found that BRN2 and SOX21 are downstream effectors of CHD7, which shapes cellular identities by enhancing CNS-specific cellular program while repressing non-CNS-specific cellular programs. Our results identified CHD7 as a regulatory hub by interaction with super-enhancer elements to orchestrate the spatiotemporal dynamics of CNS-specific transcription factors, thereby regulating NE and CNS lineage identities.

T-3101

A DISTINCT CHROMATIN AND EPIGENETIC SIGNATURE DEFINES TANKYRASE INHIBITOR-REGULATED HUMAN NAIVE PLURIPOTENCY

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The derivation of a naïve human pluripotent stem cell (N-hPSC) state allows for the study of epigenetic phenomena such as epigenomic imprinting, X-inactivation, and euchromatin/heterochromatin maintenance within a normal developmental context. Because many of these pathways are aberrantly mimicked in pathological settings, the derivation of a non-aberrant model is key to advancing our understanding of epigenetic regulation. In addition, the human naïve pluripotent state may allow for increased differentiation potential paralleling differences found between mouse embryonic stem cells and mouse epiblast stem cells. In 2016, our group established that a naïve-like state can be recapitulated in conventional hPSC through combined small molecule inhibition of MEK/ERK, WNT (2i), and TANKYRASE ½ (XAV939); along with recombinant human LIF (i.e., LIF-3i). Conventional, primed hPSC cultured in LIF-3i displayed global and allele-specific hypomethylation of CpG and non-CpG islands without compromise of normal patterns of CpG methylation on imprinted loci, increased expression of naïve transcripts (e.g., Dnmt3L), and the use of the distal Oct4 enhancer. We now further report on the chromatin landscape of our LIF3i-reverted N-hPSC state. LIF-3i-cultured N-hPSCs displayed decreased levels of H3K27me3 on bivalent promoters, yet increased abundance of total H3K27me3. LIF3i N-hPSCs also decreased levels of the heterochromatin marks H3K9me3 and H3K9me2; as well as increased levels of the poised transcription mark H3K79me2. N-hPSCs maintained similar levels of core PRC2 components, though demonstrated substantially depleted levels of JARID2. Additionally, canonical PRC1 core proteins CBX2,6, and 7 were decreased in N-hPSCs while exhibiting increased levels of BMI1 and RYBP. Finally, unlike other reports of naïve states reliant on the MEK/ERK inhibitor PD0325901, DNMT1 was insignificantly modulated in LIF-3i N-hPSCs at high passages. Protein levels of class I and II HDACs were also reduced during LIF-3i naïve reversion. These findings support the conclusion that LIF3i N-hPSCs undergo global chromatin remodeling and a shift in chromatin maintenance regulation that resembles the chromatin landscape of the naive mouse pluripotent state.

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T-3103

TARGETED GENE ACTIVATION DIRECTS TROPHOBLAST TRANS-DIFFERENTIATION USING THE NOVEL DESIGNED EPIGENETIC INHIBITOR, EEDBINDER-DCAS9

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Bifurcations in cell fates are controlled through epigenetic modifications, however the key loci regulated by PRC2 dependent H3K27me3 repressive marks are not known. To dissect the functional loci regulated by PRC2 in trophoblast to ICM bifurcation we fused a computationally designed protein, EED binder (EB) that tightly binds EED and disrupts PRC2 function, to dCas9 to direct PRC2 inhibition at precise loci using gRNA. Free of DNA manipulations or chemical inducers, EBdCas9 is able to transdifferentiate human induced pluripotent stem cells (iPSC) to human trophoblast fate using gRNA specific to two key transcription factors CDX2 and GATA3. Co-transfection of gRNA targeting GATA3 and CDX2 resulted in 40-80 fold increase of these transcripts, as well as the trophoblast markers. ChIPseq analysis showed H3K27me3 reduction in GATA3 TSS and gene body compared to untransfected samples. RNAseq analysis revealed that our EBdCas9 gRNA transfected, but not control hPSC produced gene expression signature that corresponds to human trophoectoderm and alignment against early cynomolgus monkey single cell transcriptome showed enhanced advancement towards trophectoderm. Epigenetic memory tracing of the newly trans-differentiated trophoectoderm cells confirms a transcript upregulation threshold that is maintained for at least 21 days following initial gRNA transfection. These data reveal for the first time that the first human fate bifurcation between trophoblast and ICM is solely controlled by PRC2 dependent epigenetic H3K27me3 marks in precise loci upstream of Gata3 and CDX2 TSSs. We also tested the other general applicability of EBdCas9, by using tiling to identify the regions where gRNAs induce transcription in the following bivalent genes: TBX18, p16, and Klf4. In total, we have targeted 40 sites upstream of five different genes, and observed significant transcriptional derepression in all genes, all together in 17 loci. EBdCas9 tool is broadly applicable to questions in epigenetic regulation of single locus to pinpoint critical marks for control of gene expression by PRC2.

PLURIPOTENCY

T-3105

PUMA REGULATES GENOMIC STABILITY OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (PSCs), mainly including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), hold great promise in regenerative medicine. However, genomic instability of PSCs owing to both endogenous replication stress and exogenous genotoxic agents impedes their safer applications in the clinic. P53 plays an important role in maintaining genomic integrity. Previous studies from our and other laboratories showed that deletion of PUMA, a pro-apoptotic mediator of p53, leads to better survival of multiple mouse stem cell systems under conditions of ionizing radiation or during iPSC reprogramming, but without increased genomic instability after the DNA damage. Here, we attempt to define the role of PUMA on the genomic stability of human PSCs. PUMA expression of human PSCs significantly increased under stress conditions such as differentiation to three germ-layers in vitro as well as ionizing radiation. However, there was no decrease in chromosomal stability and lineage-commitment in vivo when PUMA was deleted in human PSCs via CRISPR-Cas9. PUMA deletion gave rise to better survival and pluripotent state of PSCs after irradiation-induced DNA damage. Following irradiation, cell cycle was arrested in the G2 phase in the absence of PUMA and recovered faster than the wild type control. In addition, higher homologous recombination repair was also observed in PUMA deficient PSCs. Interestingly, inhibition of PUMA significantly reduced tumor formation potential in vivo. Finally, better survival of human PSCs without compromising genomic stability could be also achieved when PUMA inhibitor was applied in cell culture. Taken together, we demonstrate a novel role of PUMA in genomic integrity of human PSCs and thus, provides a new strategy to enhance the safety of human PSCs in regenerative medicine.

T-3107

IDENTIFICATION OF KAP1 AS A LIN28 REGULATING PROTEIN IN MOUSE EMBRYONIC STEM CELLS

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Lin28 has been implicated in mammalian development and maintenance of the pluripotency of embryonic stem cells (ESCs). Post-translational modification (PTM) of proteins plays critical roles in various biological processes, including proliferation and differentiation. However, the role of PTM in the regulation of expression and function of Lin28 in ESCs is poorly understood. Using affinity purification and mass spectrometry, in this study, I identified KAP1 (KRAB-associated protein 1) as a novel Lin28 binding protein. KAP1 specifically interacted with Lin28 in ESCs and the interaction was mediated through coiled-coil domain of KAP1. Induced overexpression of KAP1 in ESCs stimulated self-renewal and suppressed differentiation of ESCs. KAP1 overexpression led to increased protein level, but not mRNA level, of Lin28 protein, suggesting an involvement of PTM in the regulation of Lin28 expression. KAP1 overexpression significantly abolished Lin28 ubiquitination in ESCs. In contrast, short interfering RNA mediated knockdown of KAP1 promotes Lin28 ubiquitination, leading to proteasomal degradation of Lin28 protein in NIH3T3 cells. KAP1 overexpression interfered ubiquitination of Lin28 mediated by Trim71, an E3 ubiquitin ligase for Lin28. These results suggest that KAP1 plays a key role in the regulation of stability of Lin28 in ESCs by modulating Trim71-meidated ubiquitination and subsequent degradation of Lin28 protein.

T-3109

TRANSCRIPTIONAL ANALYSIS OF PLURIPOTENCY IN NORTHERN WHITE RHINOCEROS STEM CELLS: FIRST STEP TO REWIND EXTINCTION

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Earth is currently facing the sixth mass extinction, predicted to be the most devastating that we have ever experienced. In many cases, standard conservation approaches are not enough to save critically endangered species. However, new hope may come with the advancement of stem cell-based methodologies. We have pioneered the development of induced pluripotent stem cells (iPSCs) from the functionally extinct northern white rhinoceros (NWR). These cells will be utilized for gamete generation and assisted reproduction with the goal of restoring population viability, genetic diversity, and avoiding extinction. We have conducted transcriptomic analysis on different NWR fibroblasts and iPSCs lines to identify key pluripotency traits in this species. Principal component analysis and hierarchical clustering of differentially expressed transcripts showed distinct separation of the fibroblast and iPSCs compartments. Analysis of the enriched upstream regulators identified MYC, CDH1 and TFAP2C among the most overrepresented in the NWR iPSC populations. Molecular and cellular functions analysis revealed that iPSCs exhibit an overrepresentation of cellular movement, assembly and organization as well as maintenance functions. In accord with the RNA-Seq data, POU5F1, SOX2, NANOG, LIN28 and DNMT3b, key pluripotency markers in other species, including humans, were top differentially genes expressed in rhino iPSCs, and were further validated by immunocytochemistry. Additionally, NWR iPSCs correspond to a post-implantation state in development and resemble the primed state of human iPSCs. These iPSCs lines are being utilized for the generation of the primordial germ cells that hold the potential for in vitro germ cell maturation, fertilization and embryo transfer to surrogates, generating a self-sustaining population of NWR.

T-3111

GENERATION OF PLURIPOTENT FERRET IPSC-LIKE CELLS

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Ferrets (Mustela putorius furo) are an attractive mammalian model for lung disease. They share similar physiology and also emulate many clinical features associated with human lung diseases; coupled with their relatively small size this makes them an indispensable model system. Given the advantages of utilizing the ferret to model lung diseases, such as cystic fibrosis, it has the potential to be a valuable pre-clinical model for lung based regenerative cell therapy. To achieve this, we need to be able to generate ferret iPSC with the capacity for differentiation into airway stem/progenitor cells and to repair an injured epithelium. We reprogrammed ferret fetal fibroblasts using the Epi5 episomal reprogramming kit, evaluated the conditions necessary to derive and maintain iPSC, and assessed their pluripotency through gene expression, immunofluorescence, directed differentiation and teratoma formation. Under conditions where FGF2 signaling maintains pluripotency, similar to those that successfully maintain epiblast state of human iPSC, we were able to generate and expand ferret iPSC-like clonal lines. These iPSC-like cells express pluripotency markers, including Oct4, Sox2, Nanog, and Lin28A, at both the RNA and protein level. Additionally, they are capable of serum stimulated spontaneous differentiation to all three germ layers through the formation of Embryoid bodies. Teratomas containing evidence of cells from all germ layers formed after subcutaneous injection into immunocompromised mice. Using more defined directed differentiation protocols we have been able to generate smooth muscle α-actin and brachyury expressing smooth muscle-like cells of the mesoderm and PAX6 and TUJ1 expressing neural progenitor cells of the ectoderm. Of relevance to lung regeneration, we have been able to optimize endoderm differentiation to generate >60% FOXA2 and SOX17 expressing definitive endoderm cells in three independent iPSC lines. In conclusion, we have been able to successfully create an iPSC-like state in ferret cells that has the capacity for self-renewal and multi-lineage differentiation. We are able to generate early lung progenitor cells that can be pursued for evaluating engraftment into, and repair of, an injured lung epithelium.

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T-3113

FATTY ACID OXIDATION PROMOTES SELF-RENEWAL OF GROUND-STATE EMBRYONIC STEM CELLS

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Ground-state pluripotency is stabilized by inhibition of mitogen-activated protein kinase-extracellular-signal-regulated kinase (MAPK/ERK) signaling and glycogen synthase kinase-3 (GSK3), the so-called 2i condition, yet deciphering how an exquisite balance between energetic and biosynthetic demands orchestrates propagation of ground-state embryonic stem cells (ESCs) remains incompletely understood. Using RNA-sequencing and triple quadrupole metabolomics to profile metabolic pathways in conventional (serum/leukemia inhibitory factor (LIF)) and ground-state (2i/L) ESCs, we found that 2i/L ESCs exhibited a drastically higher activity of the FAO pathway than that of serum/LIF ESCs at transcriptional and metabolite levels, indicating that 2i/L ESCs harbor the capability to oxidize fatty acids more efficiently than serum/LIF ESCs. Interestingly, pharmacological inhibition of FAO pathway had a very specific effect on 2i/L ESCs, which formed smaller colony sizes, exhibited halted proliferation and reduced RNA synthesis. Nonetheless, the cells maintained expression of pluripotency markers. Our data demonstrated that FAO is required for 2i/L ESC proliferation, but dispensable for maintenance of ground-state pluripotency network.

T-3115

PARP1 INTERACTS WITH ZSCAN4 AND PARTICIPATES IN TELOMERE REGULATION

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Zinc finger and SCAN domain containing 4 (Zscan4) is a gene that is expressed exclusive in 2- cell stage embryos and sporadically in a subpopulation of embryonic stem cells (ESCs). Zscan4 participates in the regulation of telomere homeostasis and genomic stability. Poly [ADP-ribose] polymerase 1 (PARP1) is known to sense DNA damage and activate several DNA repair pathways. In the present work, we worked to investigate if PARP1 interacts with Zscan4 and participates in telomere regulation. In HEK293T cells, ZSCAN4 and PARP1 are mainly localized in the nucleoplasm and nucleolus, respectively. Immunoprecipitation experiments show that ZSCAN4 directly interacts with PARP1 when Zscan4 and parp1 are ectopically expressed in HEK293T cells. Next, we generated stable Zscan4 over-expressed (Z4-OE) NIH/3T3 cell and ESC lines. Comparing to the control, the Z4-OE ESCs show slower proliferation rate (doubling time of 27.0 ± 1.6 h vs. control 15.4 ± 0.5 h, $P<0.05$), but 1.76-fold longer telomere length, as measured by the relative telomere to single copy gene (T/S) ratio. Similarly, slower proliferation rate was also observed in Z4-OE NIH/3T3 cells (doubling time 52.3 ± 10.7 h vs. control 31.1 ± 3.9 h). Inhibition of PARP1 activity by treating 3-aminobenzamide (3-AB) led to shortened telomeres in Z4-OE ESCs (75.3% that of the control). The present work demonstrates that PARP1 interacts with Zscan4 and participates in telomere regulation, and provides novel insights on the molecular interaction between DNA damage and telomere regulation.

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PLURIPOTENT STEM CELL DIFFERENTIATION

T-3119

THE INVOLVEMENT OF ERK1/2 SIGNALING IN DEFINITIVE ENDODERM FORMATION

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Directed differentiation of human pluripotent stem cells (hPSCs) has provided exceptional potential in cell/organ replacement therapy. The definitive endoderm (DE) germ layer gives rise to key organs such as the liver and pancreas. The ability to efficiently derive SOX17+ DE in vitro involves fine tuning of signaling pathways using growth factors and small molecules. Studies from past decades have shown that Activin/Nodal signaling is undisputedly the main driver in deriving DE from hPSCs. In our study, we demonstrate that the FGF2-FGFR2-ERK1/2 signaling is indispensable for SOX17+ DE formation. The inhibition of MAPK/ERK1/2 signaling is found to ablate SOX17 expression suggesting its importance could have surpassed Activin/Nodal in deriving SOX17+ DE and hence challenging the long-standing central dogma. We have also uncover a unique interplay between Activin/Nodal-SMAD2 and FGF2-FGFR2-ERK1/2 signaling whereby the forma is involved in the early initiation of DE specification but is thereafter suppressed by FGF2-FGFR2-ERK1/2 signaling to facilitate the subsequent generation of SOX17+ DE cells. Here, we propose a two-step signaling event involving Activin/Nodal and MAPK/ERK1/2 signaling in DE formation.

T-3121

CO-CULTURE WITH HUMAN IPSC-DERIVED LUNG PROGENITORS INDUCES A TIME-DEPENDENT MODULATION OF ENDOTHELIAL CELL BEHAVIOUR TO RECAPITULATE DEVELOPMENT OF THE AIR-BLOOD BARRIER

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Bioartificial lung scaffolds recellularized with autologous induced pluripotent stem cells (iPSCs) derivatives may bypass immunogenicity leading to better tolerance after implantation of these constructs for treatment of end-stage lung diseases. In vitro maturation of iPSC-derived epithelial cells remains a major challenge for the recreation of an efficient air-blood barrier (ABB), which is critical for lung function. We hypothesized that interactions between lung progenitors (LPs) and endothelial cells (ECs) can recapitulate the formation of an ABB. Human iPSCs were differentiated into CD34+PECAM-1+VEGFR2+ iECs and NKX2.1+Sox2+Sox9+ LPs, verified by flow cytometry and immunocytochemistry. Both iECs and LPs proliferated (Ki67+) post-seeding into perfused acellular rat lung matrices, and exhibited site-specific engraftment: iECs homed to vascular tree and capillary beds, whereas LPs to denuded airways.

iECs were directly co-cultured in 2-D assays with LPs for 24-72hrs, prior to CD144 immunomagnetic separation; in parallel, iEC monoculture acted as a control. EC-associated gene and protein changes were quantified by Q-PCR and Western blots, respectively. At 24hrs of co-culture, the angiopoietin-1 (ANG1) and ANG2 ratio (ANG1:ANG2) in CD144+iECs was significantly decreased by <50%, implying vessel destabilization. This was accompanied by markedly reduced Mst1 kinase expression coupled with YAP1 activation - a key mediator of Hippo signaling involved in angiogenesis. At 48hrs, the expression of mature EC markers, VEGFR2 and CD105, were increased by ≥2.5-fold relative to control. At 72hrs, the ANG1:ANG2 was reversed (≥3.2-fold increase), with augmented expressions of PECAM and ICAM (≥2.7-fold), vascular tight junction proteins involved in ABB permeability, compared to earlier time-points or control. Collectively, our results provide insight into how LPs regulate EC behavior in a time-dependent fashion: initially, inducing vessel plasticity and angiogenesis; subsequently, enhancing EC maturation and endothelial barrier integrity. Thus, harnessing this dynamic interplay between LPs and ECs could lead to more complete alveolar re-endothelialization and improve the robustness of ABB in bioengineered lungs scaffolds, thereby producing greater functional lung grafts.

T-3123

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO NEURAL CREST CELLS

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Neural crest cells (NCCs) are multipotent stem cells that arise during vertebrate embryonic development. NCCs are formed at the neural plate border, then delaminate from the neural tube, migrate to various locations, and give rise to a wide array of derivatives including the craniofacial skeleton, peripheral and enteric nervous systems, pigment cells, as well as many other cell types and organs. Neural crest cell dysfunction can result in birth defects, for example, cleft/lip palate and Hirschsprung's Disease; furthermore, neuroblastomas and melanoma are cancers that originate from neural crest lineages. Using NCCs derived from human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells (ESCs and iPSCs) to model the NCC development and diseases is valuable because obtaining these tissues is difficult. Here we describe the STEMdiff™ Neural Crest Differentiation Medium and protocol, which promote efficient and reproducible differentiation of hPSCS to NCCs. Briefly, undifferentiated hPSCs maintained in either mTeSR™1 or TeSR-E8™ were dissociated and plated at 2 x 105 cells/cm2 on Corning® Matrigel®-coated plates in complete STEMdiff™ Neural Crest Differentiation Medium containing 10 µM Rho-Kinase inhibitor (ROCKi) for one day, followed by daily full medium changes (without ROCKi). After 6 days, differentiation was assessed by immunostaining for neural crest markers (SOX10 and CD271) and quantified using the ImageXpress® Micro 4 High-Content Imaging System. STEMdiff™ Neural Crest Differentiation Medium consistently converts hESC and hiPSC lines maintained in both mTeSR™1 (6 lines) and TeSR™-E8™ (3 lines) into SOX10+CD271+ positive NCCs (85.5 ± 1.6%; mean ± SEM; n=33; 9 different hPSC lines) with only low levels of CNS-type PAX6+ neural progenitor cells (5.6 ± 0.7%; mean ± SEM; n=33; 9 different hPSC lines). The hPSC-derived neural crest cells are multipotent and able to differentiate into downstream derivatives such as chondrocytes, osteoblasts, and peripheral neurons. In summary, the STEMdiff™ Neural Crest Differentiation Kit supports efficient generation of multipotent NCCs and is reproducible across multiple hPSC lines.

T-3125

ROLE OF GLIS3 IN THE GENERATION OF PANCREATIC BETA CELLS FROM HUMAN EMBRYONIC STEM CELLS

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Type 2 diabetes is a major health concern worldwide affecting 1 in 10 Americans while an estimated 80 million have prediabetes. Study of the Krüppel-like zinc finger transcription factor Gli-similar 3 (Glis3) in humans and knockout mice indicated that Glis3 plays a critical role in pancreatic development and in the transcriptional regulation of insulin gene expression and is implicated in both type 1 and type 2 diabetes. Recent progress with developing experimental strategies to induce differentiation of embryonic stem cells into insulin secreting b cells have been encouraging for the potential of stem cell therapy for diabetes. We therefore investigated the role of Glis3 during the differentiation of embryonic stem cells into pancreatic b cells. We developed a protocol for stepwise differentiation of human/mouse embryonic stem cells into pancreatic endocrine cells and monitored the expression of specific markers for each step of the differentiation process by real-time PCR and immunohistochemistry. In these cell models, Glis3 mRNA expression was significantly induced at the pancreatic progenitor cell stage of the differentiation process, a stage at which Ngn3, a marker of pancreatic stem cells, is also

induced and a time critical for the development of pancreatic endocrine precursors. To be able to examine the effect of Glis3 expression at different stages of differentiation, we generated a stable Glis3-inducible hES cell line that allows induction of Glis3 by doxycycline at different stages of differentiation. Induction of Glis3 in ES cells significantly increased the expression of insulin gene compared with control. To determine the effect of Glis3 deletion on differentiation, we are developing human ES cells in which Glis3 is knocked out or Knocked in by the CRISPR method. Our study indicates that Glis3 plays an important role in the generation of pancreatic ß cells and functions at an early stage of pancreatic development when pancreatic progenitors arise and differentiate into endocrine precursors. Given the critical role Glis3 plays in ß cell generation and insulin regulation, Glis3 might provide a novel therapeutic target in the management of diabetes.

T-3127

DIFFERENTIAL REGULATION OF TERRA IN THE DEVELOPMENTAL PROCESS OF PLURIPOTENT STEM CELLS INTO NEURAL LINEAGES

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Human pluripotent stem cells (hPSCs) are one of the most versatile and beneficial cell sources for translational utility as their ability to provide all cell lineages. Their unlimited self-renewal and differentiation potential is tightly regulated by genetic and epigenetic modulators. The sufficient telomere length is critical for chromosomal stability and subsequent self-renewal of hPSCs and telomeric repeat containing RNA (TERRA), a non-coding RNA encoded in sub-telomeric region, is implicated in telomere homeostasis and rejuvenation in PSCs. However, the dynamics of TERRA expression has not been fully understood in induced PSCs and their specific derivatives. Here, we demonstrate that TERRA expression is highly up-regulated in the reprogrammed PSCs. Somatic sources, used for reprogramming, exhibited minor effects on TERRA expression and the level of TERRA is not significantly varied in the clones derived from same somatic source. The chromosome end-specific RT-qPCR technique allow reliable quantification of TERRA expression but the results from conventional primers implicate that upgraded techniques are still required for some chromosome regions. Correlation with high telomerase activity and consistent TERRA expression in continuous passages (passages 20 and 30) and differentiated post-mitotic neurons further suggested that TERRA regulate telomere stability in hPSCs and their derivatives. Our study revealed the dynamics of TERRA expressions in hPSCs may provide valuable background for stem cell research and applications.

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T-3129

STEM CELL IMMUNOENGINEERING FOR UNIVERSAL CARDIAC THERAPY VIA CRISPR-CAS9

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Heart transplant is currently the only clinical option for heart failure, a common form of cardiovascular disease, which is a leading cause of death globally. Heart transplant is not an ideal solution due to reliance on lifetime immunosuppressive therapies. Additionally, the availability of donor organs is dwarfed by demand and further limited by HLA matching between donor and patient. Induced pluripotent stem cell (iPSC) technology and directed differentiation strategies have provided the potential to de novo generate theoretically unlimited quantities of cardiomyocytes (CMs) from a patient's own cells.

However, studies have shown that iPSC-derived CMs will elicit an immune response even when transplanted autologously, indicating unique immunogenic properties of CMs. To develop improved therapeutic options, we engineered universal donor stem cells (USDCs) to evade immune detection and provide decreased immunogenicity. Class I HLA molecules display intracellular proteins to cytotoxic T cells, which if unrecognized will trigger cell lysis. Using CRISPR-Cas9 technology, we removed beta 2 microglobulin (B2M), a required protein for cell surface expression of HLA molecules, via knockout in 2 human pluripotent stem cell (hPSC) lines to prevent T-cell detection. However, this strategy leaves our engineered cells vulnerable to lysis by natural killer (NK) cells, which will attack cells lacking all HLA molecule expression. To overcome this, we designed a protein made up of HLA-E, an NK cell inhibitor, fused to B2M and integrated this vector into the genome to allow functional cell surface expression only of HLA-E. Our UDSCs showed normal hPSC morphology and pluripotent marker expression and differentiated to all 3 germ layers. To test the immunogenicity of our UDSCs, we will differentiate them to ISL1+ committed cardiac progenitor cells and CMs. We will challenge these two cell types via in vitro lysis and degranulation assays with NK or cytotoxic T-cells. We expect that our UDSCs will illicit reduced or no response to both NK and cytotoxic T-cells as compared to wild type cells. Our UDSCs will have great potential for transplantation medicine for cardiovascular disease and have broad applications to other cell types affected by degenerative or autoimmune diseases.

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T-3131

COMPROMISED MESODERM DIFFERENTIATION CAPACITY IN TERC KNOCKOUT EMBRYONIC STEM CELLS

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Mammalian telomere lengths are primarily regulated by telomerase, consisting of a reverse transcriptase protein (TERT) and a RNA subunit (TERC). In somatic cells telomerase is normally turned off and as a result, telomeres continue to shorten each time they proliferate. In embryonic tissues and stem cells, telomerase activity is detectable as a mechanism to prevent excessive telomere attritions. Patients with telomerase insufficiency caused by loss of function mutations in genes such as Tert and Terc suffer from telomere syndrome signatured by premature aging. We previously reported the generation of mouse Terc+/- and Terc-/- embryonic stem cells (ESCs) by somatic cell nuclear transfer, and demonstrated that Terc-/- ESCs were telomerase insufficient, had short telomeres, and importantly, showed severely compromised capacity in supporting full term embryo development in tetraploid complementation assays. In the present work, we investigated the germlayer development competence of Terc-/-, Terc+/- and wild-type (Terc+/+) ESCs. The expression of conventional pluripotency markers is indistinguishable among ESCs of three genotypes, including NANOG, OCT4, SALL4 and SOX2. The telomere lengths measured by Southern blot-based telomere restriction fragment (TRF) analysis, as expected, are longest in wild-type (63.5kb), followed by Terc-/- ESCs (50kb), but shortest in Terc-/- ESCs (25kb), and correlate reversely with the population doubling time. Interestingly, while in vitro embryoid body (EB) differentiation assay only reveals the difference in the size of EBs (Terc-/-: 127.0μm vs. Terc+/+: 165.99 μm and Terc+/-: 162.7μm, $P<0.05$), the more stringent in vivo teratoma assay demonstrate that Terc-/- ESCs are severely defective in differentiating into the mesodermal, but not the endodermal or the ectodermal lineages. Consistently, in a directed in vitro chondrocyte differentiation assay, cells in the Terc-/- group failed in forming Collagen II expressing cells, comparing to those in the Terc+/- and wild-type groups. Our work, for the first time, demonstrate that the mesodermal lineage differentiation is more vulnerable to telomerase insufficiency than other germlayer lineages are, underscoring the significance of treating mesoderm linage cell types with priority in patients with telomere syndromes.

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T-3133

MICRORNA-MEDIATED CONTROL OVER CALLOSAL PROJECTION NEURONS DURING DEVELOPING OF THE CEREBRAL CORTEX IN THE MOUSE

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SATB2 is a DNA-binding protein that regulates chromatin organization and gene expression. Due to its interaction with several key transcriptional determinants of neocortical development such as TBR1, FEZF2 and CTIP2, SATB2 may occupy a central position in transcriptional networks significant to Autistic Spectrum Disorders. Throughout corticogenesis, SATB2 protein acts as a transcriptional factor expressed in the upper layer neurons of the cortex, callosal projection neurons, and by preventing the transcription of the deep layer genes, establishes the end of the cortex formation. However, there is a huge gap in the literature concerning the mechanisms inhibiting SATB2 positive neurons expression in the early steps of cortex development while the deeper layers are been originated. It is central to overcome the lack of information in order to recognize how neurons responsible for cognition processes are generated. We initially addressed this question by studying the involvement of RISC complex. We observed that this complex plays an important role during upper layer formation as neuronal precursor cells (NPCs) transfected with siRNA for DICER, expressed SATB2 positive neurons earlier than the normal cortex developmental timing. In addition, we demonstrate that Argonaute-1 (main component of RISC complex) binds significantly to SATB2 gene and this binding decreases as upper-layer development starts. Using a novel technique, miRCATCH, that allowed us to pool down the specific miRNAs that bind to Satb2 3'UTR we have observed two strong candidates. By in situ hybridization at E17 we demonstrate that these two microRNAs do not co-localize with SATB2 gene indicating possibly a derepression of SATB2 at this developmental stage. In utero electroporation confirms this data as SATB2 3'UTR-GFP reporter is present only in the upper layers at this same developmental stage. In what concerns SATB2 protein, mutations of miRNA binding sites in the SATB2 3'UTR, were able to rescue protein translation earlier than the control experiments, both in vitro and in vivo. All this data confirms the possibility of a post-transcriptional control of miRNA over SATB2 gene during corticogenesis that, in abnormal circunstances, can lead to absence of corpus callosum and cognition impairments in the SATB2-Associated syndrome.

T-3135

MBD3 CONTRIBUTES TO THE DIFFERENTIATION COMPETENCE OF ESC INTO EPILC VIA THE RECRUITMENT OF PRC2 COMPLEX

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Loss of MBD3, a scaffolding component of the NuRD complex compromises differentiation potential of embryonic stem cells (ESCs), i.e., ESCs lacking MBD3 remain undifferentiated even after exposure to differentiation stimulus. MBD3 is named for its prominent domain, i.e., methyl-CpG-binding domain (MBD). It is known that there are the three MBD3 isoforms (a, b, and c), and we demonstrated that, like other forms of MBD3, MBD3c lacking an entire portion of the MBD domain, were able to erase the defect of lineage commitment potential of Mbd3-knockout ESCs. In addition, our analyses revealed that MBD3 recruits PRC2 complex to its vicinity via the predicted coiled-coil domain common to all three isoforms. Comparison between wild-type and Mbd3-knockout ESCs revealed that there is significant difference in their gene expression profiles when cultured under conventional mouse ESC culture medium with LIF and serum. However, this difference was much less significant when cultured under 2i condition using two kinase inhibitors for MEK and GSK3b. Therefore, we generated Mbd3 inducible knockout ESCs and cultured at least several days under 2i condition with retention of Mbd3 expression. Then, those ESCs were induced to differentiate into Epiblast-like cells (EpiLCs) with FGF2 and activin A. During this induction, exogenous Mbd3 expression was either maintained or repressed. Our data revealed that ESCs lacking Mbd3 expression were refractory to the conversion to EpiLCs with minimum change in colony morphology. Gene expression analyses revealed that some, but not all, genes known as ESC naïve-state specific markers, were not appropriately repressed in their expression levels during induction of conversion of ESCs to EpiLCs. Tbx3 and Prdm14 genes are such representative examples. Our ChIP analyses revealed that PRC2 complex was recruited to TSS portions of these gene in Mbd3-expressing ESCs, but not in those without Mbd3 expression. These results suggest that loss of differentiation competence of Mbd3-knockout ESCs is at least in part attributed to their impairment of recruitment of PRC2 complex to naïve ESC specific gene upon induction of differentiation.

T-3137

ROBUST INDUCTION OF MULTICILIATED BRONCHIAL EPITHELIUM FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide, ranking third among the global age standardized death rates. Therefore, developing new models is mandatory for respiratory research as critical differences exist between human and animal models such as rodent models regarding airway epithelium structure and physiology. Human induced pluripotent stem cells (iPSC) are characterized by both unlimited in vitro proliferation and the ability to differentiate into any cell type. Their use would make it possible to envision an unlimited production of human bronchial epithelium, a critical step to consider innovative research programs, large-scale pharma R and D programs and putative cell therapy. We reprogramed peripheral blood from a healthy individual and three patients with severe COPD. These iPSC lines were differentiated successively into anterior primary streak (APS), then into definitive endoderm (DE), then into anterior ventralized intestinal endoderm (vAFE). The induction of DE was robust and characterized by a CXCR4 level > 95% (n = 6). The vAFE step made it possible to obtain purity levels > 80-90% of NKX2.1+ bronchial progenitors (n = 3). After polarization and passage into ALI, the immunofluorescence markings and the PCRs at 50 days of differentiation attested to the presence of mucus cells (MUC5AC), basal cells (KRT5), Club cells (CCSP) and hair cells (FOXJ1). Recent adjustment of extracellular matrix and medium parameters allowed us to obtain a potent differentiation into multiciliated cells displaying motile cilia from the normal HY03 iPSC line, as documented by video microscopy (n = 2). In conclusion, we designed a robust protocol than can differentiate iPSC into NKX2.1+ bronchial progenitors within one week with excellent purity. Then, these progenitors can be differentiated into bronchial epithelium comprising numerous functional multiciliated cells.

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T-3139

MODIFICATIONS DESIGNED TO STABILIZE THE MTESR1 FORMULATION DO NOT IMPACT DOWNSTREAM DIFFERENTIATION

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The ultimate goal of human pluripotent stem cell (hPSC) research is to generate mature cell types for use in regenerative medicine, drug screening, or disease modeling. The field has invested years developing robust and efficient protocols for hPSC differentiation to a wide variety of cell types, however, reproducibility can suffer if part of the workflow changes. mTeSR™1 (mT1) is the most widely used feeder-free hPSC maintenance medium, with many published downstream differentiation protocols. mTeSR™ Plus (mTPlus) is a related formulation which enables reduced feeding by maintaining a more consistent pH and stabilizing components including FGF2. We investigated the impact of these modifications on downstream differentiation protocols by comparing cells maintained in mTPlus for ≥ 5 passages with reduced feeding vs. mT1 with daily feeding. We used STEMdiff™ kits to generate 10 separate cell types across the 3 germ layers, without modifying the protocols originally optimized for hPSCs maintained in mT1. Ectoderm: PAX6+ neural progenitors displaying neural rosette morphology were generated using the SMADi Neural Induction Kit. Further differentiation into class III β-tubulin+ neurons, or astrocytes expressing GFAP and S100B was achieved with similar efficiencies from hPSCs maintained in either mT1 or mTPlus (n=2). Cerebral organoids with organized cortical regions were also generated from H9 cells maintained in mTPlus. Mesoderm: Brachyury+ early mesoderm was produced with 80 ± 2% purity from mTPlus cultures using Mesoderm Induction Medium (n=4). Comparable efficiency of differentiation to beating cTnT+ cardiomyocytes was observed with mean of 70% (mT1) versus 79% (mT Plus, n=2). Cells from mT1 or mTPlus cultures generated 43 ± 5% or 40 ± 5% CD34+CD45+ hematopoietic progenitor cells respectively (n=5), with comparable yields in each condition. Endoderm: SOX17+CXCR4+ definitive endoderm was produced with efficiencies of 92 ± 2% (mT1) and 90 ± 1% (mTPlus, n=3). hPSCs maintained in mTPlus also generated NKX6.1+PDX1+ pancreatic progenitors (n=3) and small intestinal organoids containing intestinal epithelium and associated mesenchyme. Overall, we observed little to no impact on the efficiency of downstream differentiation protocols developed for mTeSR™1 when used with hPSCs maintained in mTeSR™ Plus.

T-3141

REPRODUCIBLE AND SCALABLE DIFFERENTIATION OF ATRIAL-LIKE CARDIOMYOCYTES BY PRECISE MODULATION OF THE RETINOIC ACID/CYP26A1 AXIS

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Atrial-like cardiomyocytes derived from pluripotent stem cells are a promising model for developing therapeutics targeting cardiac arrhythmias and atrial fibrillation, however generation of these cells at a consistent scale and purity for routine use remains a challenge. Here we report a method for the reproducible differentiation of human ES/iPSCs into atrial-like cardiomyocytes at a multi-million cell scale through the precise modulation of the retinoic acid (RA) pathway in a 3D spheroid suspension culture. Insufficient exposure to RA yields a mixed culture of atrial and ventricular myocytes, while excessive RA concentrations divert the cardiac progenitors to a non-myocyte fate resembling early cardiac progenitors. We find that the variable modulation of RA action by the Cytochrome P450 enzyme CYP26A1 is the critical determinant of atrial differentiation efficiency, and that eliminating the counter-regulatory actions of CYP26A1 by chemical or genetic means permits the reproducible and predictable induction of the atrial fate. RNA-seq analysis of these optimized cultures at both the single cell and population level reveals a highly homogeneous culture of atrial-like myocytes which express many of the chamber-specific transcripts of the human left atrium. Finally, we leveraged this myocyte model to optimize a high throughput optical screening platform for the fine measurement of cellular action potential and calcium transients under electrical field stimulation. This new method now enables the consistent detection of small changes in the cardiac action potential and provides a valuable tool for the development of novel drugs for the treatment of atrial arrhythmias.

T-3143

EFFECTIVE CARDIAC DIFFERENTIATION AT A SUPER LOW SEEDING DENSITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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In recent years, cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) have been receiving increased attention for using in medical care. Cardiac differentiation is progressed through several stages (hiPSCs → mesoderm → cardiac progenitor → cardiomyocytes) and high density of hiPSCs is strongly recommended in initiation stage. Nonetheless, the reason why high cell density is crucial and what hidden factors in high-density cells can accelerate cardiac differentiation remains unknown. Here we successfully established a new culture condition for cardiac differentiation initiating at super low cell density to eliminate all the hidden factors present in high-density cells for clarifying key signals in cardiac differentiation. We found that low-density cells strongly promoted mesoderm differentiation; however, the cells were also inducing the anti-cardiac mesoderm genes including CDX2 and MSX1. Due to the high expression of these anti-cardiac mesoderm genes, formation of cardiac progenitor and cardiomyocytes were prevented. Downregulation of these genes by earlier treatment with Wnt inhibitor effectively improved cardiac differentiation efficiency up to 80% from 10% started at a super low density of 1% confluent. Although being seeded at an initial low density, cell population became confluent in the terminal differentiation stage as similar to yield in differentiation started at high cell density. This implies a higher productivity in the low-density differentiation. On the other hand, the results also suggested that hiPSCs at high-density might secret inhibitors (including Wnt inhibitor) to inhibit anti-cardiac mesoderm genes, which may be the mechanism to explain why high cell density is recommended for cardiac differentiation. This study provides a new and effective method for cardiac differentiation and production without depending on cell density applicable for drug screening and cell therapy.

T-3145

A NOVEL APPROACH TO MASS-PRODUCE HEMATOPOIETIC PROGENITOR CELLS FROM HUMAN IPSC USING A 3D CO-CULTURE SYSTEM WITH HUMAN MSC

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Hematopoietic progenitor cells (HPC) generated from patient-derived human induced pluripotent stem cells (hiPSC) could provide enormous potential for the treatment of hematopoietic disorders by cell-based therapy. Currently, two major approaches have been implemented for HPC generation from hiPSC: embryoid body (EB) based differentiation and OP9 co-culture system. The EB based approach can be performed in a xeno-free condition, but this system requires complicated combinations of cytokines/growth factors and is not scalable. Conversely, the OP9 co-culture system can produce HPC robustly without any additional factors. However, xenogeneic conditions are undesirable for clinical applications. To overcome the dilemma of current approaches, we developed a new co-culture system using human mesenchymal stem/stromal cells (hMSC). Initially, we substituted hMSC for OP9 in the traditional co-culture system, but hiPSC on hMSC did not develop sac-like structures and failed to generate CD34+CD43+ cells. Postulating that the sac formation might be facilitated by a more physiological microenvironment, we then attempted to co-culture hiPSC and hMSC in a 3D culture condition. When hiPSC and hMSC were co-cultured as spheroids (hereafter referred to hematopoietic spheroids), hiPSC could develop sac-like structures and produce CD34+CD43+ cells without the addition of any cytokines or growth factors. Based on hematopoietic CFU assay, hematopoietic spheroid derived HPC (hs-HPC) could differentiate into erythro-myeloid lineage cells. Definitive hematopoietic potential of hs-HPC was demonstrated by the generation of CD4+CD8+ T-lymphocytes using OP9/DLL1 co-culture system. Importantly, hematopoietic spheroids can also be cultured in stirred suspension bioreactors in xeno-free medium, allowing for the mass-production of hs-HPC in a xeno-free condition suitable for clinical application. Our new system can accelerate the translation of human iPSC-derived cell products to cell-based therapies for hematopoietic malignancies and other immunological disorders.

T-3147

IDENTIFICATION OF X-LINKED GENES THAT DRIVE SEX DIFFERENCES IN MOUSE EMBRYONIC STEM CELLS

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During early development female mammals have two active X chromosomes before they undergo X-chromosome inactivation. To ensure tight coordination of X inactivation and development, double X dosage blocks differentiation until X dosage compensation has occurred. This developmental block is likely mediated by one or several X-linked genes that, when present at a double dose, inhibit the differentiation-promoting MAPK signaling pathway and increase expression of pluripotency factors. To identify X-linked genes responsible for mediating this phenotype, we have performed a series of pooled lentiviral CRISPR Knock-out (KO) screens in mouse embryonic stem cells (mESC). We first screened a short-guide RNA (sgRNA) library targeting all X-linked genes for its ability to decrease the activity of a fluorescent MAPK reporter. In three following subscreens, the hits from this primary screen were further characterized regarding their ability to modulate (1) pMek, an intermediate components of the MAPK pathway, (2) Nanog, a pluripotency factor, and (3) ESC differentiation dynamics. In this way, we have identified several genes that, when knocked-out, recapitulate the male phenotype with regard to MAPK pathway activity, pluripotency factor expression and differentiation. We have successfully validated several hits by generating heterozygous deletion mutants in female and their overexpression in male mESCs. Among the identified hits we found Dusp9, which inhibits the MAPK pathway by dephosphorylating Erk, a central member of the MAPK pathway. Taken together we have screened all X-linked genes in a high-throughput manner to generate a list of factors that act

together in driving sex differences in mESCs. Our results bring us significantly closer to elucidating the mechanisms underlying the observed sex-induced differences in cultured mESCs, which pose a valuable model system for mammalian development.

T-3149

FUNCTIONAL CHARACTERIZATION OF HUMAN IPSC-DERIVED PULMONARY NEUROENDOCRINE CELLS

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Pulmonary neuroendocrine cells (PNECs) are the first specialized cells to appear during lung development, as early as gestational week 8 in humans. They serve as growth modulators, airway chemosensors and a possible stem cell niche. PNECs increase in many lung diseases (e.g., neuroendocrine hyperplasia of infants (NEHI) and cystic fibrosis (CF)). However, their role in disease pathophysiology is unclear. PNECs comprise <1% of lung cells making them difficult to isolate, and lack of an in vitro system to model PNECs has made them challenging to study. Here we report generation of induced PNECs (iPNECs) from human induced pluripotent stem cells (iPSCs) by adapting our published airway differentiation protocol. We examined a range of known PNEC markers over a differentiation time course by immunofluorescence (IF) and quantitative RT-PCR. During human gestation, early markers (e.g., ASCL1, ROBO2, and ENO2) appear by 8 weeks, while SYP and CHGA are expressed between 18-22 weeks. ASCL1, ROBO2, and ENO2 emerge early during in vitro iPSC differentiation (by day 13). SYP and CHGA appear later, showing robust expression by Day 25 at air-liquid interface (ALI) and are rarely detected earlier, recapitulating in vivo development. Mature iPNECs express lung marker NKX2-1 and proliferate. Exposing the apical surface of differentiating cells at ALI is crucial for iPNEC generation. Moreover, repressing NOTCH signaling using a γ-secretase inhibitor promotes iPNEC specification and improves maturation, as indicated by arborized networks at later stages. Single-cell transcriptomics confirms that iPNECs have a gene expression pattern similar to human fetal lung-derived PNECs, with an overlap of 44 common PNEC-related genes. Additionally, iPNECs are attracted to Slit2 ligand in a migration assay, confirming ROBO2 receptor expression, and express major neuropeptides. In silico pseudotime analysis reveals that basal cells predate iPNECs in our iPSC differentiation cultures. Consistent with this, primary human basal cells grown at ALI gives rise to PNECs, supporting prior mouse data showing E9.5 p63+ basal cells as PNEC progenitors. In conclusion, we demonstrate successful generation of functional human iPNECs, providing a ready source of cells for investigating their deregulation and role in lung diseases, such as CF and NEHI.

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T-3151

COMBINING COMPOUND INDUCTION AND GENETIC APPROACHES TO GENERATE ISLETS

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Type 1 diabetes mellitus (T1DM) is characterized by autoimmune destruction of insulin-producing β cells. To treat T1DM patients, the primary therapy is to control their blood glucose levels by insulin injection throughout their lives. The discovery of human pluripotent stem cells (hPSC) makes it possible to generate replacement stem cell derived β cells in vitro that could be used for transplantation. Though many differentiation protocols have been developed for this purpose, stem cell derived β cells do not yet show the full function expected for in vivo β cells. One potential reason could be the lack of other cell types in the clusters, including alpha cells, delta cells, and others. It would be promising to improve stem cell derived β cells' function by selecting purified β cells, reaggregate them to generate pure β cell clusters, or even mix different cell types to make artificial islets. In this study, we used genetic approaches to modify hPSC lines to make it possible to select pure population of different endocrine cell types and mix them in specified ratios to make artificial islets that compare more favorably to those isolated from cadavers and perhaps even improve on their natural counterpart.

T-3153

DOWNREGULATION OF A LONG NON-CODING RNA INHIBITS CARDIOMYOCYTE DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes fundamentally relies on many signaling molecules and transcription factors, however understanding long noncoding RNA (lncRNA) transcription and regulation during this process remains elusive. Recent discoveries have shown that lncRNA plays a crucial role in controlling hPSCs fate and phenotype during directed differentiation, which is

important for exploring their potential use for disease modeling, cell therapy, and drug testing. Here, we have identified a nuclear-localized lncRNA that is transiently upregulated along with a cardiac transcription factor during early stages of growth factor-mediated cardiomyocyte differentiation from hPSCs. The transcripts of both the lncRNA and the cardiac transcription factor were co-expressed in many tissues including the heart at the almost similar level. Knockdown of the lncRNA using short hairpin RNA (shRNA) did not affect undifferentiated cell morphology, growth, and expression of pluripotency markers, but reduced the expression of mesodermal, WNT-target and WNT-signaling genes upon the growth-factor induction. Moreover, knockdown of the lncRNA significantly decreased cardiomyocyte differentiation. The lncRNA shRNA differentiation culture contained no beating cardiomyocytes and expressed lower levels of cardiomyocyte-specific protein when compared with control. Overall, these data suggest a novel role of the lncRNA in controlling cardiomyocyte differentiation from hPSCs.

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T-3155

A GENE REGULATORY NETWORK GOVERNING THE EARLY SPECIFICATION OF HUMAN NEURAL CREST

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The neural crest is a multipotent stem cell-like population unique to vertebrates which migrates extensively and gives rise to numerous derivatives including neurons and glia of the peripheral nervous system, melanocytes, and much of the craniofacial skeleton. Neural crest cells are associated with a large number of human health conditions, including widely prevalent birth defects such as cleft lip and cleft palate, and aggressive cancers. Understanding the formation of human neural crest is of utmost relevance if we are to ameliorate the effects of such conditions through diagnostic and therapeutic efforts. In this work, we make use of a validated cutting-edge model of human neural crest development based on embryonic stem cells, which recapitulates developmental states well-characterized in vivo, to address the gene regulatory network governing early human neural crest cell formation. Deep transcriptome sequencing during the time course of human neural crest cell formation reveals dynamic gene expression changes and suggests a pre-neural plate border state of neural crest cell specification. We take a multi-pronged approach to assess the contribution of candidate pre-border factors to the human neural crest gene regulatory network. Loss-of-function assays via siRNA and genetic knockouts validate the contribution of these early factors to human neural crest cell formation. This work provides a novel view of the neural crest gene regulatory architecture bridging the pluripotent cell to the neural plate border and NC states.

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PLURIPOTENT STEM CELL: DISEASE MODELING

T-3159

DIFFERENTIAL SUSCEPTIBILITY OF FETAL RETINAL PIGMENT EPITHELIAL CELLS, IPSC-RETINAL STEM CELLS, AND RETINAL CUP ORGANOIDS TO ZIKA VIRUS INFECTION

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Zika virus (ZIKV) causes microcephaly and congenital eye disease. The cellular and molecular basis of congenital ZIKV infection are not well understood. Here, we utilized a biologically relevant cell-based system of human fetal retinal pigment epithelial cells (FRPEs), iPSC-derived retinal stem cells (iRSCs), and retinal cup (RC) organoids to investigate ZIKV-mediated ocular cell injury processes. Our data show that FRPEs were highly susceptible to ZIKV infection exhibiting increased apoptosis, whereas iRSCs showed reduced susceptibility. Detailed transcriptomics and proteomics analyses of infected FRPEs were performed. Nucleoside analogue drug treatment inhibited ZIKV replication. Retinal Cup organoids were susceptible to ZIKV infection. The Asian genotype ZIKV exhibited higher infectivity, induced profound inflammatory response, and dysregulated transcription factors involved in retinal cup differentiation. Collectively, our study shows that ZIKV affects ocular cells at different developmental stages resulting in cellular injury and death, further providing molecular insight into the pathogenesis of congenital eye disease.

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T-3161

NEW EX VIVO DIAGNOSTIC TOOL FOR ARTERIAL SPASM USING IPS CELL-DERIVED VASCULAR SMOOTH MUSCLE CELLS

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Although Prinzmetal's or variant angina is known to be caused by coronary artery spasm, there have been no studies explaining the exact underlying mechanism. Here, we investigated the mechanism of variant angina using peripheral blood-derived iPS cells and developed Ex vivo diagnostic tool for this disease. The peripheral blood was collected from patients who were diagnosed with variant angina after provocation test. With our new methods, we cultured multipotent stem cells from peripheral blood and generated induced pluripotent stem (iPS) cells. We differentiated these iPS cells into vascular smooth muscle cells (VSMCs). After stimulation with carbachol or acetylcholine, the VSMCs from the patients with variant angina showed a strong contraction, when compared to the normal group. In addition, we measured the intracellular calcium efflux and calcium sparks of VSMCs using laser scanning confocal microscopy with the fluorescent dye. In response to stimulation, the variant angina group showed much higher intensity of calcium efflux peak than normal group. Moreover, only variant angina group showed the secondary or tertiary peaks of calcium efflux. In addition, the presence or absence of secondary calcium peak was another diagnostic criteria for variant angina. Further experiments showed that the different level of sarco/endoplasmic reticulum calcium handling gene was a main cause of the different response of calcium peaks after stimulation. In conclusion, our study using peripheral blood demonstrated that the different response of intracellular calcium efflux could be an Ex vivo diagnostic tool for variant angina. Our new method could be used for a new drug-screening or diagnostic tool for variant angina by obtaining peripheral blood in outpatient department.

T-3163

GBETA5 P.S81L CAUSES BRADYCARDIA BY INCREASING THE ACETYLCHOLINE-ACTIVATED POTASSIUM CURRENT AND AUGMENTING CHOLINERGIC RESPONSE

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Mutations in GNB5, encoding the G-protein β5 subunit (Gβ5), have been linked to a multisystem disorder that includes severe bradycardia. We here investigated the mechanism underlying bradycardia caused by the recessive p.S81L Gβ5 variant identified in families of Latin and North African descent. Using the CRISPR/Cas9-based genome editing technique we generated an isogenic series of human induced pluripotent stem cell (hiPSC) lines that were respectively wild-type, heterozygous and homozygous for the GNB5 c.242C>T p.S81L variant. These cells were differentiated into cardiomyocytes (hiPSC-CMs) that robustly expressed the acetylcholine-activated potassium current (IKACh). Baseline electrophysiological properties of the hiPSC-CMs from the three isogenic lines did not differ. Upon application of carbachol (CCh), homozygous p.S81L hiPSC-CMs displayed an increased IKACh density, a more pronounced membrane hyperpolarization and decrease of spontaneous activity as compared to wild-type and heterozygous p.S81L hiPSC-CMs, in line with the bradycardia in homozygous carriers. Application of XEN-R07032, a specific IKACh blocker, resulted

in near-complete reversal of the phenotype. Additionally, in vivo studies in zebrafish gnb5 knockout confirmed the effect of XEN-R0703 in rescuing the CCh-induced bradycardia. Our results provide mechanistic insights in the Gβ5 p.S81L-associated bradycardia and proof of principle for potential therapy in patients carrying GNB5 mutations.

T-3165

SEQUENTIAL STIMULATION AND INHIBITION OF LYSOPHOSPHATIDIC ACID RECEPTOR 4 ARE CRITICAL FOR CARDIAC DIFFERENTIATION AND REPAIR

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The clinical application of cell therapy to repair the damaged heart needs to understand the precise differentiation process of stem cells and the characteristics of cardiac progenitor cells. We examined the cardiac-specific markers that expressed on the cell surface and determined their functional significance during cardiac differentiation. We screened cell-surface expressing proteins on cardiac progenitor cells at differentiation day 3 compared to undifferentiated pluripotent stem cells (PSCs). Among candidates, we identified lysophosphatidic acid receptor 4 (LPAR4) that is a G protein-coupled receptor. During in vitro differentiation of mouse PSCs toward cardiac cells, LPAR4 expression peaked for 3–5 days and then and declined immediately. Also in vivo, LPAR4 was specifically expressed in the early stage of heart development in embryos and disappeared completely in adults, suggesting that stimulatory signal of LPAR4 at an early stage should be shut off for further progression of differentiation. We next have identified the LPAR4 downstream signaling molecule, p38MAPK, by comparing PSCs and LPAR4 knockdown PSCs. In both mouse and human PSCs, ODP (LPAR4 specific agonist) followed by p38MAPK blocker (SB203580) treatment significantly increased cardiac differentiation efficiency. Furthermore, we investigated whether LPAR4 is the maker for adult cardiac progenitor cells. We found that LPAR4-positive cells were rarely present in normal adult mouse hearts, but LPAR4-positive cells were increased when the heart was damaged. LPAR4-positive cells from adult hearts differentiated into cardiomyocytes. After myocardial infarction (MI), the sequential stimulation and inhibition of LPAR4 with ODP and p38MAPK blocker resulted in the reduction of infarct size and improvement of left ventricular dysfunction. In summary, we demonstrated that LPAR4 is a cardiac progenitor-specific marker and its functional significance during cardiac differentiation and regeneration. Our findings provide a new insight in cell-free cardiac repair by the modulation of progenitor-specific downstream signaling.

Funding Source: Korea Health Technology R&D Project "Strategic Center of Cell and Bio Therapy" (grant number: HI17C2085) "Korea Research-Driven Hospital" (grant number: HR18C0006) (KHIDI), Ministry of Health and Welfare, and the Republic of Korea.

T-3167

TRANSCRIPTOME OF IPSC-DERIVED NEURONAL CELLS REVEALS A MODULE OF CO-EXPRESSED GENES CONSISTENTLY ASSOCIATED TO ASD

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Although the knowledge regarding genetic risk factors for Autism Spectrum Disorders (ASD) has greatly improved in recent years, the complex and heterogeneous genetic architecture associated with the disorder hampers the establishment of a molecular diagnosis. On the other hand, it is becoming clear that ASD candidate genes converge to a few molecular pathways, indicating that similar functional consequences may underlie disease pathophysiology, which could be reflected, for instance, in disturbances at transcriptional level. Based on this premise, we explored expression profile of neuronal cells derived from induced pluripotent stem cells (iPSC) from

a sample enriched by high functioning normocephalic ASD individuals and controls. Using a system biology approach, we identified that, in neuronal progenitor cells (NPC), a module of co-expressed genes (MNPC10) involved in oxidative phosphorylation and protein synthesis is upregulated in ASD individuals. In neurons, on the other hand, a network composed by synapse and neurotransmission genes (MNeur1) was found as upregulated in patients, while a module (MNeur18) related to translational initiation is inversely co-regulated with MNeur1, being downregulated in these individuals. Expression alteration of these classes of genes was validated by functional analysis in NPC and neurons. Also, a proteomic analysis in NPC revealed potential molecular links between the module deregulated in NPC and those deregulated in neurons. More importantly, comparing our results to multiple transcriptome studies of neuronal cells, conducted either with post-mortem brain or iPSC-derived neurons from ASD individuals, we found that MNeur1 has a strong overlap with ASD-associated modules identified by all these studies, revealing the consistent association of this network to ASD. Interestingly, this module has been consistently found as upregulated in iPSC-derived neurons, while downregulated in post-mortem brain tissue. Since iPSC-derived neurons have a transcriptome profile more closely related to fetal brain than to adult brain, this result suggests that the deregulation of this module might occur in different directions across development in ASD individuals. Our data reveals a network of genes whose expression levels might be used as biomarkers for ASD.

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T-3169

NEW REGULATORY INSIGHTS INTO TAKOTSUBO SYNDROME USING A PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELL-MODEL

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Takotsubo syndrome (TTS) is characterized by acute transient left ventricular dysfunction in the absence of obstructive coronary lesions. We identified a higher sensitivity to catecholamine-induced stress toxicity as mechanism associated with the TTS phenotype in our former stem cell study, but the pathogenesis of TTS is still not completely clarified. In this work, we aimed to prove the hypothesis of an altered phosphodiesterase (PDE)-dependent cAMP-signaling in TTS in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Generated TTS-iPSC-CMs from 6 patients were treated with catecholamines to mimic a TTS-phenotype. Using a cytosolic Förster resonance energy transfer (FRET) cAMP sensor, we could observe that β-adrenergic receptor (β-AR) stimulations by Isopreterenol (Iso) led to stronger FRET responses in the cytosol of TTS-iPSC-CMs as compared to controls. To analyze the interplay of β-AR subtype-signaling and PDE contribution to the cAMP signaling in TTS, specific PDE-inhibitors were used. We were able to show that after β-AR stimulation, the strong effects of the PDE4 family in the cytosol of control cells were significantly decreased in diseased TTS CMs. Instead, the contribution of the important PDE family PDE3 to cytoplasmic cAMP degradation was increased in TTS. In line, we showed a significantly increased PDE3A expression and a down-regulated PDE4D expression in TTS-iPSC-CMs compared to control. By analyzing PDE-dependent cAMP downstream effects as PKA-dependent phosphorylation, we could show an additional increase of PLN phosphorylation (PLN-S16), especially in control, when treating iPSC-CMs with a combination of iso and PDE4 inhibitor. In contrast, in TTS-iPSC-CMs the contribution of the PDE-families PDE2, 3 or 4 to phosphorylation of PLN-S16 was increased over iso alone. This suggests that different PDEs in TTS and control are involved in functional segregation of the SERCA2a microdomain from the cytosol in terms of cAMP downstream effects. Our data show for the first time alterations of cytosolic cAMP signaling and PDE contributions in healthy and diseased TTS-iPSC-CMs. This suggests an important role of PDEs, especially PDE3 and 4, in the development of TTS and provides a new therapeutic target family in the treatment strategy for TTS.

T-3171

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM A FEMALE PATIENT WITH LARGE X CHROMOSOME DELETION FOR ESTABLISHING DISEASE MODELS AND FINDING CURES

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Induced pluripotent stem cells (iPSCs) are widely used as a disease model and it is effective especially in rare diseases. We established iPSCs from a young girl who exhibited global developmental delay and intellectual disability from early in infancy and found having a hemizygous deletion of Xq27.3-q28. The deletion site on the X chromosome includes FMR1, the gene responsible for Fragile X syndrome, which may partially delineate the patient's neurodevelopmental abnormalities. iPSCs were generated from her peripheral blood mononuclear cells by

infecting with Sendai viral vector encoding four reprogramming factors (Oct4, Sox2, Kif4 and c-Myc). 11 iPSC clones were established and the expression of FMR1 gene in iPSCs was analyzed by quantitative PCR to identify the epigenotypes of iPSCs. FMR1 gene was expressed in the 6 iPSC clones while it was absent in the other 5 clones, representing random inactivation of normal and abnormal X chromosomes in her blood cells. We then differentiated iPSCs to neural progenitor cells (NPCs) which were disease relevant type of cells. The normal or absent expression pattern of the FMR1 gene was not changed when the iPSCs were differentiated into NPCs. Using two NPC clones expressing deleted X chromosome as well as two control clones expressing normal X chromosome, we attempted to reactivate repressive FMR1 gene in iPSC-NPCs using three chromosome reactivating reagents (5-Aza-2-deoxycytidine, trichostatin A and UNC0638). NPCs were exposed to these three drugs for 72 hours in two different doses. Although there was no significant change detected in FMR1 gene expression with these chemicals tested, these isogenic pairs of iPSCs will become a powerful tool to study disease mechanisms and finding potential therapeutic approaches for this rare X-linked disorder.

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T-3173

PLURIPOTENT STEM CELL-BASED MODELING OF CIGARETTE SMOKE INJURY TO THE HUMAN ALVEOLAR EPITHELIUM

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Smoking is the most important cause of chronic obstructive pulmonary disease (COPD), which encompasses chronic bronchitis and emphysema. Cigarette smoke causes oxidative stress and provokes an inflammatory response, which results in breakdown of alveolar architecture to cause emphysema. In spite of the clear implication of the alveolar epithelial response to smoke in the etiology of emphysema, there exists no system capable of modeling this response in a physiologically relevant manner. We directed the differentiation of human induced pluripotent stem cells (iPSCs) to type 2 alveolar epithelial-like cells (iAEC2s) in organoids known as alveolospheres. We dissociated alveolospheres and re-plated iAEC2s in an air-liquid interface (ALI) culture system. We characterized the makeup of the ALI cultures compared to alveolospheres by RT-qPCR for marker genes and by immunohistochemistry. Using a VitroCell VC1 smoke exposure robot, we exposed iAEC2 ALI cultures to gas-phase cigarette smoke. We assessed maintenance of epithelial integrity by trans-epithelial electrical resistance (TEER). We quantified transcriptional changes in known and novel smoke-perturbed genes by RT-qPCR, and transcriptionally compared iAEC2s to primary human bronchial epithelial cells cultured at ALI. iAEC2 ALI cultures generated from day 35 iPSC-derived alveolospheres could be maintained for at least 14 days. iAEC2s at ALI maintain transcript and protein-level expression of surfactant protein C (SFTPC), a specific type 2 alveolar epithelial cell marker, compared to their parental alveolospheres. iAEC2s at ALI also maintain mRNA expression of NKX2-1, a key lung lineage transcription factor. iAEC2s at ALI gain TEER, a quantitative measure of barrier function, over time, reaching a steady state of approximately 400Ω.cm2 after five days of ALI culture. TEER was significantly reduced in smoke-exposed iAEC2 ALIs. Interestingly, iAEC2s at ALI exhibit a unique transcriptional response to smoke compared to primary airway epithelial cells. Overall, we were able to successfully develop an ALI culture protocol for human iPSC-derived AEC2s, expose them to cigarette smoke in a physiologically relevant manner, and identify novel smoke-responsive transcriptional perturbations that are unique from those of airway epithelial cells.

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T-3175

MODELING TRANSIENT NEONATAL DIABETES MELLITUS IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Transient Neonatal Diabetes Mellitus (TNDM) is a rare genetic disorder that affects 1 in ~300,000 live births. TNDM patients display insulin secretion defects shortly after birth. Insulin treatment for the first few postnatal months normalizes glycemia, but 85+% TNDM patients relapse during adolescence and suffer from permanent diabetes. The etiology of TNDM is poorly understood as no TNDM pancreas could be carefully examined so far; it is assumed that TNDM results from defective pancreatic β-cell development and/or function. Due to the developmental and structural differences of murine and human pancreases, we favored a human model of TNDM. Seventy-five % of TNDM patients display an imprinting or cytogenetic defect of the 6q24 chromosomal region. Imprinted genes are mono-allelically expressed genes, whose expressed allele depends on its parental origin. The critical 6q24 TNDM region harbors ZAC1/PLAGL1, a paternally expressed, maternally imprinted, zinc finger transcription factor, which is the TNDM-6q24 candidate gene. We presently mimic the epimutation observed in some TNDM patients using a dCas9-SunTag-TET1 system that allows the targeted demethylation of the maternal ZAC1 allele in

hiPSCs. Maternal ZAC1 demethylation results in ZAC1 double-dosage and bi-allelic expression. WT and TNDM-like hiPSCs are then differentiated into pancreatic progenitors and β-cells using published protocols. We previously showed that ZAC1 controlled the cell cycle exit in different cell types, and regulated genes that belong to the imprinted gene network (IGN), including many extracellular matrix genes. We perform single cell RNAseq experiments to identify deregulated genes and biological processes in TNDM-like vs. WT pancreatic progenitors, with a special focus on IGN members and ECM genes.

T-3177

POSTTRANSCRIPTIONAL MODULATION OF TERC BY PAPD5 INHIBITION RESCUES HEMATOPOIETIC DEVELOPMENT IN DYSKERATOSIS CONGENITA EMBRYONIC STEM CELLS

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Telomere attrition causes bone marrow failure in dyskeratosis congenita (DC) patients. Some of the most severe mutations in these patients are in dyskerin (DKC1), a component of the telomerase complex, responsible for the stability of TERC (telomerase RNA component). Due to a lack of adequate models of study, therapeutic alternatives for DC remain inefficient. To circumvent that, we engineered human embryonic stem cells (hESCs) carrying disease-associated mutations in DKC1 (DKC1_A353V) to study TERC stability during hematopoiesis. Utilizing serum-free differentiations we were able, by stage-specific modulation of WNT, to independently derive extra-embryonic and intra-embryonic-like definitive hematopoietic progenitors from WT and DKC1 hESCs. We show that in DKC1_A353V derived hematopoietic progenitors TERC is rapidly degraded by the exosome RNA degradation complex, dependent on its 3' polyadenylation by the non-canonical poly(A) polymerase (PAPD5). This led to telomere attrition and accumulation of DNA damage in these cells. Moreover, DKC1_A353V cells showed reduced erythro-myeloid and T-lymphoid definitive hematopoietic output, indicating that the hematopoietic differentiation of DKC1 mutant hESCs recapitulates key aspects of bone marrow failure observed in DC patients. We next examined if inhibition of PAPD5 could restore hematopoietic output in DC. We expressed, from the AAVS1 safe-harbor locus, shRNA constructs against PAPD5 in WT and DKC1_A353V hESCs. Targeted RNA sequencing at the 3' end of TERC showed that DKC1_A353V_shPAPD5 hESCs have a reduction in the percentage of oligo(A) species in TERC, with increased TERC expression, when compared to DKC1 mutants. This led to higher telomerase activity, telomere elongation and reduced DNA damage accrual. Moreover, silencing of PAPD5 significantly restored definitive hematopoietic erythro-myeloid and T-lymphoid potential, indicating that inhibition of PAPD5 restores multilineage potential in DC. Silencing of PAPD5 had no toxic effects in WT or DKC1 mutants. Combined, our data provides the first evidence that modulation of PAPD5 restores hematopoiesis in DC, through direct regulation of the 3'-end maturation of TERC. We propose that the posttranscriptional regulation of TERC by PAPD5 might represent a novel avenue for the management of DC.

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T-3179

GENERATION OF HYPOTHALAMIC NEURON SUBTYPES FROM HUMAN PLURIPOTENT STEM CELLS

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Several neuron subtypes of the paraventricular nucleus of the hypothalamus (PVH) are critical for the regulation of body weight. Of particular importance are the melanocortin-4 receptor (MC4R)-expressing neurons which are part of the leptin-melanocortin feeding circuitry. Heterozygous loss-of-function mutations in the melanocortin-4 receptor (MC4R) are the most common cause of human monogenic obesity. Rodent models and non-neuronal human cell lines have greatly contributed to our understanding of the impact of mutations in MC4R on food intake and energy expenditure. However, the molecular mechanisms underlying human MC4R deficiency are still not well understood. Therefore, we have been developing a MC4R patient-specific hypothalamic models system featuring differentiation into the MC4R neuron subtype. To generate such neuron subtype in vitro, we utilized a transcription factor approach. Specifically, 1) MC4R neuron-specific transcription factors were identified using single-cell RNA sequencing (10X Genomics) of the PVH region isolated from 5-weeks old C57BL/6Tac mice, 2) this transcription factor signature was confirmed in PVH sections from mice and human post-mortem hypothalamus using RNAscope, 3) transcription factors were tested for their capacity to induce the conversion of mouse embryonic fibroblasts into Mc4r neurons, 4) overexpression of transcription factors in human pluripotent stem cells generates functionally mature MC4R neurons. Our studies reveal that cell identity-defining transcription factors of Mc4r neurons of the PVH can drive the differentiation of human stem cells into MC4R-expressing neurons resembling those of the PVH. We will use this human neuron subtype-specific model system to investigate the pathogenetic molecular aspects of MC4R mutations in our patients as well as to perform drug testing in vitro.

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T-3181

NETWORK-BASED PREDICTION AND VALIDATION THROUGH SYSTEMATIC PROFILING OF CAUSAL SCHIZOPHRENIA GENES AND VARIANTS IN HUMAN NEURONAL AND GLIAL CELLS

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The recent increase in genetic discovery for psychiatric disorders has uncovered an undisputed genetic basis for schizophrenia (SCZ) and more than 100 robustly associated loci. However, the driver genes, variants, and mechanism of action of these loci remain uncharacterized, hindering the ability to translate genetic findings into novel drug targets and treatments for SCZ patients. To overcome this limitation, we integrate genetic, transcriptional and epigenomic evidence to prioritize driver genes and regulatory regions for gene-regulatory and neuronal/glial phenotypic assessment using in vitro derived human neurons and glia. First, we defined the “Brain regulatory genomic space” (NRGS) as the union of the genomic loci with putative gene-regulatory roles in the brain, by integrating epigenomic, chromatin interaction, genomic and evolutionary evidence. We next intersected these regions with 108 SCZ-associated genetic loci, resulting in 1062 putative disease-associated regulatory regions, corresponding to 0.12% of the genome. By linking each of these regions to their downstream target genes using chromatin conformation and eQTL genetic evidence we identified a total of 300 novel SCZ candidate target genes. Finally, we harnessed adult human brain single-cell expression profile data from 10,000 cells and intersected those with global human interactome maps using the Single-Cell Imputation and NETwork (SCINET) algorithm, to generate cell-type specific interactome maps and score the candidate genes through their connectivity profiles. We are currently conducting experiments to test the functional impact of perturbing high-scoring candidate genes using both neurons and glial cells, through the use of a programmable CRISPR-Cas9 system in neurons and microglia derived from human induced pluripotent stem cells (iPSCs) and assessing morphological and electrophysiological parameters indicative of functional synaptic transmission aberrations. Overall, our computational integration of multi-omic and single-cell datasets helps elucidate non-coding loci associated with SCZ and enables prioritization of novel candidate therapeutic targets for 2D and 3D human culture systems, which can help elucidate the mechanism of action of neuropsychiatric genes and enable new therapeutic avenues.

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T-3183

A HUMAN STEM CELL BASED MODEL FOR STUDIES OF CARDIAC HYPERTROPHY

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Cardiac hypertrophy is an important and independent risk factor for the development of heart failure. To better understand the mechanisms and regulatory pathways involved in cardiac hypertrophy, there is a need for improved in vitro models of the condition. In this study, we investigated how hypertrophic stimulation affected human iPSC-derived cardiomyocytes. The cardiomyocytes were stimulated with endothelin-1 for 8, 24, 48, 72 or 96 hours. To characterize the hypertrophic response, we performed transcriptional profiling using RNA sequencing to identify differentially expressed genes. Moreover, additional parameters such as cell size, ANP levels, NTproBNP levels and lactate concentration were analyzed. The gene expression data showed significant upregulation of key genes known to be related to a hypertrophic response but also novel genes were identified that have previously not been associated with cardiac hypertrophy. Interestingly, a number of these genes showed different transcriptional profiles over time. The same patterns could also be observed when the ANP and NTproBNP concentrations were analyzed in the conditioned cell culture medium. Notably, the NTproBNP concentration was 9-fold higher after 24 hours. The cardiomyocytes did also increase in size by approximately 15% when exposed to endothelin-1 for 48, 72 and 96-hours. Furthermore, lactate concentration in the media was significantly increased which demonstrates that the cardiomyocytes consume more glucose. The increase in glucose consumption is an important observation as it is a hallmark of cardiac hypertrophy. Taken together, these results show that hiPSC-derived cardiomyocytes stimulated with endothelin-1 display a hypertrophic response. The results from this study also provide new molecular insights about the underlying mechanisms of cardiac hypertrophy and may help accelerate the development of new drugs against this condition.

T-3185

PATIENT IPS CELL DERIVED NEURONAL CELLS AND ORGANOIDS AS DISEASE MODELS FOR NIEMANN PICK DISEASE TYPE-2

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Niemann Pick disease type C2 (NPC2) is a lysosomal storage disease caused by mutations in the NPC2 gene that encodes NPC2 protein. Deficiency in NPC2 in patients results in cholesterol accumulation in lysosomes and neurodegeneration. We have generated iPS cells from NPC2 patient dermal fibroblasts and differentiated them into neural stem cells and neurons. Both NPC2 neural stem cells and neurons exhibited the disease phenotype of lysosomal cholesterol accumulation and enlarged lysosomes, similar to these found in NPC1 cells. Treatments of the NPC2 cells with 2-Hydroxypropyl-β-cyclodextrin (HPBCD) and delta-tocopherol significantly reduced cholesterol accumulation and normalized the size of lysosomes. We are in the progress to develop the NPC2 iPS cells into neuronal organoids for a better model system for this disease to further study disease pathophysiology and to evaluate drug efficacy and toxicity.

T-3187

SMOOTH MUSCLE-LIKE LYMPHANGIOLEIOMYOMATOSIS CELLS MEDIATE ENDOTHELIAL CELL APOPTOSIS AND VASCULAR DISRUPTION BY RELEASE OF CYTOTOXIC MITOCHONDRIA AND EXOSOMES

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Smooth muscle (SMC)-like cells, harboring mutations in the mTOR signaling pathway, invade lungs in lymphangioleiomyomatosis (LAM) disease disrupting airway and vascular structures leading to respiratory failure. Mechanistic insights into how LAM-SMCs destroy the lung vasculature could lead to novel treatments. We posit that, unlike healthy SMCs which stabilize fragile vessels, LAM-SMCs promote disruption of lung microvasculature by

inducing endothelial cell (EC) apoptosis. iPSCs derived from LAM or healthy subjects were differentiated into SMCs using a teratoma protocol. The co-culture of healthy iSMCs with ECs on Matrigel enhanced network persistence for ≥72H, while LAM-iSMC co-culture led to rapid EC network collapse in <15H, and decreased EC expression of eNOS, TIE2, CD31 protein at 24-72H. LAM-iSMC conditioned media (CM) increased EC apoptosis at 24-72H, assessed by Annexin V/PI flow cytometry and increased cleaved caspase (Cas)-3, -9 protein expression. Observation that LAM-iSMCs spontaneously shed MitoTracker-labelled mitochondria (Mt) into the CM suggests that secreted Mt could adversely affect EC. Indeed, Mt isolated from LAM-iSMC reduced EC gene and protein expression, increased expression of the vessel-destabilizing, ANGPT2 and induced EC apoptosis. Conversely, apoptotic ECs released exosomes (Exo) containing translationally-controlled tumor protein (TCTP), an upstream positive regulator of the mTOR cascade. Uptake of TCTP-containing Exos by LAM-SMCs evidenced by immunofluorescence studies, was associated with increased levels of proliferation-associated phospho-mTOR-and-S6k protein. Inhibition of Exo secretion from ECs with GW4869 decreased phospho-mTOR-and-S6k protein expression in LAM-iSMCs, implicating uptake of TCTP in mediating the hyperproliferative state of LAM-iSMC. Notably, GW4869 also partially reversed the deleterious effects of LAM-iSMC-CM on EC protein expression, viability and apoptosis, in line with an Exo-mediated mechanism. In conclusion, LAM-iSMCs-derived Mt and Exo cooperate to disrupt vascular networks by inducing EC apoptosis. Conversely, the reciprocal uptake of TCTP-containing Exo released by apoptotic ECs enhances LAM-iSMC proliferation. These findings may provide insights into novel therapeutic targets for treating LAM disease.

T-3189

SCALE UP OF HUMAN PLURIPOTENT STEM CELL CULTURE FOR SC-ISLET DIFFERENTIATION (PANCREAS)

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Human pluripotent stem cell (hPSC)-derived pancreatic islets (SC-Islets) have the potential to treat insulin-dependent diabetes by replacing the missing insulin producing beta cells. To produce SC-Islets in quantities required for clinical trials, Semma has optimized 3D hPSC culture and directed differentiation in a multi-liter controlled system bioreactor. Semma's 3D hPSC scale-up optimization has resulted in a robust process that maintains pluripotency with consistent expansion, cluster size, and morphology. Parameters optimized include seeding density, RPM and dissolved oxygen. These optimal conditions resulted in differentiation efficiency similar to small-scale spinner flasks. Scaling up the process of producing stem cell derived tissues is essential for bringing stem cell-derived therapies to the clinic.

REPROGRAMMING

T-3191

COMPUTATIONAL ANALYSIS OF TIMESERIES SINGLE-CELL RNA-SEQ DATASETS TO UNRAVEL REPROGRAMMING DYNAMICS

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With the prevalence of single-cell RNA-sequencing (scRNA-seq), we now have an unprecedented access to high-resolution reprogramming trajectories. However, the interpretation of these timeseries reprogramming scRNA-seq datasets can be difficult due to the high dimensionality in both the number of cells and genes. Thus, many dimension reduction and pseudotime algorithms have been established to represent the data in a concise manner as well as describe the ordering of each single cells in the trajectory. To further elucidate the reprogramming dynamics, one can also identify the master transcriptional regulators driving the cellular identity of different cell populations. To this end, we have modified the Mogrify algorithm, a network-based algorithm to predict transcription factors driving cell state conversion, for use in timeseries single-cell datasets. In particular, we applied the Mogrify algorithm to identify key transcriptional regulators driving the different stages

of reprogramming into primed and naive pluripotency. Overall, we hope to better understand the mechanisms underpinning reprogramming through a better clarification of the gene regulatory dynamics.

T-3193

SINGLE CELL SEQUENCING REVEALS THE EXPRESSION CHANGES UNDERLYING REPROGRAMMING TO IPSCS AND CONSERVED MECHANISMS OF REPROGRAMMING PROCESSES

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Reprogramming somatic cells to induced pluripotent stem cells (iPSCs) by overexpression of Oct4, Sox2, Klf4, and cMyc (OSKM) is an inefficient process and leads to heterogeneous cell populations. Here, we performed single cell gene expression profiling on fibroblast-to-iPSC reprogramming time courses, and found that OSKM expression induced a continuum of cell states between MEF and pluripotent-like cell identities.These data revealed gradual transcriptional changes across the entire reprogramming culture over time, regardless of reprogramming progression, and that alternative cell identities unrelated to the cell type of origin only rarely form during the reprogramming process, and that a post-implantation epiblast-like state can emerge from established iPSCs. Reprogramming to iPSCs entails the step-wise silencing of the starting cell program starting with silencing of the most MEF-specific genes, induction of an intermediate state with expression of genes from various lineages in the same cell, prompted by the reprogramming factors in collaboration with AP1 TFs, and the installment of a strong proliferative signature linked to the upregulation of RNA-processing and chromatin regulators. As the silencing of the MEF program is finalized concomitant with the repression of AP1 TFs, pluripotency genes are strongly induced. The mesenchymal-to-epithelial transition (MET), previously identified as a critical reprogramming step, does not represent a sharp transition, but instead represents a continuous and prolonged process that starts with the expression of E-cadherin in the aforementioned intermediate state and only finishes when the pluripotency gene expression program is strongly induced. Regardless of cell type of origin, reprogramming to iPSCs occurs through an intermediate state in which genes from various lineages are co-expressed in the same cell. We show that a similar intermediate state occurs in direct reprogramming events, such as MEFs to induce neurons (iNs), albeit the co-expressed lineage genes differ and are reprogramming system-dependent, demonstrating that studies of iPSC reprogramming uncover general principles underlying TF-induced reprogramming processes.

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T-3195

HARNESSING P53 TO STABILIZE ACCELERATED REPROGRAMMING

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The tumor-suppressor protein p53 regulates proliferation and transcription, binds nucleosome-dense regions, triggers apoptosis, and maintains genomic integrity, serving as a guardian of cellular identity. In reprogramming, inhibition and knockdown of p53 enhances reprogramming suggesting that p53 functions as an inhibitor to cell fate transitions. However, we find that maintaining p53 levels enhances reprogramming by supporting a rare population of hypertranscribing, hyperproliferating cells. HHCs reprogram near-deterministically across diverse protocols. Conversion with a p53 mutant lacking a DNA-binding domain (p53DD) expands the HHC population and reduces signs of genomic stress that manifest as chromatin bridges and micronuclei. Addition of an shRNA targeting wildtype p53 reduces HHCs and reprogramming while increasing signs of genomic stress. In other systems, p53 activity controls genome stability via Topoisomerase I (Top1). We find that loss of Top1 phenocopies loss of p53, reducing HHCs and reprogramming while increasing genomic stress. Together our data suggest a model in which p53DD co-opts wildtype p53 functions to promote genome stability via Top1 in a privileged population of reprogramming cells. Our work highlights a more nuanced role for p53 in facilitating the transition of cells from one identity to another which can inform our understanding of cellular plasticity.

T-3197

CAUSE AND EFFECT OF ALTERED KLF4 STOICHIOMETRY ON SOMATIC CELL REPROGRAMMING

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Forced expression of the Yamanaka factors (OCT3/4, SOX2, KLF4, and c-MYC) reprogram somatic cells to induced pluripotent stem cells (iPSCs). Reprogramming provides a model for studying cell fate conversion as fibroblasts undergo a mesenchymal-to-epithelial transition (MET) and gain pluripotency. However, differences in reprogramming methodology impact Yamanaka factor stoichiometry, confounding comparative studies of the underlying mechanisms. Employing transposons, we assessed various 2A-peptide polycistronic reprogramming systems with a wide range of KLF4 expression levels. We reveal that high KLF4 induces transiently-expressed and functionally-undefined MET genes, yet achieves high-purity reprogramming. We identified the cell-surface protein TROP2 as a marker for cells with transient MET gene expression, and observed the emergence of cells expressing the pluripotency marker SSEA-1 mainly from within the TROP2+ fraction. Using TROP2 as a marker in CRISPR/Cas9-mediated candidate screening of MET genes, we identified the transcription factor OVOL1 as a potential regulator of an alternative epithelial cell fate. OVOL1+ cells express an array of non-iPSC MET genes and proliferate slowly. Thus, expression of OVOL1 improves reprogramming by inhibiting the proliferation of partially reprogrammed cells. Moreover, we demonstrate a technical aspect of polycistronic gene expression using 2A-peptides which alters protein sequence and may influence stoichiometry. Our study sheds light on how reprogramming factor stoichiometry alters the spectrum of cell fates, ultimately influencing reprogramming outcomes.

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T-3199

BECLIN1 MODULATES CARDIOMYOCYTE FATE INDEPENDENT OF ITS ROLE IN AUTOPHAGY DURING CARDIAC REPROGRAMMING

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Direct reprogramming of fibroblast to alternative cell fate by forced expression of transcription factors offers a unique platform to explore fundamental molecular events governing cell fate identity. The discovery and study of reprogrammed induced cardiomyocyte (iCM) not only provide alternative therapeutic strategies for heart disease but also shed lights on basic biology underlying CM fate determination. Recent advances in iCM field primarily focus on the early transcriptome and epigenome re-patterning, little is known about how reprogramming iCMs remodel, erase, and exit fibroblast program to acquire its final cell status. Here we show that autophagy, an evolutionarily conserved self-digestion process, was induced and required for iCM reprogramming. Surprisingly, autophagic factor Beclin1 (Becn1) was found to suppress iCM conversion in an autophagy independent manner. Depletion of Bcn1 resulted in much improved iCM generation. In a genetic mouse model of becn1 haploinsufficiency, delivery of reprogramming factors further improved heart function and decreased scar size upon myocardial infarction. Mechanistically, loss of Becn1 upregulated Lef1 and downregulated Wnt inhibitors, which subsequently activated canonical Wnt/b-Catenin signaling pathway. Furthermore Becn1 interacts with classical PI3K III complex to repress iCM conversion and Becn1-Lef1 interplay was positively regulated by ULK1. Collectively, our study revealed a previously unrecognized role of Bcn1 beyond autophagy to shape iCM cell identity during reprogramming.

T-3201

RESOLVING CELL FATE DECISIONS DURING SOMATIC CELL REPROGRAMMING BY SINGLE-CELL RNA-SEQ

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Reprogramming of somatic cells to a pluripotent state should be regarded as a breakthrough for basic biology. Reprogramming generates a spectrum of cell fates between somatic and pluripotent states. As such, investigation of this process could provide valuable insight into how cell fate and cell fate transitions are regulated. Indeed, by analyzing the cellular and molecular processes associated with reprogramming, it is clear that the process goes through a series of intermediate states with distinct molecular signatures, that have been mapped by comprehensive transcriptomic, proteomic and epigenetic studies, based on populations of cells. Despite progress made through bulk analyses as outlined above, very little is known at the single-cell level. The averaging of populations of cells tends to mask infrequent events during reprogramming, thus obscure very rare essential cellular transitions, or overemphasize irrelevant biological processes not required for reprogramming. The fact that only a small fraction of the starting cells eventually become pluripotent demands a vigorous reappraisal of principles and mechanisms at single-cell resolution. In this report, we mapped the cell fate changes during reprogramming generated by three approaches: Yamanaka factors, chemicals, and a seven-factor (7F) system at single-cell resolution. We show that the starting cells bifurcate into two broad categories of cells, reprogramming potential (RP) or non-reprogramming (NR). The RP cells go through mesenchymal-epithelial transition (MET) and then

acquire pluripotency along the reprogramming path in all three systems. The NR cells are diverse and dependent on culture conditions and factors employed. Preliminary analyses reveal previously unknown NR fates marked by Cd34+/Fxyd5+/Psca+ and Oct4+/Dppa5a-, Dcn+/Cdkn2a+ and Sox17+/Gata6+, and Ins2+ and Cdh2+/Sall4+/Oct4- in these three systems respectively. Mechanistically, we show that Klf4 contributes to Cd34+/Fxyd5+/Psca+ keratinocyte-like NR and IFN-γ impedes the final transition to chimera competent pluripotency during Oct4/Sox2/Klf4 mediated reprogramming. Our work reveals a general model for somatic cell reprogramming that may provide guidance for other cell fate decision process.

T-3203

IDENTIFICATION OF A NOVEL RECEPTOR WITH IMPLICATIONS IN INDUCTION OF STEMNESS IN ADULT AND CANCER CELLS

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In the recent years, active efforts are being made to increase the success of ex-vivo stem cell expansion and transplantation efficiency, by optimizing the culture conditions, as well as exploring alternative sources of stem cells. The alternative stem cell sources include mesenchymal stem cells (MSCs), dental pulp immature stem cells (hIDPSCs), embryonic stem cells (ESCs), hair follicle bulge cells and oral mucosal epithelium, with only the MSCs having reached the stage of a few clinical or pre-clinical studies. In each of these, there are safety concerns of potential transmission of contagious agents, tumorigenesis, or immune rejection owing to the presence of xenobiotic elements including murine derived-3T3 feeder cells, fetal calf serum and various animal-derived growth factors. We have developed a new method for reprogramming of adult cells called Serine Protease Induced Reprogramming (or SPIR) to generate stem cells. This reprogramming method is simple, rapid (24-72h), economic and efficient (without exogenous supplements or virus mediated transcription factors). The stemness was characterized by expression of stemness markers, arrest in G0/G1 stage of cell cycle, low proliferation (Ki 67), drug efflux, RNA sequencing, microarray analysis as well as ability for trans-differentiation into different lineages. The receptor associated with SPIR was also identified and called as SPR (serine protease receptor). The expression of SPR was observed to be upregulated in biopsy samples of triple negative breast cancers. Kaplan-Meier survival analysis using the RNASEQ data from ~10,000 patients in the The Cancer Genome Atlas (TCGA) dataset indicated that high level of expression of SPR gene correlates with poor survival profile in the breast invasive carcinoma (BRCA). This analysis also showed that the expression of this gene is significantly elevated in triple negative breast cancer as compared with non-triple negative breast cancers. These results suggest i) that the SPR gene is an independent predictor of poor overall survival; and ii) the SPR can be a potential therapeutic target of breast invasive carcinoma.

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T-3205

COMPARATIVE ANALYSIS OF DIFFERENT MEDIA CONDITIONS FOR GROWTH AND MAINTENANCE OF INDUCED PLURIPOTENT STEM CELLS

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The discovery of induced Pluripotent Stem Cells (iPSCs) had a great impact on fundamental stem cell studies and disease modeling; moreover, iPSCs have provided a new perspective on precision and regenerative medicine. Accordingly, several types of xeno-free and feeder-free media and matrixes were developed in the past decade to support culture of iPSCs. All these media facilitate continuous growth of Pluripotent Stem Cells (PSCs) in an undifferentiated state providing powerful models to improve our understanding of disease mechanisms and develop therapies to treat these diseases. Currently, international standards for the validation and characterization of PSCs are based on expression of self-renewal and pluripotency markers. However, in our view there is a lack of in depth molecular information regarding the true character and uniformity of PSCs. To do this, we reprogramed three different human healthy dermal fibroblasts using a CytoTune iPS 2.0 Sendai Reprogramming Kit. Transduced fibroblasts were plated on feeder plates, Geltrex, Matrigel, Vitronectin XF and Vironectin coated culture plates and cells were grown in human ES medium+ bFGF, StemFlex, mTeSR1, TeSR-E8, and Essential 8 medium, respectively. Four colonies from each condition were picked propagated till passage 8. Three colonies from each condition were selected based on the proper karyotype and elimination of CytoTune™ 2.0 Sendai vectors2.To gain more insight into differences or similarities between xeno-free and feeder-free media, we performed comparative transcriptome and methylome analysis of three iPS clones grown under different media conditions. DNA methylation was studied

genome wide using MeD-seq 1. lThis technology is based on a methyl dependent restriction enzyme LpnPI, which recognizes 50% of all methylated CpGs, and generates 32bp fragments for sequencing. This in depth molecular characterization of iPSCs generated under distinct media conditions will shed light onto the quality of each culture condition and its consequent ability to generate stem cells suitable for use in regenerative medicine and disease modeling.

T-3207

STELLA DOES NOT IMPROVE REPROGRAMMING EFFICIENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human embryonic stem cells (hESC) and human induced pluripotent stem cels (hiPSC) differ at the epigenomic level and in their differentiation potential. This points towards differences in resetting the genome-wide pluripotent epigenetic marks and suggests a superior reprogramming capacity of the oocyte when compared to iPSC reprogramming. We investigated the capacity of STELLA (DPPA3), a protein involved in epigenetic chromatin reprogramming in the zygote, to improve hiPSC reprogramming. In the mouse, supplementation of STELLA to the classical reprogramming factors significantly enhanced reprogramming kinetics and yielded iPSC with high-grade chimera competence. In this project, we generated hiPSC lines by lentiviral transduction of BJ fibroblasts with C-MYC, KLF4, SOX2 and POU5F1 and combined this with STELLA mRNA transfection during the first four days of reprogramming. We obtained 7 control iPSC lines and 6 STELLA iPSC lines and compared their transcriptional profile to that of 5 male control hESC lines and BJ cells using whole genome RNA sequencing. Expression analysis revealed no differentially expressed genes (DEGs) between control and STELLA iPSC. When comparing the control and STELLA iPSC directly to hESC, 23 and 26 DEGs, respectively, were detected. Of these, 18 genes were overlapping between these two groups. Similarly, when comparing the hPSC groups to the BJ fibroblasts, 8041 DEGs were detected in STELLA iPSC, 8113 in control iPSC and 7490 in hESC. Of these, 6969 genes were common between STELLA iPSC and hESC, 7026 between control iPSC and hESC, and 7649 between control and STELLA iPSC. In conclusion, we observed no effect of adding STELLA to the hiPSC reprogramming cocktail at the transcriptome level. Transcriptomes of both control and STELLA iPSC are highly similar to that of hESC, but show a common iPSC-specific DEG signature.

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TECHNOLOGIES FOR STEM CELL RESEARCH

T-3209

THE ENHANCED THERAPEUTIC EFFECT OF HUMAN DENTAL PULP STEM CELLS VIA CO-TRANSPLANTING WITH HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS ON CRITICAL HINDLIMB ISCHEMIA MOUSE MODEL

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Critical hindlimb ischemia (CLI) is the most severe clinical symptom among peripheral artery diseases (PADs), which are caused by narrowed arteries reducing blood flow to legs. CLI leads to ulcers on the leg and feet, which are difficult to be cured. Current therapies to treat CLI include artery stent

and artery bypass surgery in clinics, but there are side effects such as high restenosis. Dental pulp stem cells (DPSCs) are located within dental pulp, which have similar characteristics to mesenchymal stem cells (MSCs). Recently, DPSCs are reported to have perivascular characteristics suggesting the potential application as perivascular cell source. In this study, we investigated whether therapeutic effects of DPSCs could be enhanced co-injecting with human umbilical vein endothelial cells (HUVECs) on CLI animal model. The angiogenic potential was determined by in vivo Matrigel plug assay. The therapeutic effects in CLI model were evaluated by blood flow using laser doppler and ischemia damage scoring. In the results of in vivo Matrigel plug assay, DPSCs alone or HUVECs alone could not make microvessel-like structures. However, in co-injection of DPSCs and HUVECs, there were functional microvessel-like structures containing host red blood cells. This phenomenon was also confirmed in CLI animal model. Injection groups of DPSCs alone or HUVECs alone into CLI animal model showed better blood flow than HBSS injection group. However, co-injection group of DPSCs and HUVECs showed the highest blood flow among them. Moreover, in the results of the ischemia damage scoring, co-injection group of DPSCs and HUVECs showed the lowest scoring compared to other groups. In conclusion, it is expected that co-injection of DPSCs and HUVECs will enhance therapeutic effects in CLI.

T-3211

HIGH-LEVEL GENE EDITING IN HUMAN PRIMARY HEMATOPOIETIC STEM/PROGENITOR CELLS AND T LYMPHOCYTES

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The CRISPR-Cas9 system has revolutionized the field of genome editing. While precise gene editing of human hematopoietic stem/progenitor cells (HSPCs) holds great promise for curing life-threatening diseases such as severe immunodeficiencies and hemoglobinopathies, low levels of editing efficiency hamper the advancement of genome editing technologies toward clinical therapies. Our lab has been interested in identifying approaches that increase precise gene knockin efficiencies (Genome Biology, 2017; Nucleic Acids Research, 2018). In the current study, we focus on identifying optimal nucleofection protocols and culture conditions. We transfected human cord blood CD34+ cells with a RNP complex of Cas9 protein (IDT) and synthetic sgRNA (Synthego). When comparing several nucleofection programs of the Lonza 2b or 4D nucleofector, we observed up to 80% indels using the program DO-100 of the 4D nucleofector, compared to ~20% when using the program U-008 of the 2b nucleofector. For precise editing via homology-directed repair (HDR), we used an adeno-associated virus type 6 (AAV6) donor template carrying 600 bp homology arms. The HDR efficiency was determined by FACS analysis after precise insertion of the promoterless GFP cassette at the stop codon of EEF2. CD34+ cells were transduced with AAV6 immediately after nucleofection. We observed up to 60% precise genome editing (HDR) efficiency. Association analysis showed that 1) high-level editing was positively linked to greater proliferative capacity of the cord blood samples, and 2) indels and HDR efficiencies were closely correlated ($R^2 = 0.88$, $n = 7$). Using the same editing system, we obtained precise genome editing of 65% at the EEF2 locus and gene disruption efficiency of 95% at the CD326 locus in primary T cells from human peripheral blood. Ongoing transplantation studies in immunodeficient NSG mouse will determine the editing efficiencies in long-term HSCs. Currently, we are also screening small molecules and exosomes in hopes to identify optimal conditions for high-level HSC expansion and gene editing. We believe that further investigations in the same vein will contribute to successful clinical gene therapies based on HSCs or T cells.

T-3213

MULTIWELL MICROELECTRODE ARRAY TECHNOLOGY FOR THE EVALUATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTE AND NEURON DEVELOPMENT, OPTIMIZATION, AND VALIDATION

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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 and neuron 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, whereas the HESI NeuTox consortium represents a collective interest in the development and application of human iPSC-derived neuron models. An important element of these initiatives, and others, is the continued improvement and optimization of the commercial cell products, including the differentiation protocol, the manufacturing process, and the consumables required for development. Furthermore, advanced cells preparations, such as spheroids or organoids, are under intense investigation with aims towards establishing mature human phenotypes in vitro. Here, we present data supporting the use of multiwell microelectrode array (MEA) technology as an efficient approach for the development, optimization, maturation, and validation of human iPSC-derived neuron and cardiomyocyte models. A planar grid of microelectrodes embedded in the substrate of each well interfaces with cultured networks, such that the electrodes detect the raw electrical activity from the cells. Recent advances in MEA technology afford the quantification of the shape and propagation of the cardiomyocyte action potential, as well as the mechanical contraction of the cardiomyocytes. By comparison, the organization of the cellular activity across neurons within a network and across time can be described by measures of activity, synchrony, and oscillations to quantify phenotypes of network electrophysiology. For both neurons and cardiomyocytes, the discrete nature of the MEA technology easily adapts to the localized cell populations associated with advanced preparations like spheroids. These results support the continued development and use of human iPSC-derived cardiomyocyte and neural assays on multiwell MEA technology for high throughput drug discovery and safety assessment.

T-3215

SELECTIVE CELL DEATH OF HUMAN PLURIPOTENT STEM CELLS BY YM155 IS DUE TO HIGH EXPRESSION OF SLC35F2

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Application of human pluripotent stem cells(hPSCs) to cell therapy is being hampered by its riskiness of teratoma formation. Varied approaches including small molecules has been attemped to resolve this problem. Previously, we have reported that treatment of YM155, a Survivin inhibitor, selectively eliminates hPSCs by inducing p53 mitochondrial translocation and following apoptosis. However, it remained unclear how YM155 selectively eliminates hPSCs while leaving differentiated cells intact. Herein, we took advantage of bulk cancer cell line gene expression data and drug response data from the Cancer Cell Line Encyclopedia (CCLE) and Cancer Therapeutics Response Portal (CTRP), respectively to identify the mechanism underlying selective cell death of hPSCs. Using data from gene expression omnibus (GEO), we identified a hPSC signature and calculated hPSC scores for human cancer cells. By correlating the hPSC scores with the sensitivities of each cell line to each compound from database, we identified YM155 as the most effective drug in cells with high hPSC scores. Based on correlation of drug and hPSCs score, SLC35F2 was the transcript most correlated with YM155 sensitivity suggesting that cellular import of YM155 by SLC35F2 is responsible for the highly selective cytotoxicity of the drug in hPSCs. We could conclude SLC35F2 is responsible for YM155 mediated cell death in hPSCs by seeing both knocking down and knocking out SLC35F2 induced resistance on YM155 in hPSCs.

T-3217

OPPORTUNITIES FOR SCIENTIFIC SYNERGY ACROSS REGENERATIVE MEDICINE, CELL THERAPY, AND THE CELL-BASED MEAT INDUSTRIES

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The concept of cell-based meat, where animal stem cells are grown into muscle and fat tissues in vitro for consumption, has recently been catapulted into the public sphere thanks to advances in stem cell biology, tissue engineering, and bioprocessing. Here, we discuss the opportunity to address shared challenges across the fields of regenerative medicine, cell therapy, and cell-based meat. These challenges, such as the development of low cost, serum-free medium formulations, the creation of edible or biodegradable scaffolds to replicate multicellular architecture, and cell harvesting, may share similar methodologies, equipment, and legal frameworks under which they are performed. Additionally, the reproducible production of hundreds of billions of cells will involve exploration of new scale-up methodologies and bioprocessing strategies that may translate to other industries. Thus, interdisciplinary research leading to the development of new tools, methods, and materials across these industries promises to provide synergistic outcomes for all.

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T-3219

MECHANOBIOLOGICAL CONDITIONING OF HMSCS INTO A HYBRID ENDOTHELIAL-PERICYTE PHENOTYPE ENHANCES THERAPEUTIC ACTIVITY IN TREATING ISCHEMIA

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Stem cell therapies have great promise for treating cardiovascular disease. However, these therapies have yet to reach their full potential due to poor efficacy in clinical trials. Here, we created a high throughput screening system allowing the application of mechanical load to cultured cells in 576 individual culture wells simultaneously. We used this novel screening system to study mechanical conditioning of human MSCs towards vascular cell phenotypes using combinations of pharmacological inhibitors and biomechanical forces. We investigated various dynamic strain waveforms for their ability to activate the Hippo and TGF-β signaling pathways and found the optimal activation occurred when the cells were treated with 7.5% strain waveform at a frequency of 0.1 Hz using a complex physiological loading waveform. In addition, we screened a library of small molecule kinase inhibitors and found a set of compounds that synergistically increased nuclear Yap/Taz localization and Smad2/3 phosphorylation. Specific combinations of these pharmacological inhibitors and a complex dynamic mechanical waveform dramatically increased the simultaneous expression of endothelial cell (EC) and pericyte markers in MSCs as measured by flow cytometry and immunostaining. Simultaneous expression of EC and pericyte genes were also observed through by RNA Seq, and we also saw enhanced in vitro angiogenic activity of the cells. In addition, these mechanically and pharmacologically conditioned cells had enhanced angiogenesis in subcutaneous implantation model and increased revascularization in mice with hind limb ischemia. Our results demonstrate a new phenotype in MSCs that has combined properties of ECs and pericytes, and which has increased regenerative capacity for treating peripheral ischemia.

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T-3221

PRECONDITIONING OF CANINE ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS WITH DEFEROXAMINE POTENTIATES ANTI-INFLAMMATORY EFFECTS BY DIRECTING/ REPROGRAMMING M2 MACROPHAGE POLARIZATION

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Preconditioning with hypoxia or hypoxia-mimetic agents has been tried with mesenchymal stem cells (MSCs) to improve the secretion of anti-inflammatory factors. These preconditioning procedures upregulate hypoxia inducible factor (HIF) 1-alpha leading to the transcription of HIF-dependent tissue protective and anti-inflammatory genes. Due to the limited number of studies exploring the activity of deferoxamine (DFO)—a hypoxia-mimetic agent—in MSCs, we aimed to determine whether DFO can enhance the secretion of anti-inflammatory substances in canine adipose tissue-derived (cAT)-MSCs. Furthermore, we investigated whether this activity of DFO could affect macrophage polarization and activate anti-inflammatory reactions. cAT-MSCs preconditioned with DFO exhibited enhanced secretion of anti-inflammatory factors such as prostaglandin E2 and tumor necrosis factor-α-stimulated gene-6. To evaluate the interaction between DFO preconditioned cAT-MSCs and macrophages, RAW 264.7 cells were co-cultured with cAT-MSCs using the Transwell system, and changes in the expression of factors related to macrophage polarization were analyzed using the quantitative real-time PCR and western blot assays. When RAW 264.7 cells were co-cultured with DFO preconditioned cAT-MSCs, the expression of M1 and M2 markers decreased and increased, respectively, compared to co-culturing with non-preconditioned cAT-MSCs. Thus, cAT-MSCs preconditioned with DFO can more effectively direct and reprogram macrophage polarization into the M2 phase, an anti-inflammatory state.

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T-3223

SELECTION OF HIGHLY CHARACTERIZED INDUCED PLURIPOTENT STEM CELL CLONES USING HIGH THROUGHPUT ROBOTIC SYSTEM FOR CELL SOURCE QUALITY CONTROL IN MANUFACTURING

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This paper demonstrates the phased implementation of the integrated Cell X Platform to develop rigorously documented, repeatable, and reproducible iPS cell manufacturing using critical quality attributes (CQAs) based on quantitative image analysis parameters. Induced pluripotent stem (iPS) cells are being developed for a broad range of research and therapeutic applications. However, reprogrammed iPS clones are inevitably heterogeneous. Manual clone selection based on subjective visual inspection is commonly used. These methods are prone to large variation performance, and lack the standardization and reproducibility that is needed for scaling and clinical translation. This work is utilizing the Cell X Platform in an automated platform to enable standardized iPS clone selection and expansion for cell fabrication. The DF6-9-9T.B hiPS cell line, and iPS reprogramming of skin fibroblasts and peripheral blood mononuclear cells was used to develop our image processing parameters for front end clone selection and downstream "weeding" of differentiated cells during clone expansion. The Cell X Platform is integrated with an automated quantitative cell and colony analysis software. Large field of view quantitative live cell image analysis enable attributes (potential CQAs) of "completely" reprogrammed iPS cells to be defined and tested in systematic repeatable and reproducible manner. The principles and nomenclature enabling this approach are outlined in ASTM Standard F2944-12. To automate manipulation of iPS cells in rapid, precise, repeatable and rigorously documented manner, the Cell XTM device utilizes an automated Olympus IX83 microscope and Zernike phase contrast imaging, and Parker Hannifin automation components to prove cell-manipulation (biopsy, pick, weed), fluid handling, and motion control systems with micron and microliter level precision. Automated image processing provides quantitative metrics (size, area, density, as well as shape, perimeter and surface texture attributes), as well as x,y,z coordinates at a cell, colony, cell region, or well level. Based on these metrics, we demonstrate that a user can quantitatively define iPS clones or regions of interest and selectively biopsy local cells, pick and transfer the cells for expansion, or remove undesirable cells.

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T-3225

AN INDUCIBLE BICISTRONIC SUICIDE GENE CONSTRUCT IMPROVES UPON THE IN-VITRO AND IN-VIVO EFFICIENCIES OF SINGLE CASSETTES

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Inducible suicide genes are designed to provoke cellular death upon activation. This property is especially useful in therapeutical applications in which exogenous, ex-vivo modified cells are introduced into patients. If the transplanted cells are provided with inducible suicide constructs, the activation of the transgenes could eliminate harmful effects arising from their malignant transformation. The Herpes Simplex Virus (HSV) deoxythymidine kinase (TK) gene and the iCaspase 9 inducible construct are the two most used of such cassettes. Although both exhibited promising results in a considerable number of cell types, none has shown complete elimination of the targeted cell. In order to improve those results, we have devised a bicistronic construct in which HSV-TK and iCAspase9 are joined by a 2A element in a same coding unit. The construct has been tested in vitro in human colon carcinoma HCT116, human placenta choriocarcinoma JAR, human prostate adenocarcinoma PC3 and human iPS cell lines, and in vivo upon subcutaneous injection of the cells into nude mice. In all cases, activation of the two suicide genes produces significantly better results that each one of them alone. We have also observed that serial activation of the two genes improves upon the efficiency of simultaneous activation, but it is dependent on the order in which the genes are set into action.

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T-3227

A NOVEL QUALITY CONTROL METHOD FOR REMOVAL AND MONITORING OF RESIDUAL PLURIPOTENT STEM CELLS WITHIN A LARGE NUMBER OF DIFFERENTIATED CELLS BY MULTI-STEP NEGATIVE SORTING

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In the field of regenerative medicine involving embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, differentiated cells are implanted to patients. However, studies have shown some remaining undifferentiated cells after implantation may lead to tumors, hindering the advancement in patient treatment. Therefore, there is a demand for a method that efficiently removes such undifferentiated cells prior to implantation while quantitatively monitoring the number of the remaining cells from the quality control perspective. To remove undifferentiated cells, we have developed a novel method called the multi-step negative sorting. This uses a disposable microfluid chip-based cell sorter, On-chip Sort (On-chip Biotechnologies Co., Ltd). The advantages of the system include damage-free and aseptic sorting, and full sample volume analysis. The entire sample can be analyzed without any dead volume, and thus it enables absolute quantification of residual iPS cells. In this method, the sample is loaded to sample reservoir of the chip, and undifferentiated cells are removed by the formation of gentle pulse flows into waste reservoir, while differentiated cells flow into collection reservoir located downstream. These cells are then recovered and reloaded to the sample reservoir for further clean-up. This is repeated until all the undifferentiated cells are removed. Undifferentiated cells were fluorescently labelled with anti-Tra-1-60 antibody, and the fluorescence signals are used as triggers for the detection and sorting pulses. As an assessment of undifferentiated cell removal efficiency within a large number of cells, samples containing 10^8 MOLT4 cells spiked with known number of iPS cells were sorted. The time required to completely remove 10^4 iPS cells was about 1 hour after 5 sorting cycles, and about 60% of MOLT4 cells remained. The flow rate of MOLT4 cells was estimated at over 150,000 cells/sec. Handling of cells in such high speed is not attainable using standard cell sorting systems. Number of residual iPS cells can be monitored from the flow cytometry data of full sample volume analysis during each sorting steps. Therefore, the multi-step negative cell sorting is the optimal quality control method for removal and monitoring of residual iPS cells within a large number differentiated cells.

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T-3229

SINGLE NUCLEOTIDE POLYMORPHISM-INDUCED ALTERATION OF CYP ENZYME ACTIVITY IN HUMAN IPS CELL-DERIVED HEPATOCYTE-LIKE CELLS

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Drug-induced liver injury is a main cause of drug attritions in pharmaceutical development. Recent studies have demonstrated that human pluripotent stem cell (hPSC) could be an alternative source of human hepatocytes for drug development and toxicity screening tests. However, single nucleotide polymorphism (SNP) in cytochrome P450 (CYP) genes contribute to interindividual differences in hepatic metabolism of drugs, affecting individual drug efficacy and potentially causing adverse effects. Here, we generated human induced pluripotent stem cell (hiPSC) lines with pharmacologically important traits (CYP3A5*3C), which are highly polymorphic in Asian from patient cells. The CYP-polymorphic hiPSCs were differentiated into a highly enriched population of hepatocyte-like cells (CYP-hiPSC-HLCs) and were characterized by evaluating gene and protein expressions and drug metabolizing activities. Our results showed that CYP-hiPSC-HLCs displayed multiple morphological and biochemical features of hepatocytes and expressed key hepatic markers, including albumin and hepatocyte nuclear factor-4α. SNP genotyping assay revealed that the differentiated CYP-hiPSC-HLCs retained the same genetic polymorphism with that of iPSC line after hepatic differentiation. Importantly, CYP-HLCs exhibited a significantly low CYP3A5 activity, reflecting the genetic polymorphism of patients. Our study demonstrates that establishment of iPS cell lines with different SNPs in CYP genes, and generation of hepatocytes from these cell lines may allow the prediction of metabolism and potential toxicity of new drug candidates for personalized drug therapies.

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T-3231

EFFECTS OF SMALL MOLECULES ON MICROHOMOLOGY-MEDIATED END JOINING AND GENOME EDITING SPECIFICITY IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Efficient and precise genome editing is essential for fulfilling the promise of human induced pluripotent stem cells (iPSCs) in translational research involving disease modeling and therapy development. Until recently, site-specific genome engineering in primary and non-transformed human cells has been challenging. However, recent developments in editing technologies have facilitated genome editing in these cells through the creation of double strand breaks (DSBs) at pre-defined genomic locations. DNA repair templates (homology donors) are often co-delivered into the cells along with a nuclease. When the homology-directed repair (HDR) machinery is active, the donor template can be integrated at the site of the DSB as a result of cellular DNA repair. Studies have identified various classes of small molecular weight drugs that can affect the balance between HDR and its competing pathway of non-homologous end joining, which often leads to donor-independent error-prone DNA repair. The effects of these drugs on overall fidelity of nuclease-mediated editing, however, have not been systematically investigated. More recently it has been shown that another DSB repair mechanism, microhomology-mediated end-joining (MMEJ) can be exploited to generate defined edits at sites of DSBs generated by nucleases. The advantages of this alternative end-joining mechanism include smaller homology regions required for repair, significantly simplifying the process of donor template construction. In this study, we aimed to examine the efficiency of creating reporter knock-in iPSC lines using an MMEJ approach and to determine the effect of chemical treatment on nuclease specificity. A MMEJ GFP-reporter donor vector targeting the RUNX1 gene was co-delivered with CRISPR/Cas9 into human iPSCs in the presence or absence of small molecules, including L755507 and brefeldin A (BFA). Genomic DNA was isolated from expanded individual drug-resistant clones and analyzed by PCR for targeted integration (TI) events. Our results indicated that TI efficiency could be significantly improved by BFA and L755507, which have been shown to enhance HDR-mediated repair, in a concentration-dependent manner. Studies are currently under way to determine the effects of these small molecules on nuclease specificity and overall genomic integrity.

T-3233

HUMAN PLATELET LYSATE FOR THE CULTURE OF DENTAL PULP MESENCHYMAL STROMAL CELLS

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Some preclinical and clinical studies are using human platelet lysate (hPL) instead of FBS to expand mesenchymal stem/stromal cells in vitro in order to eliminate the risk of xenogeneic immune reactions and transmission of bovine prion and viral pathogens during cell therapy. Human dental pulp mesenchymal stromal cells (DPSC's) have been considered a potentially good source for cell therapy since they present immunomodulatory and osteogenic properties. But, almost all published in vitro studies used FBS to expand DPSC's. In the present study DPSC's from third molars of three patients were isolated and expanded using a culture medium with FBS or hPL (obtained by processing a pool of six apheresis-derived platelets), then cell molecular signatures were evaluated to check whether hPL significantly modified DPSC's features. Cell expansion until P5 allowed to obtain a minimum amount of cells which have been required for some stem cell-based therapy in humans. Both groups (FBS and hPL) showed similar immunophenotypes (CD34, CD45, CD90, CD73, CD19, HLA-DR, and CD146), cell differentiation potential (osteogenic, adipogenic and chondrogenic), normal karyotypic profile and no senescence signal. A genome-wide DNA methylation analysis (RRBS - Reduced Representation Bisulfite Sequencing) showed only 2,620 differentially methylated

cytosines (DMCs) from a total of 914,542 evaluated. Comparing DMCs from FBS x hPL samples, 1,369 were hypermethylated and 1,252 were hypomethylated. Both groups had the DMCs enriched for intergenic regions, open sea, shelf and shore ($p < 0.001$). Only hypomethylated DMCs were enriched at enhancers regions ($p< 0,004$). When compared FBS x hPL samples, RNA-seq data analysis showed 159 differentially expressed genes (DEG) and Ingenuity Pathway Analysis indicated 10 pathways enriched for DEGs, with inhibition trend toward NRF2-mediated Oxidative Stress Response and NF-κβ Signaling pathways. Mass spectrometry-based proteomics revealed 378 differentially expressed proteins (FBS x hPL), of which 211 were highest expresses in FBS samples and 167 in hPL samples. Taken together, the results indicated that hPL, in substitution to FBS, does not cause significant changes in DPSC's biology in the evaluated parameters and hPL is a good choice to expand these cells for clinical purposes.

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T-3235

HIGH THROUGHPUT AMENABLE GENE EDITING TOOLS FOR FUNCTIONAL GENOMIC AND ENGINEERED CELL LINE DEVELOPMENT

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Latest advancements in genome editing technologies has revolutionized gene therapy and opened up new opportunities for cancer therapy and treating diseases related to mutations in the genome. With editing tools like CRISPR-CAS9 researchers are able to efficiently knockout genes of interest and study its functions, or knock in a specific change into the genome of interest and correct a disease relevant mutation. However there is a significant need for easy-to-use and efficient high throughput (HTP) gene editing workflows which will enhance the capability of these tools for genome wide screening applications or disease model generation in challenging cell types. The work described here includes 1) HTP amenable tools and optimized workflows including multiplexed gene editing using pre-complexed CRISPR-Cas9 ribonucleoprotein (RNP) and 2) comparison of pre-complexed CRISPR/Cas9 RNP formats to the existing genome-wide functional knockout screening tools such as LentiArray CRISPR and siRNA libraries for challenging cell types including primary T Cells and iPSC cell lines. To date, we have observed up to 80% editing efficiency with multiplexed RNP complex targeted to simultaneously edit six different genes in T Cells. Data also shows that the pre-complexed CRISPR- Cas9 RNP complex described here is stable for at least six months without compromising the editing efficiency when tested in T-Cells giving the user the flexibility to reuse the mix for subsequent experiments. The HTP tools and protocols developed through this work will expand the toolbox capability for disease modeling and drug discovery by enhancing overall productivity thereby accelerating biotherapeutic research.

T-3237

PERFUSION CULTURE FOR EXPANSION OF INDUCED PLURIPOTENT STEM CELL

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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 300 folds the volume of culture from 5mL to 1500mL. These our bioreactors are suitable for cell aggregates formation and proliferation of iPS cells, growth and maturation of organoids. The iPS cells need to change the medium at a predetermined schedule to maintain undifferentiated state. The process of exchanging the medium is simple and labor intensive, but risks contamination. In the case of small-scale culture, automation of the process by robot is an option, but in the case of large-scale tank culture, a method of continuous perfusion in a closed system is preferred. Continuous perfusion by membrane separation is the mainstream in bioprocessing, but in iPS cell culture there is a risk of interfering with the growth of aggregates due to shear stress during liquid transfer. We propose a method of continuously separating cell aggregates and culture medium by a separation tube installed in the headspace of a culture tank. The cell aggregates settle in the separation tube and the medium is continuously withdrawn from the top of the separation tube. This method does not affect the growth of cell aggregates, and safe and reliable medium exchange is possible. The separation tube we designed in this way is extremely simple. In this meeting, we introduce a case of continuous perfusion of human iPS cell aggregates culture using 500 mL reactor.

T-3239

ENGINEERED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED BLOOD BRAIN BARRIER-CHIP FOR DISEASE MODELING

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Specialized brain microvascular endothelial cells (BMECs) form a tight monolayer of cells lining the brain vasculature. These cells express specific tight junction and transport proteins that help create the blood brain barrier (BBB), a multicellular neurovascular unit composed of endothelial cells, astrocytes, pericytes, and neurons that closely regulates the entry of solutes and molecules from the blood into the CNS. Using a micro-engineered poly(dimethylsiloxane)-based Organ-Chip (Emulate, Inc.), we have developed an entirely human BBB-Chip with iPSC-derived iBMECs, astrocytes, and neurons. The BBB-Chip exhibits physiologically relevant transendothelial electrical resistance, has spontaneous neuronal activity, responds to relevant inflammatory cues, and accurately predicts blood-to-brain permeability of pharmacologics. Using this system, we can model the response of iBMECs to various levels of shear stress and show that the capillary wall formed by iBMECs in the BBB-Chip protects neural cells from plasma-induced toxicity when the BBB-Chip is perfused with whole human blood. Furthermore, by combining the BBB-Chip with iPSC-derived tissues from patients with neurodegenerative disease, we can accurately model the disruption of barrier integrity and the specific loss of transporters that occurs with these illnesses. In summary, we have developed a novel platform that recapitulates complex BBB functions, provides a new way to model inheritable neurological disorders, and advances drug screening capabilities.

Funding Source: California Institute for Regenerative Medicine grant ID DISC1-08800, the Sherman Family Foundation, and the Israel Science Foundation (grant 1621/18).

T-3241

PRECISE CREATION AND CORRECTION OF A KATP-CHANNEL MUTATION USING MICROHOMOLOGY-ASSISTED SCARLESS GENOME EDITING IN HUMAN IPSCS

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The combination of induced pluripotent stem cells (iPSC) and nuclease-mediated genome editing holds great promise to study human genetics and the basis of genetic disease, with eventual therapeutic outcomes. Using a recently reported editing method, involving the enrichment for targeted clones with selection markers and the subsequent scarless excision of the markers through endogenous microhomology mediated end joining (MMEJ) repair, it is possible to more precisely investigate single-base mutations in human cells. We have derived iPSCs from a Japanese male who has a severe form of neonatal diabetes caused by a single 878C>A base change in the KCNJ11 gene, which encodes for a subunit of the ATP-sensitive potassium channel. Initial targeting of this gene in the patient iPSCs or wildtype PSCs was performed to insert a selectable transgene with overlapping homology arms containing a corrected or mutated base and CRISPR-Cas9 protospacer elements. Following clonal isolation and validation, a second targeting step was performed to excise the transgene at the protospacers, activating MMEJ repair and depositing the altered base. Differentiation of these engineered stem cells and their isogenic controls into beta-cells is expected to reveal the developmental and functional consequences of the mutant channel and serve as a tool to probe disease progression and therapeutic strategies.

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LATE-BREAKING ABSTRACTS

T-4001

RESPONSES OF HUMAN NEURAL PROGENITOR CELLS TO TRAUMATIC BRAIN INJURY-LIKE DAMAGE

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Stem cell technology has opened new avenues for studying a variety of human pathophysiological processes, in particular modeling disease progression in vitro in living cells. Traumatic brain injury (TBI) is one neurological pathology that occurs frequently, even in everyday settings such as falls and car accidents. Although traumatic brain injuries can be chronically debilitating for patients and their caregivers, negligible clinical regenerative medicine treatments exist to help patients recover. To help identify potential targets for future cell or molecular therapy design, our objective is to identify specific molecular pathologies of TBI-like injuries and correlate these with different types of cell damage (chemical or mechanical). We mimic TBI using two in vitro models that produce cell tearing or transient excess nitric oxide production, respectively, and study the molecular consequences of injury using a representative human neural progenitor cell line (ReNcell VM, Millipore). Control cells proliferate over multiple passages with monolayer adherent cell morphology and persistent expression of neural stem cell transcription factors Sox2 and Nestin, detected via immunofluorescence and flow cytometry. We can routinely extract RNA suitable for quantifying gene expression levels from moderate cell numbers (~0.5 million cells; 3.25 ± 0.5 ng total RNA/cell). Ongoing studies are measuring cell viability and gene expression related to differentiation and acute cell stress state to compare between control groups, mechanically-induced injury, and chemically-induced injury. Genes selected for analysis have been chosen from a custom computational meta-analysis we performed of PubMed-indexed TBI literature. Results from these molecular mechanism studies may ultimately help identify potential therapeutic targets to treat debilitating brain injuries.

Funding Source: Fellowship to OMK via CIRM Bridges Program from California State University-Channel Islands. URCA support for RS from the University of California Santa Barbara.

T-4003

GENERATION OF FUNCTIONAL LIVER ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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Organoids, a miniaturized three-dimensional (3D) organ-like structure which is self-organized hold a great potential for both disease modeling and drug screening. Previous outstanding studies described the generation of organoids recapitulating actual organ morphogenesis and development such as brain, intestine, and lung. In this study we described the step-wise protocol for robustly generating 3D liver organoids which could stably be maintained over several months. Our organoids display a homogenous structure consisting of mostly hepatocytes expressing multiple mature hepatic markers as well as CK19-positive cholangiocytes. Moreover, our liver organoids are superior to hepatocytes which were typically differentiated via 2D condition in terms of their functionality. Our step-wise approach for robustly generating 3D liver organoids might provide as a useful platform for understanding various liver diseases as well as drug discovery.

T-4005

SELF-ASSEMBLY OF HUMAN NEO-VASCULARIZED SKIN AND SKIN ORGANOIDS

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Stem/progenitor cells bear the potential to self-organize under appropriate conditions forming organoids that are considered to resemble organ functions in vitro. Here we established a humanized skin regeneration mouse model, based on self-assembly of adult as compared to iPSC-derived skin cell lineages under xeno-free conditions resulting in organized neo-vascularized human skin. Adult inter-follicular epidermal keratinocytes (KC), skin fibroblasts (FB) and endothelial cells (EC), were isolated and propagated in 2D under xeno-free conditions. Umbilical cord blood-derived induced pluripotent cells (iPSC) were differentiated into iPS-KC, -FB and -EC in addition. Cell viability, identity and purity were confirmed by microscopy, flow cytometry and clonogenicity indicating their stem/progenitor potential. In vitro triple cell type skin organoid formation was used to establish the role of human platelet-derived growth factors during self-assembly. Life cell tracking revealed sequential organoid assembly starting from stromal-

vascular aggregation and followed by superficial anchorage of KC. Xeno-free human cell grafts, containing a mixture of KC, FB and EC in human platelet lysate were transplanted onto full-thickness wounds of NSG mice using an artificial transplant chamber to circumvent lateral murine skin contraction. Two weeks after transplantation, histological analysis demonstrated appropriate cell organization into layered skin. Dermal analysis showed a regular distribution of collagen fibers and ground substance. Immune-histochemistry confirmed the human origin of the grafts as well as a combination of murine and human neo-vasculature. Quantification showed significantly increased vessel numbers upon co-transplantation of EC compared to limited murine in-sprouting angiogenesis after transplantation of KC+FB in the absence of human EC. These data show that self-assembly of human KC+FB combined with co-transplantation of EC can create complex organoids in vitro and neo-vascularized human skin in vivo. This technology has broad applicability in studies of skin regeneration and skin pharmacology, but also builds the basis for novel therapeutic strategies.

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T-4007

POLYGENIC ARCHITECTURE INFORMS POTENTIAL VULNERABILITY TO CHOLESTATIC DRUG-INDUCED LIVER INJURY

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Drug-induced liver injury (DILI)-related studies have received increasing attention worldwide, since DILI is one of the leading side effects of drug withdrawal resulting from unpredictable high mortality in both children and adults. However, it is still difficult to generate preclinical cellular models that accurately describe relatively rare diseases such as DILI. Genome-wide association studies (GWAS) in a dish strategy is an evolving approach to study phenotypic variation, potentially advanced by modelling more complex inherited traits. This proof-of-concept study showed that in vitro human disease models coupled with polygenic risk score (PRS)-based stratification reflects a phenotypic liability for unpredictable DILI. Based on the GWAS using more than 800 DILI patients for 150 or more drugs, we developed the PRS to predict DILI vulnerability by aggregating the effects of tens of thousands of common SNPs. The predictive capacity of the PRS for cholestatic DILI was confirmed in an independent human clinical test. Furthermore, we validated that multi-donor primary hepatocytes and iPS cell-derived liver organoids showed a significant association between the PRS and hepatotoxic response by multiple drugs. Enrichment pathway analysis from GWAS and cell-based assay highlighted diverse vulnerable cascades including reactive oxygen species, potentially alleviated by a potent antioxidant. Furthermore, from high-risk transcriptomic signatures, in silico 2,546 compound screening indicated two potential cholestatic drugs (either commercial or under trial) with clinical DILI evidence. Thus, we have also found the therapeutic potential for targeting DILI vulnerability-related pathways. This genetic-, cellular-, organoid- and human-level evidence underscored the vulnerable mechanisms in polygenic architectures, thus facilitating future mechanistic toxicology studies. Moreover, the proposed "polygenicity-in-a-dish" strategy potentially contributes to prospective designs of safer, efficient, and robust clinical trials.

T-4009

INTER-CELLULAR COMMUNICATION FACILITATES COAGULATORY FUNCTIONS IN HUMAN LIVER BUD ORGANOIDS

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Recent advances in multicellular organoid cultures have demonstrated promise as a human tissue replica to study human biology with a future potential to treat diseases. Transcriptomic studies highlight the activation of diverse gene signatures specific to organoids; yet, it remains elusive how inter-cellular communications impact more complex human physiology at functional level. We herein reports multi-cellular liver bud organoid (LB) culture supernatant from human induced pluripotent stem cell (iPSC) corrected multiple coagulation defective phenotype with decrease of activated partial thromboplastin time in plasma from coagulation factor 2, 5, 8, 9 and 11 deficient patients, respectively. Among these coagulation factors, LB highly secreted coagulation factor 8 (CF8) and had CF8 activity by using human chromogenic activity assay. Surprisingly, its CF8 activity was higher and was more persistent than those of mature hepatocyte (MH) and endothelial cell (EC) from iPSC. Moreover, CF8 secretion from EC was significantly increased by adding hepatocyte. In this condition, EC expressed both CD31 and LYVE-1, indicated that EC might be changed to be liver sinusoidal endothelial cell. Therefore, hepatocyte-endothelial interactions were considered to be important to secrete CF8. Furthermore, we showed that supplementation of LB supernatant rescued coagulation function of hemophilia A mice in vivo. Thus, we showed interactive functional protein cascades can be modeled in multicellular organoids, facilitating further mechanistic study of human physiology. More broadly, given that plasma product entails critical risks for infection such as HBV, HCV and HIV, defined organoid supernatant product might serve as potentially safer and effective therapeutic proof-of-principle.

T-4011

ASSESSMENT OF METFORMIN EFFECTS ON INVASION OF PANCREAS CANCER BY USING PATIENT DERIVED PANCREATIC CANCER ORGANOIDS AND PANCREATIC STELLATE CELLS

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Pancreatic cancer occurs from uncontrolled abnormal cells growth and forms a tumor placing its notoriety at the top among cancers. Pancreatic ductal adenocarcinoma (PDAC) is the highest malignancy case of the pancreas and has difficulty in treating due to a complicated tumor microenvironment surrounding the tumor. The early diagnosis of pancreatic cancer is difficult since this cancer is concealed under the rapid and rough invasion to the surrounding organs. As a concept of drug repurposing, one of the type 2 diabetes mellitus treatments, metformin, has been risen to the surface of reducing a chance to occur the pancreatic cancer through meta-analysis and else. Current studies suggest that activated pancreatic stellate cells (PSCs) might be involved in local invasion and progression of PDAC. PSCs synthesize desmoplasia with an excessive production and accumulation of the extracellular matrix (ECM). Recently, three-dimensional (3D) structure organoids are considered as an important tool for studying biology field. Pancreatic organoids are derived from duct-structure clusters and have characteristics of pancreas. In our study, the complex of pancreatic organoids and PSCs are utilized to study the effect of metformin which might be a candidate drug to inhibit PSCs invasion. We established the co-culture system of pancreatic organoids and PSCs to analysis the effect of metformin on local invasion of pancreatic cancer.

T-4013

BIOPRINTED PLURIPOTENT STEM CELL-DERIVED KIDNEY ORGANOIDS PROVIDE OPPORTUNITIES FOR HIGH CONTENT SCREENING

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Recent advances in the directed differentiation of human pluripotent stem cells to kidney organoids advances the prospect of drug screening, disease modelling, and even restoration of renal function using patient-derived stem cell lines. Here, we demonstrate the successful adaptation of our directed differentiation protocol to the NovoGen Bioprinter® MMX technology to achieve automated, rapid fabrication of self-organizing kidney organoids. Bioprinted organoids were found to be equivalent to those previously reported via manual generation at both the level of morphology and component cell types, as well as gene expression patterns and cell clusters revealed by single cell transcriptional profiling. Utilization of a bioprinter allows for the generation of large numbers of uniform and highly reproducible organoids in reduced time (approximately 20x faster) compared to manual processes. Treatment of bioprinted kidney organoids cultured in conventional 6-well format with doxorubicin exhibited concentration-dependent morphological changes consistent with cell injury and degeneration. Consistent with clinical observations, doxorubicin showed distinct glomerular toxicity with marked increases in cleaved caspase 3 mRNA and protein, accompanied by loss of podocyte-specific cell markers. Proof of concept high-throughput toxicity screening was achieved with bioprinted kidney organoids in 96-well Corning® Transwell® plates treated for 72 hours with a range of doxorubicin concentrations (0.2 to 25 μM). Analysis of 6-well and 96-well cell viability data suggested that organoids printed in both multi-well plate formats were similarly sensitive to doxorubicin. The doxorubicin IC50 for organoids bioprinted in 6-well plates was 3.9 ± 1.8 μM (value ± S.E.), while the calculated IC50 for organoids bioprinted in a 96-well plate was 3.1 ± 1.0 μM. Collectively, these results suggest that bioprinted kidney organoids are functionally equivalent to those prepared manually and thus are likely to be useful for toxicity screening, the development of iPSC-based approaches for the interrogation of complex disease phenotypes, and the scaling needed for clinical restoration of renal function with patient-derived iPSCs.

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T-4015

AN ORGANOID CULTURE SYSTEM FOR EXPLORING SKELETAL STEM CELL NICHES

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An organoid culture system for exploring skeletal stem cell niches. Stem cell niches are local tissue microenvironments that maintain stem cells and regulate their function by producing factors that act directly on stem cells. Recent discoveries of stem cells in mice and human skeletal system indicated their potential role in skeletal development. Investigations of these stem cell niches and its regulatory mechanisms offer the opportunity to better understand tissue homeostasis and regeneration in skeletal development, as well as provide new insights of treatments to a series of bone-related diseases. Current isolation methods for stem cells are mainly based on genetic labels and/or specific combinations of cell surface markers, disturbing the specific in vivo microenvironment around stem cells and limiting the representativeness of stem cell analysis, thereby leaving the cellular and molecular mechanisms that regulate stem cells niches poorly understood. By utilizing organotypic section culture and Clarity technology, we have established a new skeletal organoid culture system mimicking the in vivo cellular transitions and stem cell niches of the growth plate area, which provides a vital new tool for on the spot elucidation of cellular mechanisms and key niche signals of bone development and maturation. Applying this system to long bone growth plates of Actin-Cre Rainbow mice we observed differential clonal formation and proliferation

with bone slides embedded in Matrigel using specific cytokines (BMP2, VEGF and WNT1) and varying stiffness. The results showed that both cytokines and mechano-responsiveness were essential for proper proliferation of bone stem cells (SSCs) in the growth plate. We observed that mechanical loading promoted SSC clones to form a columnar distribution, and both BMP2 and VEGF promoted cell proliferation while WNT1 induced rapid differentiation. In conclusion, our findings describe a new approach for the in situ study of skeletal stem cell niches and the mechanism of their crosstalk with surrounding cells with a tunable microenvironment. The presented model also has the potential to be utilized in the implementation of preclinical, toxicological and therapeutic investigations.

T-4017

INDIVIDUAL BRAIN ORGANOIDS REPRODUCIBLY GENERATE THE CELLULAR DIVERSITY OF THE HUMAN CEREBRAL CORTEX

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Stem cell-derived brain organoids hold great promise for studying the development and function of the human brain and provide an invaluable experimental tool to model neurological diseases. However, their use as experimental systems has been limited by their poor characterization and inherent reproducibility. Here, we show that a newly optimized organoid model pre-patterned to form the dorsal forebrain generates a rich diversity of cell types appropriate for the human cerebral cortex. Using single-cell RNA-sequencing of 166,242 cells isolated from 21 individual organoids derived from multiple stem cell lines, we found that 95% of the organoids generated a virtually indistinguishable compendium of cell types, through the same temporal trajectories, and with organoid-to-organoid variability comparable to that of individual endogenous brains. The data demonstrate that reproducible development of cerebral cortex cellular diversity is a highly constrained process that does not require the context of the embryo. The work paves the way for modeling aspects of human cortical development and disease that have never been experimentally accessible outside the embryo.

T-4019

HIGHLY PURE SENSORY NEURON FOR ENGINEERING 2D AND 3D CONSTRUCTS: TOWARD INDUSTRIAL USE AND FUTURE REGENERATIVE MEDICINE

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Human sensory neurons (SNs) are of great interest in the field of tissue engineering for developing artificial human-skin tissues for screening new analgesics or replacing damaged skin. A few studies have been introduced for inducing SNs from embryonic stem cell (ES)/ induced pluripotent stem cells (iPSC), but it is difficult for current methods to induce SNs to meet the necessary standards for industrial applications due to low purity, unknown factor usage during differentiation and toxic reagents employed for neuronal cell selection. Here, we developed a simple and efficient method to induce SNs from iPSCs using small chemical inhibitors. The obtained cells were then processed with magnetic sorting by SN progenitor cell (SNPC) surface marker CD271/ p75. The sorted cells were seeded onto several subtypes of laminin-coated substrates with and without chemical inhibitors. The purity and function of SNPCs were then evaluated using cell morphology, quantitative real-time PCR, immunostaining with specific markers, and SN-positive chemical stimulators. Finally, we confirmed that the improved method enabled us to obtain highly pure SNs without proliferating biproduct cells compared with previously developed methods. Using these highly pure SNs, we were able to achieve spatially controlled SNs growth on the printed micropatterns using neurophilic materials so that the neurites of SNs were elongated following shape of the micropatterns. Consequently, the highly pure SNs can be useful to engineer SNs within 3D hydrogels where the biproduct cells or antibiotic-fixed cells cannot be removed. We are now applying these methods to fabricate 3D constructs using a bioprinting technique with appropriate biomaterials where the SNs could successfully elongate and differentiate within 3D conformation. Therefore, we expect that this method finds industrial use for creating artificial human-skin constructs containing functional SNs that will be useful in future regenerative medicine.

T-4021

FOLLISTATIN-RESISTANT ACTIVIN A PROVIDES MORE STABLE AND SUSTAINED SIGNALLING ACTIVITY IN HUMAN STEM CELL CULTURING

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Activin A, a member of TGF-beta superfamily, is widely used in human stem cell culturing to maintain the undifferentiated state and to induce cell differentiation. Its signalling activity also induces the cells to produce follistatin, a secreted antagonist of activin A, which binds to activin A and inhibits its signalling in a negative-feedback loop. This leads to gradual reduction of activin A bioactivity during the stem cell culturing. With the aim to develop an engineered form of activin A with more sustained activity in cell culture, we have designed a number of activin A mutants that preserve the wild-type signalling activity, but

resist inhibition by follistatin. We have used biophysical method and cell-based luciferase assay to confirm that these mutants have reduced binding affinity to follistatin and show resistance to follistatin inhibition. We have also analysed the human stem cells under treatment of wild-type and engineered activin A. For stem cell maintenance, the engineered activin A greatly reduced activity fluctuation in the daily culturing. It has also shown more sustained signalling activity in prolonged cell culturing. Moreover, the engineered activin A showed higher efficiency in inducing stem cells to differentiate to definitive endoderm compared to wild-type activin A at low protein concentrations. Overall, the engineered activin A could potentially be a more economical and robust alternative for activin A in stem cell research.

Funding Source: Biotechnology and Biological Sciences Research Council

T-4023

COMPFORCE: A COLLABORATIVE COMPUTATIONAL PLATFORM FOR CELL FATE ENGINEERING TOOLS

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Identifying sets of core transcription factors controlling cell identity is key to understanding developmental processes and to advance direct conversion and reprogramming protocols. With hundreds of cell types and roughly 2,000 transcription factors, experimental efforts have been understandably narrow, focusing on well-studied, specific cell types and utilizing exhaustive approaches to test transcription factor combinations. Recently, in an effort to create scalable, genome-wide approaches, a number of complex computational tools have been proposed to predict candidate factors for cell fate engineering experiments. However, several barriers have limited the adoption of these methods by the community and their integration and comparison. These barriers include non-standard input requirements, a lack of comprehensive benchmarking of the methods' predictive power, and the absence of a set of guidelines dictating which methods are most suitable for a user's aims. Here, we describe our collaborative platform, CompForce, which addresses these issues by integrating multiple prediction tools in one publicly available R package. In doing so, CompForce standardizes input requirements, allowing multiple user-chosen methods to be run through a single command. We establish the ease-of-use of CompForce and compare predictive power of included methods through gold standards that can be divided into two categories. Literature-based gold standards include experimentally validated cell-fate determinants per conversion, while synthetic-based in silico gold standards were generated and extracted via Dyngen, a gene regulatory network simulation tool. We demonstrate that methods integrating transcription factor network information recover a higher percentage of the gold standard-defined factors. Additionally, we show that relatedness between source and target cell type should be taken into consideration when choosing an appropriate approach, as methods focused on comparing targets to common progenitors lose predictive power when source and target cell type come from distinct lineages. Finally, with the intent of continual expansion and community collaboration, CompForce's code structure allows for easy addition of new methods as they are developed.

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T-4025

SECRETOME ANALYSIS FOR THE IDENTIFICATION OF HIGH POTENCY AND QUALITY MSCS

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Adult mesenchymal stromal cells (MSCs) hold the potential for the cure of numerous diseases. However, their large-scale clinical applications are still hindered by a limited expansion capacity due to physiological aging. MSCs extracted from older donors exhibit reduced replicative lifespan and impaired differentiation capacity. Furthermore, the expansion of MSCs leads to progressive in vitro aging. In the pre-senescent state, aged MSCs appear indistinguishable from young, highly-potent cells and retain the ability to self-renew and express MSC markers. Yet, they rapidly lose the ability to differentiate. This is a critical barrier for tissue engineering and cellular therapies using adult MSCs since cells must be able to self-renew, but also to differentiate efficiently. Moreover, studies have shown that some of the benefits of MSC therapy could be attributed to their secretion of bioactive factors. Therefore, methods to screen for high quality and potent human MSCs and methods to identify therapeutic secretagogues are essential for the scale-up needs of clinical applications and for regenerative medicine. In this study, we compare the secretome of both young, highly potent human MSCs and compare them to aged MSCs. Our goal is to understand the landscape of secretion pattern changes in MSCs preceding loss of potency and understand how these intercellular cues and signals may affect cellular lifespan and multipotency. Our data reveal unique secreted signatures which may serve as potential therapeutic factors, as well as predictive markers for the screening of highly potent MSCs prior to clinical applications.

T-4027

EVALUATION OF CELL PRESERVATION CONDITIONS FOR TRANSPORTATION OR ANALYSIS OF INDUCED PLURIPOTENT STEM CELLS BY SINGLE CELL RNA SEQUENCING

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The potential of iPSC-derived therapies is widely recognized. However, identification of Quality Attributes for such products remains a major challenge. Preserving the analytical characteristics of cell therapy products such as iPSCs to allow transportation between laboratories for comparisons, or to facilitate sophisticated analyses of various types, or for evaluation of multiple time points is an important question requiring further exploration. A related issue is evaluation of stability of such products upon storage, or transportation from manufacturing site to the point of care. In addition to standard and modified cryopreservation protocols, fixation methods intended to preserve cell transcriptomes to allow single cell RNA sequencing (scRNAseq) have been evaluated for a few specific situations. To extend these observations, we evaluated various conditions using iPSCs, which are more delicate than many cell types, and thus a more realistic surrogate for potential cell therapy products. We tested three different methods of preserving iPSCs-: cryopreservation, methanol fixation, and fixation using DSP [dithio-bis (sucinimidyl propionate)] in comparison with fresh iPSCs. Cells were subjected to these treatments and compared by assessing viability using trypan blue dye exclusion method and scRNAseq immediately after reversing the preservation procedure (Day 0) or after 4 days. All methods compromised viability immediately: in comparison with fresh cells (92% viability on Day 0) the values for each of the methods was as follows: cryopreservation (66%), methanol fixation (50%), and DSP (70%).. Viability on Day 4 was as follows: cryopreservation (45%), methanol fixation (28%), and DSP (46%). We will present the transcriptomic analysis and an evaluation of the data from the standpoint of both utility in supporting analytical studies and design of a more comprehensive evaluation of stability to storage and transport conditions.

T-4029

USING SMALL MOLECULE COMBINATIONS TO PROMOTE CRISPR-HOMOLOGY DIRECTED REPAIR OF DONOR DNA INSERTIONS IN INDUCED PLURIPOTENT STEM CELLS

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While the CRISPR-Cas9 programmable system is commonly used to edit the genome of iPSCs in a sequence-specific manner, inserting donor oligonucleotides in between blunt-end cuts of double-stranded DNA breaks remains a highly inefficient process. At an estimated less than 1 percent success rate, this approach requires screening through hundreds of clones in order to identify a single desired modification. Through poorly understood mechanisms, double-stranded breaks preferentially favor error-prone non-homologous end-joining (NHEJ) DNA repair pathway over the error-free homology directed repair (HDR) pathway, which contributes to the inefficiency of donor DNA being incorporated into a target site by homologous recombination. Previous chemical screens have identified three small molecules SCR7, L755507, and Resveratrol that enhance the HDR pathway. In this study, we tested the hypothesis that co-treating iPSCs with combinations of small molecules will synergistically increase donor DNA to incorporate into the genome. To test this hypothesis, we targeted a DNA cut and introduced a single-stranded 80bp oligonucleotide into Exon 4 of the NFATc1 gene (Nuclear Factor of Activated T-Cells 1) of iPSCs. DMSO, the solvent used to dissolve compounds, and individual small molecules were cytotoxic, causing mass collapse of iPSC colonies, precluding the flow sorting of single-cell clones. The least cytotoxic combination of molecules were Scr7 (6.3uM) and L755507 (3.6uM) dissolved in 1ul of DMSO, keeping more cells alive for expansion post-electroporation. Despite no HDR events being detected, 69% of iPSC clones sequenced exhibited indel mutations at the target site. In contrast to published studies, preliminary evidence from our lab suggests small molecules Scr7 (6.3uM) and L755507 (3.6uM) do not increase HDR efficiency.

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T-4031

DEVELOPMENT OF ANTIBODY-FREE BARCODING IN INDUCED PLURIPOTENT STEM CELL AND DIFFERENTIATED CULTURES FOR FLEXIBLE NEXT-GENERATION HIGH-THROUGHPUT SEQUENCING

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Development and integration of DNA barcoding into next-generation sequencing has allowed for multiplex analysis correlating specific cell populations with a carefully designed unique molecular identifier analyzed in parallel producing a transcriptomic analysis technique with both high-throughput and high-resolution at the single-cell level. This strategy employs antibody-based ssDNA oligonucleotide (oligo) barcode labeling necessitating covalent attachment through click-chemistry functionalization for each antibody/barcode pair, a conjugation scheme both time consuming and expensive. Therefore, an antibody-free strategy using cationic polymer nanoparticles based on poly(ethylene glycol) diacrylate backbones for rapid, easy, and inexpensive barcode labeling is being developed. These synthetic polymers bind to oligo sequences in solution without the need for direct conjugation and ubiquitously interact with iPSC, human liver organoids (HLOs), and differentiated endoderm. Visualization and quantification of nanoparticle targeting efficiency following direct fluorescent dye conjugation was completed by confocal microcopy and flow cytometry respectively. Targeting efficiency was found to be dependent upon polymer composition; highly targeting formulations following incubation with HLOs achieved a targeting efficiency of 98 %. Moreover, importantly, spheroids created from either separately labeled induced pluripotent stem cell progenitors or differentiated anterior and posterior foregut demonstrated successful polymer labeling with total cellular targeting between 75 – 90 % and less than 5 % cross-labeling following co-incubation; labeling was maintained for 7 days in culture. Oligos were successfully identified post barcoding by PCR and Sanger sequencing. These results indicate the potential for inexpensive polymers synthesized with commercially available reagents to mediate barcode-labeling of pooled progenitor populations for next-generation sequencing applications without the need for conjugated antibodies, presenting a considerable cost and time savings as oligo barcode labels can be quickly mixed with these polymers and introduced to cells without the need for direct, covalent conjugation.

T-4033

RAPIDLY PROLIFERATING STOMACH EPITHELIAL STEM CELL IS REGULATED BY MAPK PATHWAY

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We reported earlier that long sought-after stem cells in the isthmus of mouse stomach epithelium were identified by Runx1 enhancer element, eR1. Runx1 is known to be essential to generate hematopoietic stem cells. The eR1, which is 270 bp enhancer element located within the two promoters of Runx1, is responsible for driving the expression of Runx1 in hematopoietic stem cells. The fact that isthmus stem cells express Runx1 suggests the possibility that Runx1 is probably responsible for inducing tissue stem cells not only in hematopoietic system but also in other tissues. We found the protein, which we call isthmus factor (IF), is exclusively expressed in isthmus stem cells in stomach. Most of the cells expressing Isthmus Factor also express proliferation marker Ki67. Isthmus factor interacts with Ras protein but interaction with constitutively-activated form of Ras is stronger than with normal Ras, suggesting that GTP bound form of Ras is referentially interacting with Isthmus Factor and induces higher levels of phospho-ERK. Isthmus Factor is not only expressed in stomach isthmus but also in other rapidly growing tissues, such as duodenum and intestine. Interestingly, Isthmus factor is widely expressed in rapidly proliferating parts of gastric cancer together with Ki67. To clarify whether isthmus factor expressing cells are related to cancer stem cells, we co-stained Isthmus Factor with CD44v antibody, which has been shown to represent cancer stem cells in gastric cancer. We found that there are three staining patterns, Isthmus factor alone, CD44v alone and the mixture of the two. We hypothesize that CD44 single positive cells may represent dormant or slow growing cancer stem cells, whereas single Isthmus Factor expressing cells are rapidly growing cancer stem cells. By separating these fractions, we will examine exact nature of these fractions.

T-4035

MOLECULAR REGULATION OF WOUND EPITHELIUM FUNCTION AND MATURATION IN LIMB REGENERATION

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The wound epidermis is a transient epithelial structure that is necessary for salamander limb regeneration. Successful limb regrowth relies on the maturation of this structure from a thin epithelium during early regeneration to a thick structure called the apical epithelial cap (AEC). Yet, little is known about both the function of the early wound epidermis as well as factors that are important for its maturation. Here, we examined how the early gene expression programs of blastemal progenitors and their surrounding tissues change in the absence of the wound epidermis. By transcriptionally profiling limbs in which we prevented wound epidermis formation, we discovered that the wound epidermis likely plays large roles in orchestrating early inflammatory responses and tissue histolysis. We further investigated the role of the pleiotropic growth factor cytokine midkine (mk), which exhibited decreased expression in the absence of the wound epidermis. We demonstrate that mk is strongly expressed throughout regeneration. Both chemical and genetic perturbation of mk impairs wound epidermis maturation, which either completely inhibits or delays regeneration. Finally, overexpression of mk in regenerating limbs leads to uncontrolled growth of the wound epidermis. In all, our results provide molecular insight into the mechanisms governing wound epithelium function and maturation.

T-4037

HEMATOPOIETIC STEM CELLS DEPEND ON LOW PROTEIN SYNTHESIS TO MAINTAIN PROTEOME QUALITY AND HOMEOSTASIS

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Precise translational control has recently emerged as a fundamental mechanism in stem cell regulation. Low protein synthesis is a broadly shared feature of somatic stem cells that promotes regeneration in multiple tissues. However, why stem cells depend on low protein synthesis and how increases in protein synthesis impair stem cell function remain largely unknown. Here, we determined that low protein synthesis within hematopoietic stem cells (HSCs) was associated with increased proteome quality. HSCs contained less ubiquitylated and unfolded proteins as compared to restricted myeloid progenitors, and modest increases in protein synthesis caused an accumulation of defective translational products within HSCs in vitro and in vivo. These data indicate that HSCs depend upon low protein synthesis to maintain the integrity of their proteome. To test how proteome quality affects stem cell function, we examined Aarssti/sti mice that harbor a mutation in the alanyl-tRNA synthetase, which causes a tRNA editing defect that increases errors during translation. Aarssti/sti mice exhibited impaired HSC maintenance and significantly diminished serial reconstituting activity in vivo, but did not exhibit defects within restricted progenitors. The accumulation of ubiquitylated protein within HSCs overwhelmed the capacity of the proteasome, which disrupted the turnover and increased the stabilization of c-Myc. Conditional deletion of a single copy of Myc was sufficient to significantly rescue serial reconstitution defects in Aarssti/sti mice. HSCs are thus dependent on low protein synthesis to maintain proteome quality and homeostasis.

T-4039

HUMAN LIVER ORGANOIDS WITH MULTIPOTENT HEMATOPOIETIC CELLS

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During development, the fetal liver is a major site of hematopoiesis in which hematopoietic stem cells (HSCs) give rise to all the different lineages of blood cells in the body. The HSC in the fetal liver are produced from hemogenic endothelium that undergoes endothelial to hematopoietic transition (EHT) in a Notch-dependent manner, however, the full mechanisms of EHT remain to be clarified as Notch signaling has been shown to be necessary but not sufficient. We have developed a new fetal liver organoid system consisting of both hepatic and immune cells that co-differentiate in a three dimensional layout. This system mimics what is seen during fetal development, including the presence of multipotent hematopoietic progenitors arising through Notch dependent EHT. Histological analysis showed formation of hepatic organoids expressing AFP, ALB, and EpCAM located adjacent to CD34 endothelial cells undergoing

EHT. Hematopoietic cells in our organoid culture were dependent on Notch regulation as shown by lack of hematopoietic cell formation after Notch inhibition with DAPT treatment. Analysis of downstream targets of Notch by qPCR including HES1 showed an expression level peak just prior to a peak in hematopoietic colony forming cells. CFC assay revealed that our organoid culture contains multipotent progenitors that can give rise to erythroblasts, macrophages, monocytes, and granulocytes. Addition of Notch ligand DLL4 increased the presence of CD45+ cells in our culture. These CD45+ cells were collected by FACS and identified as macrophages and granulocytes by Giemsa stain. Co-culture with MS-5 mouse stromal cells resulted in a CD45+/CD19+ B cell population. Future studies will use this model to determine other essential signaling pathways involved in EHT and examine the interaction of immune and hepatic cell types during early liver development.

T-4041

EXPERIMENTAL STRATEGIES OF MESENCHYMAL STEM CELLS PROPAGATION; ADVERSE EVENTS AND POTENTIAL RISK OF FUNCTIONAL CHANGES

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For most therapeutic applications, mesenchymal stem cell (MSC) propagation in vitro is often required. However, cell culture condition is not fully physiological process and it may affect biological properties of MSCs including their regenerative potential. In our study we focused at the different aspects of MSC propagation that might influence cell properties: proliferation and differentiation ability as well as the potential of stem cells aging. Thus, the factors that influence stability of MSCs following their long-term expansion need to be clarified before cultured MSCs are employed for clinical application. The aim of our study was to investigate the potential of human MSCs (hMSCs) isolated from neonatal or adult sources and adopted methods that could increase MSC differentiation and proliferation ability and cytokine secretion profile. In our studies different culture media, 3D cell cultures, chemical and epigenetic reagents were employed. Senescence associated changes were analyzed by β-galactosidase staining and pro-inflammatory cytokine expression has been evaluated. The potential risk of transformation was verified by MSC morphological changes, chromosomal aberrations and gene expression. We demonstrated that hMSCs is heterogeneous fraction significantly varying in regenerative potential which mainly depends on the patient's medical history of the disease, age and even BMI. Our studies showed that we can increase MSC lineage differentiation, proliferation or cytokine secretion in specific in vitro condition. MSCs derived from neonatal sources comprised better proliferation however, one sample of hMSCs derived from umbilical cord blood exhibited altered cell morphology, revealed chromosomal duplications and expressed telomerase and cMYC. All samples of hMSCs derived from different adult sources entered senescence and did not show any signs of spontaneous transformation. Our studies demonstrate that we can increase hMSC regenerative properties although, we should consider the susceptibility of hMSCs to spontaneous transformation during long-term culture. This could be taken into consideration due to biosafety issues of future cell-based therapies and regenerative medicine regimens.

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T-4043

GENERATION AND CHARACTERISATION OF REGIONALISED SPINAL CORD PROGENITOR CELLS AND NEURAL CREST FROM HUMAN PLURIPOTENT STEM CELLS

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Neuromesodermal progenitors (NMPs) contribute to the elongating spinal cord and the adjacent paraxial mesoderm. They are localised to the node-streak border (NSB) in the anterior primitive streak at E7.5 and subsequently in the rostral caudal lateral epiblast of the E8.5 mouse embryo. The formation and maintenance of NMPs requires Wnt and FGF signals from the node and primitive streak, and these factors have been used in vitro to differentiate human pluripotent stem cells (PSCs) into a transient NMP-like population (hPSC-NMPs). These are typically characterised by co-expression of SOX2, BRACHYURY and CDX2, and exhibit progressive, full collinear HOX activation. During this limited period, hPSC-NMPs can be differentiated into region specific cells e.g. motor neurons, but regionalised progenitors cannot be maintained over prolonged periods of time. Here we report the generation of hPSC-NMPs derived trunk neural progenitor cells (NPCs) which are stable over 10 passages (2 months). NPCs down regulate BRACHYURY and

TBX6, indicating a loss of potency towards the mesodermal lineage, but maintain SOX2 and CDX2 expression. In addition, NPCs express PAX3, NEUROG2 and a thoracic HOX(6-9) gene signature and can be differentiated into spinal cord specific cells including motor neurons and glia. We also show that NPCs can undergo an epithelial to mesenchymal transition to give rise to neural crest-like cells, in a process resembling the delamination of trunk neural crest from the developing neural tube. We have completed RNAseq analysis during the generation NMPs, NPCs and neural crest-like cells to identify the transcriptional and signalling pathways which underpin the transition from NMP to neural progenitor cells.

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T-4045

BIOPROCESSED WATER EXTENDS LIFE-SPAN VIA MYOGENIC POTENTIALS

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The physical properties of BCP were evaluated by measuring the number of water molecules and length of the hydrogen bond between water molecules surrounding the purified and crystallized protein, and beta-amyloid protein stability was also assessed. Additionally, BCP-related microRNAs were identified using human hepatocellular carcinoma, colorectal cancer, and gastric cancer cell lines. Satellite cells grown in BCP medium displayed increased expression of Notch1, Pax7, AMP-activating protein kinase-alpha (AMPK-alpha), and anti-oxidant enzymes and significant resistance to hydrogen peroxide-induced cell death. In vivo, BCP enhanced muscle regeneration and reduced muscle fibrosis. Long-term BCP administration to MDX mice decreased muscle injury and had a muscle-protective effect in part by downregulating and upregulating the expression of genes related to immune response and cell death and genes related to oxidation-reduction, energy production, and cell proliferation, respectively. The life-span of SMP30 KO mice was significantly extended by administering BCP. Protein treated with BCP contained the most water molecules and longest hydrogen. These results suggest that BCP improves protein hydration and antioxidant status through upregulating antioxidant enzymes and AMPK and thereby provides a favorable niche for satellite cells, increasing muscle regeneration and reducing oxidative stress.

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T-4047

MATURATION OF HIPSC-CM FUNCTION FOR IMPROVED PREDICTIVITY OF IN VITRO CM-MEA ASSAYS

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have significantly advanced in vitro assays for disease-in-a-dish modeling and evaluation of cardiac safety risk, yet remain an immature representation of human ventricular myocytes. Recent literature has demonstrated a variety of techniques, ranging from electrical pacing over chronic timescales to patterned substrates, to mature aspects of hiPSC-CM structure and function. Here, we characterize the contractile and electrophysiological maturation of hiPSC-CMs elicited through chronic electrical pacing. The hiPSC-CMs were electrically paced at 2Hz for 48 hours, and microelectrode array (MEA) technology was used to measure the cardiomyocyte action potential and excitation-contraction coupling. Following the 48 hours pacing, the “matured” cardiomyocytes displayed shortened repolarization timing relative to measurements taken before chronic pacing (baseline: 423 +/- 21 ms; “matured”: 316 +/- 15 ms), without a significant change in beat period (baseline: 1255 +/- 40 ms; “matured”: 1314 +/- 84 ms). The beat amplitude, a surrogate measure of strength of contraction, was measured using impedance technology from the same MEA plates. The beat amplitude was measured during spontaneous beating and in response to increasing pacing rates (1, 1.2, 1.5, 2, and 2.5 Hz). Before chronic pacing, the beat amplitude decreased as the pacing rate increased, whereas the same wells displayed an increase in beat amplitude as the pacing rate increased after the 48 hours of chronic pacing. In addition, the “matured” wells displayed increase sensitivity to positive inotropes, such as isoproterenol, digoxin, omecamtiv mecarbil, and dobutamine. In summary, this abstract demonstrates that some aspects of hiPSC-CM electrophysiology and contractile function can be matured after 48 hours of chronic pacing.

T-4049

MESENCHYMAL STEM CELL CONDITIONED MEDIUM PROMOTES DIFFERENTIATION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS INTO EARLY ENDOTHELIAL PROGENITOR CELLS WITH DEFINED FUNCTIONAL CHARACTERISTICS

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In the promotion of blood vessel regeneration, circulating endothelial progenitor cells (EPCs) play important roles as building blocks through maturation into endothelial cells and also exerting paracrine effect. One of the most important factors involved is vascular endothelial growth factor (VEGF). It is known that mesenchymal stem cells (MSCs) naturally secrete various trophic factors such as growth factors and cytokines into their environment which also known as conditioned medium. MSCs which are cultured in hypoxic condition secrete several higher levels of growth factors, such as VEGF. The objective of this study was to investigate the ability of conditioned medium as supplement to in house EPC growth medium compare to commercial endothelial growth medium. Mononuclear cells (MNCs) were isolated from healthy human peripheral blood. The MNCs were cultured in hematopoietic medium (X-Vivo 15, Lonza), formulated with Heparin, Pravastatin, Ascorbic Acid and supplemented with conditioned medium derived from Human Umbilical Cord Mesenchymal Stem Cells (hUCMSCs-CM) containing VEGF 2,795 pg/mL. As a control, we also cultured the MNCs in Endothelial Growth Medium (EGM). The functional characteristics of early endothelial progenitor cells (eEPCs) culture were observed after 7 days. Comparison was made from the functional characterization results by the ability of the eEPCs to uptake LDL and endothelial specific surface epitopes. The obtained results showed that eEPCs were cultured in hUCMSC-CM supplementation showed as CD31+, CD144+, and KDR+ cells. In particular, CD31 expression of eEPCs in conditioned medium supplementation more than 80%. Moreover, the LDL uptake functionality results showed similar results in hUMSC-CM supplementation compared to EGM. These findings indicated that hUCMSC-CM supplementation defines characteristics of eEPCs associated with its VEGF content.

T-4051

HEALTH ECONOMICAL RESEARCH FOR CELL THERAPY AGAINST STROKE

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Stroke is still a leading cause of death and disability, and despite intensive research, few treatment options exist. A recent breakthrough in cell therapy is expected to reverse the neurological sequelae of stroke. Since June 2017, we have also started the novel clinical trials, Research on advanced intervention using novel bone marrow stem cell (RAINBOW) study. It is a phase 1, open label, uncontrolled, dose response study. The primary purpose is to determine the safety of autologous BMSC product, HUNS001-01, when administered to acute ischemic stroke patients (Shichinohe H, et al. BMC Neurol. 2017;17:179). However, there are some problems to be solved before the clinical application, for examples, Ethical, Legal and Social Implications (ELSI) including Health Technology Assessment (HTA) for cell therapy. If the cost of cell therapy would be too expensive, should it be justified? National Institute for Health and Care Excellence (NICE) in UK proposed that less than £30,000 (about $40,000) per one quality-adjusted life year (QALY) would be appropriate. In our present study, we analyzed QALY using EQ-5D-5L and the medical cost in subjects of RAINBOW study. Because we obtained the preliminary data of QALY from 5 subjects, we will report them.

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T-4053

AUTOLOGOUS STEM CELL TRANSPLANTATION PROMOTING IN VIVO MECHANICAL STRETCH INDUCED SKIN REGENERATION

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Mechanical stretch, in term of skin expansion, can induce effective but limited in vivo skin regeneration for complex skin defect reconstruction. Often times in large area of tissue reconstruction, more skin is needed for reconstruction than tissue expansion can provide, because skin does not have the growth capacity to be expanded beyond two to three times its original area. We collected expanded skin tissues from different expansion statues (good and poor) and found that the dermal structure of the poor expanded skin was obviously changed. The collagen fibers were loose, the basement membrane flattened, and the skin lose the papilla-like shap of the dermal-epidermal junction. PCNA + cells and the produce of type I collagen was decreased. All these characteristics were similar to aging skin, indicating that skin lost growth capacity after long term mechanical stretch.To explore a treatment that could promote expanded skin regeneration, we first evaluated the effect of adipose derived stem cell (ADSC) using a rat model. The results showed that transplanted ADSC can be recruited and implanted in expanded skin. Compared to control group, ADSC treated skin was thickened, higher in ColI expression and contained more PCNA+ cells. The results indicated that ADSC can effectively promote expanded skin regeneration. Based on these preclinical researches, we designed a randomized clinical trial to evaluate the efficiency of autologous stromal vascular fracture (SVF), a group of cells separated from adipose tissue containing ADSCs, in promoting in vivo expanded skin regeneration. This trial recruited 22 patients undergoing skin expansion presenting with signs of exhausted regenerative capacity. After randomization, 11 patients received intradermal SVF injection and 11 patients received saline. At the end of 12-weeks follow-up, the SVF group had significantly thicker dermis (mean difference 0·08 [95% CI, 0·04-0.11]; p<0·001) and more expander volume growth (0.64[0.18-1.09]; p=0.010). Patients in the SVF group gained more regenerated skin. No severe adverse events occurred. Our research demonstrated that skin loses regenerative capacity after long term of stretch. Intradermal transplantation of autologous stem cells is an effective strategy to promote in vivo mechanical stretch induced skin regeneration.

Funding Source: Funded by the National Natural Science Foundation of China No. 81501678 and No. 81620108019.

T-4055

EVALUATION OF HIGH-FIDELITY CRISPR CAS9 ENZYMES FOR CELL THERAPY

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CRISPR-based gene editing proteins, such as Cas9, have fundamentally transformed our ability to manipulate the human genome. One application for Cas9 systems has been editing DNA in stem and other cells, to enhance and broaden the capacities of cell therapy. However, there are several concerns and limitations when using the current CRISPR systems, such as unintended off-target cleavage of the genome, as well as large deletions and rearrangement at the target site as a result of inaccurate repair. In order to make CRISPR systems more safe and predictable, these effects must be mitigated, otherwise these unwanted genotypic changes could result in unintended side effects. There is a demand for a CRISPR system that can with high precision and efficiency. We have chosen to characterize and compare several previously published and in-house high-fidelity CRISPR candidates that showed decreased off-target while retaining high on-target activity. We cloned and purified the Cas9 variants, and all tests were done using a Cas9 RNP system. 24 on-target sites were selected to compare the Cas9 variants. The Cas9 ribonucleoprotein (RNP) complexes were transfected into HEK293 and iPSC cells, and on-target efficiency was determined by NGS in-house analysis software. From the initial set of Cas9 variants, we found two that had relatively high activity (62%-81%) relative efficiency to the wild type. We then used these two Cas9 variants and measured off-target activity for five targets using Tag Enriched GUIDE-seq (TEG-seq) and Targeted Amplicon Validation sequencing (TAV-seq). Across the multiple targets, both variants had a relatively low amount off-target activity in comparison to wild type. Further comparisons in are ongoing in iPSC cells, but the initial result identify 2 potentially superior high fidelity Cas9 variants that are functionally useful for cell therapy.

T-4057

ANKEF1 IS ESSENTIAL FOR ZONA PELLUCIDA PENETRATION AND MALE FERTILITY

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Almost 50 million couples worldwide suffer from infertility and a male factor is involved in 50% of these cases. Genetic etiologies are heavily implicated in these conditions, but the fertilization process is poorly defined and many candidate genes remain to be identified. In this study we investigate the role of Ankef1 during fertilization. Ankef1 is an uncharacterized protein that contains eight ankyrin repeat domains and a calcium binding EF-hand domain. We found that Ankef1 is predominantly expressed in the testes where its transcription increases

during germ-cell differentiation. In addition, protein analysis demonstrated Ankef1 expression in mouse caudal epididymal sperm. To elucidate the functional role of Ankef1 in vivo, we generated Ankef1 knockout mice by CRISPR-cas9 technology and discovered that despite normal sexual behavior males homozygous for Ankef1 mutations are sterile. Detailed sperm analysis demonstrated that Ankef1–deficient sperm have normal morphology, motility and capacitation ability. However, they are unable to penetrate the zona pellucida (ZP) and to fertilize wild-type eggs in-vitro. Remarkably, full fertilization capacity could be restored in IVF assays when using zona-free oocytes. Since this phenotype echoes that of unexplained male infertility (UMI), and as defects in sperm-ZP binding and penetration defects are the predominant reason for fertilization failure in IVF, this model provides an exciting opportunity to elucidate the role of a novel gene essential for fertilization that could be a potential infertility treatment target.

T-4059

LINEAGE SEGREGATION AND MOLECULAR ARCHITECTURE OF MOUSE EARLY EMBRYO REVEALED BY SPATIAL AND SINGLE CELL TRANSCRIPTOME

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The blueprint of embryonic development is first visualized in the context of regionalization of cell fates in the germ layers of the postimplantation mouse embryo. Knowledge of the genetic and signaling activities that underpin lineage specification and tissue patterning spanning gastrulation is instrumental for stem cellbased translational study, and has been gleaned from embryological experimentation and phenotypic analysis of genetic animal models. However, a comprehensive genomewide molecular annotation of the progenitor cell organization of postimplantation embryo development has yet to be undertaken. Here, we reported the findings of a systematic transcriptome study of discrete cell populations at defined positions in the epiblast, ectoderm, mesoderm and endoderm of the pregastrulation and gastrulation stage embryos. This developmental spatial transcriptome has defined the molecular genealogy of cells in the germ layers in real time and real space resolution. The transcriptome further identifies the molecular determinants such as the interactive transcriptional factors, the wiring of signaling pathways and the transcriptional networks that drive lineage specification of the epiblast cells and the development of germ layer precursors. Our study also established the spatial correlates which can be used as a reference positioning system for single cells and in vitro stem cells differentiation.

Funding Source: This work was supported by the "Strategic Priority Research Program" of the Chinese Academy of Sciences (XDA16020501,XDA16020404,XDA01010303, XDB19020301).

T-4061

NEXT-GENERATION UNNATURAL MONOSACCHARIDES REVEAL THAT ESRRB O-GLCNACYLATION ENHANCES PLURIPOTENCY

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O-GlcNAc, the attachment of β-N-acetylglucosamine onto serine or threonine residue of intracellular proteins, is essential for pluripotency maintenance. Unnatural monosaccharides that can be metabolically incorporated into cellular glycans are currently used as a major tool for investigating O-GlcNAcylation. As a common practice to enhance membrane permeability and cellular uptake, the unnatural sugars are per-O-acetylated, which, however, can induce a long-overlooked side reaction, non-enzymatic S-glycosylation. Herein, we developed 1,3-di-esterified-N-azidoacetylgalactosamine (GalNAz) as the next generation chemical reporters for metabolic glycan labeling (MGL). Both 1,3-di-O-acetylated GalNAz (1,3-Ac2GalNAz) and 1,3-di-O-propionylated GalNAz (1,3-Pr2GalNAz) exhibited high efficiency for labeling protein O-GlcNAcylation with no artificial S-glycosylation. Applying 1,3-Pr2GalNAz in mouse embryonic stem cells (mESCs), we identified ESRRB, a critical transcription factor for pluripotency, as an O-GlcNAcylated protein. We showed that ESRRB O-GlcNAcylation is important for mESC stemness. Mechanistically, ESRRB is O-GlcNAcylated by O-GlcNAc transferase (OGT) at serine 25, which stabilizes ESRRB and facilitates its interactions with two master transcription factors, OCT4 and NANOG. Taken together, our data not only usher a new epoch of MGL, but firstly exploit its application in revealing O-GlcNAc regulation on mESC pluripotency.

T-4063

DELINEATING THE CIS-REGULATORY ROLE OF LTR12C IN HUMAN EMBRYONIC STEM CELLS

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Endogenous retroviruses (ERVs) constitute approximately 8% of the human genome. Although originally thought to be "junk DNA", in recent years, ERVs have been shown to exert diverse effects on normal physiological functions. Moreover, dysregulation of these elements are associated a plethora of diseases. We have previously observed that elements of the LTR12C subfamily are enriched with epigenetic features of potential tissue-specific cis-regulatory elements. Interestingly, these sequences were reported to cause upregulation of nearby genes in testicular cancer. However, the general function of LTR12C is in distinct tissues remains unclear. In this study, we employed human embryonic stem cells (hESCs) as a model system to investigate the role of LTR12C in transcriptional regulation and genomic architecture. We found that LTR12C has potential promoter activity in hESCs and identified individual LTR12C elements, which initiate transcription of other repetitive elements and single copy genes. The molecular functions of these LTR12C elements were further investigated by CRISPR knockout experiments and 3D genome approaches. Taken together, we aim to elucidate the role of LTR12C in transcriptional regulation and genomic architecture in the context of cellular differentiation.

Funding Source: University Grant Council

T-4065

DEFINING THE SEQUENCE REQUIREMENTS FOR XIST FUNCTION IN X INACTIVATION

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The lncRNA Xist provides a remarkable model to investigate the function of lncRNAs in gene regulation, as it induces chromosome-wide silencing in cis in the process of X-chromosome inactivation (XCI). XCI is fundamentally important for female mammalian development but, despite its critical role, the mechanisms by which Xist carries out the various tasks associated with XCI still remain largely unclear. Xist is thought to fulfill different roles during XCI, such as gene silencing, chromatin association, spreading, recruitment of repressive chromatin regulators, through different RNA domains, which in turn recruit different proteins. To identify functional Xist domains required for the initiation of XCI, Using CRISPR/Cas9 genome editing, I have engineered a unique XCI assay in female mouse ESCs taking advantage of the observation that XCI allelic choice becomes non-random when one Xist allele is rendered non-functional. Specifically, I generated heterozygous female F1 ESCs lacking a 6.5 kb region containing the F, B, C, and D repeats and the intervening non-repeat regions on the 129 allele XcasX129ΔBCDF. I found that these Xist mutant cells inactivate the wildtype (CAST) allele exclusively during differentiation. Although small 129 allele Xist clouds can be observed on day 2 of differentiation, these can no longer be observed at day 5 of ESC differentiation. Using the restoration of 129 Xist cloud as a readout for rescue of Xist function, I am screening for sequence requirements needed to rescue Xist function of the 129 allele. I have also generated several additional heterozygous Xist mutants with smaller deletions, to systematically identify sequences required for Xist function. Through characterization of these Xist mutants, I will be able to dissect the molecular mechanisms of specific Xist-protein interactions, to reveal the means by which Xist, through its RNA domains, integrates different functions.

Funding Source: UCLA Whitcome Pre-Doctoral Fellowship in Molecular Biology

T-4067

CHROMATIN ACCESSIBILITY IN CANINE STROMAL CELLS AND ITS IMPLICATIONS IN CANINE SOMATIC CELL REPROGRAMMING

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Use of spontaneous disease in dogs as a platform to conduct impactful and realistic translational regenerative medicine research is very attractive given their large size, longevity, heterogenous genetics and similarity to human pathophysiology. Culture conditions for canine pluripotent stem cells are elusive and not comparable to the established murine and human models. A lack of mechanistic understanding of canine-specific reprogramming and pluripotency regulatory networks hinders robust and reproducible approaches for canine somatic cell

reprogramming to induced pluripotent stem cells (iPSC). We have reprogrammed canine fetal stromal cells to iPSC with a lentiviral system containing the 4 Yamanaka factors, in the presence of LIF and bFGF. Nevertheless, adult stromal cells resist such an approach. Generation of iPSC depends on chromatin and transcriptome remodeling, entailing the inactivation of somatic enhancers and activation of pluripotency ones. We propose that a failure in chromatin remodeling constitutes a barrier to reprogramming. Through the determination of global chromatin accessibility by ATAC-seq, and gene expression by RNA-seq, in adult and fetal fibroblasts and syngeneic ciPSC we have identified loci that are differentially accessible before and after reprogramming. Analysis of ATAC-seq data shows a high number of nucleosome free regions (NFR) shared between fetal fibroblasts and iPSC, but not adult fibroblasts. We identified these genes only open in adult somatic cell, and sorted them by gene class and biological process using the PANTHER (protein annotation through evolutionary relationship) classification system. Initial data analysis shows13 candidate genes as possible reprogramming barriers: BSX, CEBPE, ELK4, ETV3L, GATA5, GBX2, HES6, MYOG, OLIG3, SPRY1, TBX21 and TCF3 and TSHZ1. Further testing of these genes by RT-qPCR and siRNA experiments in our adult stromal cells and established ciPSC will reveal if and which ones of these candidates presents a true reprogramming barrier. We have identified possible reprogramming barrier genes in canine somatic cells; manipulation of the pathways they are involved in will enable deeper understanding of canine specific reprogramming regulators and provide a mechanistic rationalle for enhanced protocols.

Funding Source: Funding for this project was provided by the University of California Davis, Center for Companion Animal Health, #2018-4-F Defining the epigenetic barrier to canine adult stromal cell reprogramming.

T-4069

BMP4 RESETS PRIMED TO NAIVE PLURIPOTENCY BY REPROGRAMMING CHROMATIN ACCESSIBILITY

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Mouse naive and primed pluripotent stem cells, ESC and EpiSC, represent two distinct stages of pluripotency. Here we report that BMP4 drives primed to naive transition or PNT by reprogramming chromatin accessibility. ATAC-seq reveals that a short pulse of BMP4 triggers EpiSCs to close 26409 and open 6428 loci to reach an intermediate state that continue to open 18744 and close 7042 loci under 2iL until reaching a naive state, following with a dramatic reactivation of the silenced X chromosome. Among loci opened by BMP4 are those encoding Id1, Tfap2c/2a and Zbtb7b that synergistically drive PNT without BMP4. Tfap2c-/- ESCs or EpiSCs self-renew normally, while the former capable of differentiating to the latter but the latter fails to undergo PNT, a defect rescuable by exogenous Tfap2c. Our results link BMP4 to PNT through a binary logic of chromatin closing and opening, revealing the intrinsic power of extracellular factors to reorganize nuclear architecture in development.

POSTER II - EVEN
19:00 – 20:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

T-2002

ROLE OF VGLL1 IN THE SPECIFICATION AND MAINTENANCE OF THE TROPHOBLAST LINEAGE USING MULTIPLE HUMAN STEM CELL-BASED MODELS

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The placenta is an understudied organ that sustains embryo development throughout gestation. Abnormal placental function affects pregnancy outcome as well as maternal and child health long after birth. Early placental development, specification of the trophoblast lineage and the trophoblast stem cell niche in human are currently poorly understood. In a recent mouse-human comparative study of placentation across gestation, we identified vestigial-like protein 1 (VGLL1) as a human-specific transcription co-factor highly expressed in placental villous cytotrophoblast (CTB), the proliferative and multipotent compartment of the placenta. VGLL1 is expressed specifically in the trophectoderm layer of the pre-implantation blastocyst, which will give rise to the trophoblast cells of the placenta. We have started to investigate the role of VGLL1 in trophoblast cells using multiple in vitro stem cell-based models. We differentiated human pluripotent stem cells (hPSC) into CTB-like cells using a BMP4-based protocol to study trophoblast lineage specification. In this system, VGLL1 was up-regulated by 24h of BMP4 addition, after GATA3 and before TP63 induction, necessary for trophoblast specification and maturation, respectively. Expression of VGLL1-targeting shRNA caused impaired expression of TP63, suggesting that VGLL1 might act upstream of this trophoblast marker. VGLL1 is also highly expressed in human trophoblast stem cells (hTSC), a novel in vitro system isolated from first trimester placenta to investigate trophoblast maintenance and differentiation. VGLL1 contains a highly conserved region binding the TEA domain-containing family of transcription factors. In primary CTB, hPSC-derived CTB-like cells and in hTSC, VGLL1 co-localized with TEAD4 in the cell nuclei and the two factors can be co-immunoprecipitated. We hypothesize that, in human, VGLL1 acts in combination with TEAD4 to drive trophoblast lineage specification and trophoblast stem cell maintenance. Future studies include identification of direct down-stream targets of the VGLL1/TEAD4 complex by ChIP-seq and the investigation of the transcription factor cascade necessary for trophoblast specification and maintenance using both hPSC and hTSC models.

Funding Source: NICHD grant R01-HD096260 to Dr. Soncin.

T-2004

CELL SURFACE MARKERS THAT ENABLE DEFINITIVE DISCRIMINATION BETWEEN MESENCHYMAL STEM CELLS OF DIFFERENT ORIGIN AND FIBROBLASTS

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Suzanne AM Sober, Dana Alhattab, Abdalla Awidi, Homa Darmani Mesenchymal stem cells (MSCs) have been isolated from a number of tissues including bone marrow, adipose tissue, Wharton's jelly, placental tissue, dermis, muscle and dental pulp. However, MSCs only make up a very small percentage of all the cell populations found within these tissues. This has presented a challenge to their isolation from various tissues, which in turn has led to the need for methods to authenticate the identity of the isolated cells. Identification and purification of MSCs expanded in culture for therapeutic use is often hampered by contaminating fibroblasts - the most common cell type in connective tissue. Since there has been no consensus as to which markers are truly specific for MSCs and which are definitely specific for fibroblasts, this study examined the expression of a panel of 14 different markers in MSCs isolated from Wharton's jelly, adipose tissue, bone marrow and placental tissue and fibroblasts from foreskin, using multiplex flow cytometric analysis. Our results indicate that the following markers can be used to differentiate between fibroblasts and MSCs derived from: adipose tissue - CD79a, CD105, CD106, CD146, CD271; Wharton's jelly - CD14, CD56 and CD105; bone marrow - CD105, CD106, CD146; placental tissue - CD14, CD105, CD146. In conclusion, the results of our study provide a basis for discriminating between fibroblasts and MSCs of different origins which can also be used to authenticate the identity of the isolated cells.

Funding Source: This work was supported by the Deanship of Research at Jordan University of Science and Technology (Grant Number: 20160245).

ADIPOSE AND CONNECTIVE TISSUE

T-2006

PROTEIN PATHWAY ANALYSIS OF VALPROIC ACIDS EFFECT IN INITIATING NEURAL DIFFERENTIATION IN HUMAN ADIPOSE DERIVED STEM CELLS

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Regenerative medicine is a rapidly expanding area. Research involving the use of small molecule chemicals aim to simplify a way to create specific drugs for clinical applications in neural related diseases and injury. Adipose Derived Stem Cells have recently shown their capacities to differentiate into interesting cells for regenerative medicine, specifically neural cells, using chemicals. Valproic Acid was an ideal candidate due to its clinical stability and relevance to primary neural cell development. Furthermore, it has been implicated in the promotion of neuronal differentiation, however the mechanism and the downstream

events were not fully elucidated. In this study, presents the use Valproic Acid on Adipose Derived Stem Cells and the effect on initiating neural differentiation within 24 hours. The protein mass spectrometric analysis revealed an up-regulation in the expression of SOCS5 and FGF21 without increasing the potential death rate of the cells. Through this, protein expression in JAK/STAT pathway was downregulated, and the MAPK cascade is activated. The bioinformatics analyses revealed the expression of specific neuron markers as well as a range of functional and structural proteins involved in the early formation and development of neuronal cells.

T-2008

EFFECTS OF ALPHA-LIPOIC ACID(ALA) AND GLUTATHIONE (GSH) ON HUMAN TURBINATE-DERIVED MESENCHYMAL STEM CELLS(TMSC): THEIR ROLE OF ANTI-OXIDATIVE EFFECTS

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The rapid and effective proliferation of mesenchymal stem cells is critical for their clinical application. It has been reported that reactive oxygen species(ROS) prevent the proliferation of various mesenchymal stem cells. But effects of ROS to hTMSCs are not still known. The objective of this study is to evaluate the effect of the oxidative stress on hTMSCs and to evaluate the anti-oxidative effect of glutathione(GSH) and alpha-lipoic acid(ALA) on the proliferation of hTMSC. -MTT assays to evaluate cell viability activity of H2O2 on hTMSCs. -MTT assays to evaluate cell viability activity of GSH and/or ALA in presence of H2O2 on hTMSCs. Flow cytometry for effect of GSH and ALA on hTMSC. Fluorescent staining for effect of GSH and ALA on hTMSC. Fluorescent staining of CD90, CD105 positive cells for effect of GSH and ALA on hTMSC- Chondrogenic differentiation. hTMSCs-H2O2 cell viability activity. In 0μM-500μM H2O2, cell viability activity of hTMSCs decreased as increasing concentrations of H2O2. GSH/ALA-hTMSC cell viability activity. To GSH 5mM, cell viability activity increased, but over GSH 5mM it decreased. To ALA 250uM cell viability activity increased but over ALA 250uM it decreased. GSH/ALA-hTMSC cell viability activity in the treatment of H2O2. H2O2 suppressed cell viability and GSH and ALA reversed and the combination of GSH and ALA revealed weak synergic effects. Flow cytometry results with and without CD90/CD105 in the treatment of ALA and/or GSH were corresponding to results of MTTs. Fluorescent staining results in the treatment of ALA and/or GSH were corresponding to results of MTTs. Glutathione and alpha-lipoic acid facilitate the proliferation of hTMSCs and have some synergic effects.

T-2010

DEFINING LINEAGE RELATIONSHIPS IN THE SKELETOGENIC NEURAL CREST OF ZEBRAFISH

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Craniofacial tissue plays important roles for daily behaviors as eating and talking. Abnormalities during craniofacial development can impair such fundamental behaviors while bring lots of inconvenience. A better understanding of how craniofacial tissues develop from cranial neural crest cells (CNCCs) can help shed light on novel therapies for craniofacial disorders. For the multipotency of CNCC, however, in vivo cell lineage relationships are not yet well investigated, particularly the hierarchy and cell potency of progenitors during CNCC differentiation into mature connective tissues. Using zebrafish (Danio rerio) as a convenient platform to study development, we aim at constructing a comprehensive lineage tree of CNCC-derived cell types by combination of single cell analysis and cell barcoding techniques. So far, cell trajectory analysis of our single cell transcriptomes along developing craniofacial tissue had shown distinct matured cell types as cartilage, joint, bone, and tendon cells. We also observe some potential cells that could be oligo-potent or bipotent progenitors for these cell types.

MUSCULOSKELETAL TISSUE

T-2012

NANOG REVERSES THE HALLMARKS OF AGING IN HUMAN SKELETAL MUSCLE PROGENITORS

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Skeletal muscle loss due to aging or sarcopenia is a major medical problem facing the elderly. Adult skeletal muscle regeneration relies on the activity of resident satellite cells in skeletal muscle niche. However, intrinsic and environmental factors decrease the myogenic differentiation potential of senescent progenitors. Here we show that expression of an embryonic transcription factor, NANOG, in senescent skeletal muscle progenitors reversed their senescent morphology and ameliorated the aging hallmarks including their epigenetic profile, DNA damage and cellular energetics and ultimately restored the myogenic differentiation potential which was impaired due to cellular senescence. This reversal in the myogenic differentiation was shown at the functional level, by formation of myotubes in 2D and 3D, and at the molecular level by restoring the expression level of myogenic regulatory factors (Myf5, Myod, Myogenin, MRF4, members of myocyte enhancer factor 2 family). DNA methylation and RNA sequencing revealed epigenetic changes as well as changes in myogenic gene regulatory networks by NANOG. In addition, mapping of RNA-seq data onto metabolic pathways revealed the rewiring of metabolic networks by NANOG to restore the energy state of senescent cells. Indeed, measurements of individual metabolites revealed the effects of NANOG on oxidative phosphorylation and amino acid metabolism. Current experiments focus on the effects of NANOG in vivo using a progeria mouse model expressing NANOG in a doxycycline dependent manner. In conclusion, these results shed light on the mechanisms through which NANOG reversed cellular senescence and restored the myogenic differentiation potential of aged skeletal muscle cells in vitro and in vivo.

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T-2014

DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS INTO FUNCTIONAL CHONDROCYTES BY SOX9-INDUCED SMALL MOLECULE

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Osteoarthritis (OA) is a common joint disease that results from the disintegration of joint cartilage. Upon damage to the cartilage, chondrocytes differentiate into hypertrophic chondrocytes and release matrix-degrading enzymes leading to cartilage disintegration and calcification. Although chondrocytes have no self-renewal, there have been efforts to treat OA using stem cells, but it is difficult for stem cells to induce differentiation into chondrocyte-like cells without inducing hypertrophic chondrocyte characteristics. Therefore, an optimized method to differentiate stem cells into chondrocytes that do not display undesired phenotypes is needed. Previous studies have shown that overexpression of sox9, which is expressed during cartilage development, increases the expression of chondrocyte factors and has succeeded in differentiating stem cells into cartilage cells. Therefore, this study focused on differentiating adipose-derived stem cells (ASCs) into functional chondrocytes using a small molecule that regulated the expression of Sox9 and then explored its ability to treat OA. First, the expression of Sox9 was evaluated in chondrocytes and stem cells, and a drug capable of increasing the expression of Sox9 was then selected using a GFP-Sox9 promoter vector. Drug 138(D138) was selected because it increases the Sox9 expression in candidate drugs and did not induce hypertrophic chondrocyte characteristics. Next, the recovery rate of cartilage regeneration in ASCs-differentiation into chondrocytes by D138 was confirmed in a collagenase-induced animal model of OA. The group injected with ASCs-differentiated into chondrocytes by D138 recovered damaged cartilage faster than compared to the group injected with untreated ASCs. Taken together, these data confirm that D138 induces ASCs to differentiate into mature chondrocytes that do not exhibit the characteristics of hypertrophic chondrocytes, thus overcoming a problem encountered in previous studies. Additionally, the mechanism by which chondrocytes are differentiated by D138 and the recovery-inducing effect of D138-differentiated ASCs in OA was confirmed. These results indicate that D138 is a novel chondrocyte differentiation-inducing drug that shows potential as a cell therapy for OA.

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T-2016

NEURAL CREST-DERIVED MSC AUGMENT CRANIAL ALLOGRAFT INTEGRATION

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Current attempts to revitalize allografts for cranial regeneration using bone marrow-derived mesenchymal stem cells (BM-MSCs), which are mesodermal in origin, show limited success. While parietally calvarial bone is mesodermal, frontal bones originates from the neural crest. Neural crest cells (NCCs) are rare in adults. Induced pluripotent stem cells (iPSCs) can be reprogrammed to iNCCs and then to MSCs. We aimed to evaluate the integration of iNCC-MSC-coated allografts compared to BM-MSC-seeded allografts in mouse cranial defects. Induced PSC lines from healthy human fibroblasts were differentiated into iNCCs and characterized via immunofluorescent staining and flow cytometry. Differentiation of iNCCs into iNCC-MSCs was confirmed by MSC consensus marker expression, osteogenic and adipogenic differentiation. Tumorigenic potential of iNCC-MSCs using soft agar and teratoma formation assays was compared to BM-MSCs. No differences were detected in the MSC markers' expression and differentiation potential of iNCC-MSCs vs BM-MSCs. To analyze allograft integration, a calvarial defect was created in NOD/SCID mice and implanted with allografts coated with Luciferase reporter gene-transduced iNCC-MSCs or BM-MSCs in fibrin or with fibrin only. Cell survival, tracked with bioluminescent imaging, showed that both BM-MSCs and iNCC-MSCs survived on the allograft for at least 8 weeks. To evaluate bone volume and quality post-treatment, μCT analysis was performed. An increase in connectivity density in the allograft+iNCC-MSC group vs allograft only was demonstrated at week 2 post-surgery ($p<0.05$), but not in BM-MSC-coated allografts. Post sacrifice at week 8, histology showed an improved integration of iNCC-MSCs compared to BM-MSCs and control. Immunostaining indicated an increased expression of osteocalcin and bone sialoprotein in cell-seeded allografts vs control. Our results indicate that iNCC-MSCs are multipotential and can respond to osteogenic signals comparable to BM-MSCs in vitro and in vivo. In our calvarial defect model, of iNCC-MSC-coated allografts show superior integration compared to BM-MSC seeded and control allografts. Further investigation is needed to reveal the mechanism of the integration iNCC-MSC-seeded allografts in an environment of neural crest origin.

Funding Source: Musculoskeletal Transplant Foundation

T-2018

IDENTIFICATION OF METABOLICALLY DISTINCT MUSCLE RESIDENT FIBRO/ADIPOGENIC SUB-POPULATIONS REVEALS A POTENTIALLY EXPLOITABLE MECHANISM OF MOUSE SKELETAL MUSCLE AGING AND DISEASE

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Fibro/Adipogenic Progenitors (FAPs) make up a heterogeneous population of skeletal muscle resident stem cells thought to be the local contributor of fibrotic and adipose tissue. In healthy conditions following injury, skeletal muscle regenerates with little fibrosis, but larger insults and diseased conditions can result in intramuscular scarring, ectopic lipid deposition, and loss of tissue function. However, the role and mechanism of FAPs in these muscle pathologies is poorly understood. Here, we describe the identification and characterization of two functionally discrete subpopulations of Sca1+ PDGFRα+ CD31/45- FAPs in mouse hindlimb muscle. These two sub-populations can be discriminated and prospectively identified by aspects of metabolic activity. In vitro, these two sub-populations display distinct differences in mitochondrial oxidative metabolism and in lineage specificity, toward fibrotic or adipogenic fates. In transplantation experiments, we found that both sub-populations have comparable engraftment ability and maintain their identities but can also transition between sub-populations. In vivo, in response to muscle injury, the proportions of both sub-populations shift and then re-adopt original homeostatic distributions in regenerated muscle. However, fibrotic injuries and aging significantly alter the homeostatic balance between the two sub-populations. Collectively, our data suggest that these inter-convertible FAP sub-populations have differential roles in muscle fibrosis and aging. Further work on the cellular and molecular mechanisms that regulate and distinguish these populations will lead to new insights and better understanding of muscle aging and disease.

T-2020

MESENCHYMAL STEM CELLS (MSCS) and TRANSCRIPTION FACTOR/CO-FACTOR REPORTER SCREENING AS ROBUST PLATFORM FOR SELECTION OF OSTEOGENIC COMPOUNDS

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Rapid increases in the incidence of osteoporosis are due to the aging of worldwide populations. While multilineage mesenchymal stem cells (MSCs) appear to be excellent cellular agents to reverse the bone loss that occurs with this disease, actual implementation of cell therapy is not feasible due to the systemic nature of osteoporosis. On the other hand, the 'gold-standard' in vitro osteogenic assay of calcium deposition for MSCs are well established but lengthy, requiring at least 1 month of time for completion. We therefore took advantage of in vitro MSC osteogenesis and, in combination with reporter assays for multiple relevant osteogenic transcription factors (TFs) and/or co-factors, establish a rapid and robust system of screening for osteogenic small molecules, including phytoestrogens which are natural compounds with selective estrogenic agonistic and antagonistic effects. Using this combination screening system, we can not only shorten the selection process for osteogenic compounds from 3~4 weeks to a few days, but also simultaneously perform comparison screenings of multiple compounds to assess relative osteogenic potency. Using a panel of 10 plant-derived compounds as an initial test, we performed the TF/co-factor luciferase reporter assay in the mouse MSC line, C3H10T1/2. Classical in vitro and in vivo osteogenesis assays were performed using primary murine and human bone marrow MSCs to validate the robustness of this rapid screening platform. We found nearly absolute correlation of the TF/co-factor luciferase reporter platform to classical functional assays. Our findings therefore demonstrate that TF/co-factor reporter assays performed in MSCs efficiently and robustly screen for candidate osteogenic compound including phytoestrogens, and thus be relevant for therapeutic application in osteoporosis

CARDIAC TISSUE AND DISEASE

T-2024

THE PARADIGM OF ENDOGENOUS MAMMALIAN HEART REGENERATION BASED ON MONONUCLEAR DIPLOID CARDIOMYOCYTE PROLIFERATION EXTENDS TO THE NEONATAL MOUSE HEART

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Fetal and neonatal hearts are regenerative, whereas the mammalian heart has historically and pessimistically been considered to be postmitotic and nonregenerative. Fetal and neonatal cardiomyocytes (CMs) are mononuclear and diploid, but almost all mammalian CMs become polyploid during the first postnatal week. We recently showed that the relatively small subpopulation of mononuclear diploid CMs in the adult mouse heart retains proliferative and regenerative competence, and furthermore, that the frequency of these CMs in the adult heart is variable and can be surprisingly high in some inbred mouse strains. Here, we use manipulation of IGF2 as a tool to extend these insights to neonatal mouse heart regeneration. We show that IGF2, an important mitogen in embryonic heart development, is required to support neonatal heart regeneration. Following injury (apex resection) on postnatal day 1, IGF2 accounts for all induced CM cell cycle entry activity (as indicated by phospho-histone H3 staining) during the early part of the first postnatal week, when most CMs are still mononuclear diploid and still proliferative. In the absence of IGF2, heart regeneration fails despite the later presence of additional activities that support robust cell cycle entry (pH3+ labeling) 7 days following injury, a time when most CMs have become polyploid and are no longer able to complete cytokinesis. However, regeneration was rescued in IGF2-deficient neonates in three independent experimental contexts that retain an elevated percentage of mononuclear diploid CMs through postnatal day 7 and beyond. These results demonstrate the relevance of mononuclear diploid CMs to heart regeneration in both the neonatal and adult heart. In the neonatal heart, IGF2 uniquely acts as a paracrine mitogen during the early period when a high number of proliferation-competent CMs are normally present. Cardiac IGF2 expression ceases after the neonatal stage, but interestingly, becomes reexpressed in the adult heart after injury. This pathway could be amenable to intervention to further boost natural regeneration in the injured adult heart.

T-2026

CONTRACTILE DEFICITS IN ENGINEERED HUMAN IPSC-CARDIAC MICROTISSUES AS A RESULT OF MYBPC3 DEFICIENCY AND MECHANICAL OVERLOAD

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Combining tissue engineering, human induced pluripotent stem cell (hiPSC) technology and genome-editing tools allows for the enhanced interrogation of physiological phenotypes and recapitulation of disease pathologies. However, non-genetic, environmental factors, including ECM modulus and tissue mechanics, also contribute to disease progression. In this work, we used two-photon polymerization (TPIP) of the UV curable organic–inorganic hybrid polymer to create a highly organized and quantitatively reproducible filamentous matrix that resembled collagen fibers in the ECM. We used this matrix together with genetically defined hiPSC to model contractile deficiency. Matrices were populated with cardiomyocytes derived from healthy wild-type (WT) hiPSCs (WT hiPSC-CMs) or isogenic hiPSCs deficient in the sarcomere protein cardiac myosin-binding protein C (MYBPC3 null hiPSC-CMs). Tissue mechanical loading was controlled by varying the diameter of fibers within the matrix. Compared to their counterparts on compliant fibers, WT microtissues temporally adapted to a more rigid mechanical environment by increasing contraction force. In contrast, MYBC3 deficient microtissues exhibited impaired force development kinetics regardless of matrix stiffness and deficient contraction force only when grown on matrices with high fiber stiffness. Under mechanical overload, the MYBPC3 null microtissues had a higher degree of calcium transient abnormalities including Early After Depolarizations (EADs) and exhibited an accelerated decay of calcium dynamics as well as calcium desensitization. Calcium transient decay and EADs were exacerbated when the MYBPC3 deficient tissues contracted against a stiff environment. Our findings suggest that sarcomere deficiency and the presence of environmental stresses synergistically lead to contractile deficits in cardiac tissues, suggesting that environmental factors including tissue rigidity must be a critical consideration for in vitro models of heart disease.

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T-2028

PHYSICAL DIFFERENCES BETWEEN AND WITHIN PATIENT-DERIVED IPSC-VSMCS IDENTIFY CELL PHENOTYPE HETEROGENEITY WHICH CORRELATES WITH GENOTYPE-ASSOCIATED DISEASE RISK

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Polymorphisms that occur in non-coding loci have variable expression, exist in linkage disequilibrium, and/or have variable penetrance, which limit our understanding of mechanisms that enhance disease risk. To understand the extent that they exacerbate variance and disease, we chose to study 9p21, i.e. the locus with strongest association with vascular disease. Patient- and induced pluripotent stem cell (iPSC)-derived vascular smooth muscle cells (VSMCs) switch from a contractile phenotype, where cells are larger and less migratory, to a synthetic phenotype, where cells are smaller, more proliferative, and migratory. To understand the effect of risk variants (R/R) on phenotype, we designed and built a microfluidic device to sort cells based on the strength of their adherence. We found that the presence of risk variants (R/R) globally reduced adhesion and contractility and promoted a proliferative, synthetic phenotype compared to counterparts lacking such variants (N/N). However, substantial variance existed between clones and within a given clonal population. We hypothesize that this is the result of stochastic expression and splicing of long non-coding RNAs associated with the presence of 9p21 polymorphisms, e.g. ANRIL, consistent with our prior interpretation of the VSMC transcriptome and ANRIL's regulation of disease-causing gene

networks (Lo Sardo et al, Cell 2018). These data demonstrate that physical, rather than biochemical, differences in cell phenotypes can identify heterogeneity between and within patient-derived iPSC-VSMCs that correlate with disease risk.

Funding Source: NIH National Heart, Lung and Blood Institute

T-2030

TRACKING THE PHYSIOLOGICAL STATE OF HUMAN EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTE USING CRISPR/CAS9 KNOCK-IN HK1-EGFP METABOLIC REPORTER TO UNCOVER THE MECHANISMS OF MATURATION/DISEASE PROGRESSION

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During cardiac development, glycolysis is the predominant source of energy for proliferating cells. As cardiomyocytes mature, mitochondrial oxidative capacity increases, with fatty acid β-oxidation becoming the major source of energy for the heart. On the other hand, during the development of cardiac hypertrophy and progression to heart failure, the most notable change in metabolic profile is a reversion from fatty acid β-oxidation to glycolysis; the preferred fetal energy metabolism process. In a quest to manipulate the unique dynamics of cardiac metabolism that accurately correlates with the physiological state of cardiomyocyte during maturation/disease progression, we generated a human ES derived metabolic reporter cell line, H7 HK1-EGFP using the CRISPR/Cas9 technology targeting on hexokinase 1, an enzyme in the glycolytic pathway. In this study, we made use of this reporter to track the physiological status of cardiomyocytes at different stages of development. Here, we demonstrated that the EGFP expression accurately correlated with the metabolic profiles and maturation status of cardiomyocytes. Using purified cells, we performed RNA sequencing to identify key molecular mechanism that governs maturation or disease progression in cardiomyocytes.

T-2032

A PHOTOSENSITIZER FOR ENHANCED PHOTODYNAMIC CANCER STEM CELL(CSC) THERAPY BY REDUCING INTRACELLULAR GSH LEVELS

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Carbonic Anhydrase 9 (CA9) has been suggested an intrinsic hypoxia as well as cancer initiating cells (CICs) marker in various solid tumors. Previously, our group reported that an acetazolamide (AZ) conjugated BODIPY photosensitizer (AZ-BPS) as a photodynamic cancer therapy (PDT) targeting CA9 positive cancer cells. Here, we reported that a metal–organic hybrid system provides a new approach to reduce intracellular GSH levels and improve the efficiency of photodynamic therapy. T-PDT+Cu showed specific affinity to aggressive human breast cancer stem cells (CD44- and ALDH-positive cells in MDA-MB-231 cells) that overexpress CA9 with benefits of anti-angiogenic effects by PDT. Importantly, our T-PDT+Cu displayed enhanced depletion of intracellular mitochondrial GSH, which significantly increased photo-cytotoxicity and decreased tumor spheroid formation, stemness and epithelial to mesenchymal transition (EMT) related genes (TGF-beta, Vimentin, Snai2, and CLDN1) compared to a reference compound, T-PDT without Cupper. Considering all, our results strongly suggest that T-PDT+Cu could be a clinical applicable therapeutic agent to targeting CA9-overexpressing breast cancer stem cells.

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T-2034

RAPID TRAVELING WAVES ENHANCE THE MATURATION OF HIPSC-DERIVED CARDIOMYOCYTES IN SELF-ORGANIZED TISSUE RING

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The immature state of hiPSC derived cardiomyocytes (hiPSC-CMs) may limit their applications in drug screening and regenerative therapy. Multiple stimulation methods such like rapid electrical pacing have been used for promoting hiPSC-CM maturation. Here we show a simple device in modified culture plate on which hiPSC-CMs can form three-dimensional self-organized tissue rings (SOTRs). Within the ring, traveling waves (TWs) of action potential spontaneously originated and ran robustly at a frequency up to 4 Hz without using any external stimulation. The CMs are rapidly paced by the TWs. After two weeks, SOTRs with TWs training showed matured structural organization (e.g., sarcomere length = ~2 um), increased cardiac-specific gene expression, enhanced Ca2+ handling properties, increased oxygen consumption rate as well as contractile force in the scale of mN/mm2. A mathematical model was also used to interpret the origination, propagation and the long term behaviour of TW within SOTRs. Taken together, our results demonstrated a unique tissue engineering approach for fast and spontaneous maturation of hiPSC-CMs, holding high potential for production of matured electrically active cells for drug screening and regenerative applications.

T-2036

UNRAVELLING THE POTENTIAL OF ADULT MURINE CARDIAC STEM CELLS

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The regenerative potential of the heart is limited, if existing at all. Nonetheless, its necessity is obvious, as heart-related diseases are still the leading cause of death in developed countries. The spotlight has turned on cardiac-residing stem cells and whether they are able to at least contribute regenerating a failing heart. Despite the controversy whether cardiac stem cells (CSCs) exist in the adult heart, we isolated and successfully propagated CSCs from murine hearts by establishing specific niche conditions, which keep the cells in an indefinite self-renewal and phenotypically stable state. This enables us to characterize and understand the potential of adult CSCs. By generating embryoid body (EB)-like cardiac bodies (CBs) from these cells, we clearly demonstrated that they differentiate solely into cells of the cardiac lineage – cardiomyocytes, endothelial cells and smooth muscle cells – in vitro. In addition, transcript expression analysis revealed a distinct gene regulation program in CSCs, which significantly differs from embryonic stem cells (ESCs) during early in vitro differentiation. One newly identified important modulator of cardiomyogenesis is the extra cellular matrix component Secreted Protein Acidic and Rich in Cysteine (SPARC). SPARC does not only promote early cardiomyogenesis during heart development in EBs; it is also abundant in murine adult CSCs, and further increases during CSC differentiation to somatic cardiac cells. Our data suggest that SPARC induces differentiation, at least partly, via the upregulation of the intermediate filament protein Desmin, which, surprisingly, promotes SPARC expression itself in a dose dependent and paracrine manner. These in vitro data lead to the assumption that SPARC and Desmin synergistically advance cardiomyogenesis via a self-reinforcing circuit. With these novel cardiac stem cell lines in hand, we are convinced that adult CSCs are capable of differentiating to functional cardiomyocytes if provided with the right environmental cues. This includes not only well-described transcription and growth factors but also genes, like SPARC and Desmin, which were not directly linked to differentiation until now.

T-2038

MRNA-ENGINEERED HUMAN MESENCHYMAL STEM CELLS PROMOTE CARDIOVASCULAR AND RENAL REGENERATION

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Cell-based strategies for tissue regeneration and repair continue to be touted as an emerging technology that holds great promise for organ-wide therapies. For prevention of organ injury and repair after an injury, mesenchymal stem/stromal cells (MSCs) have been widely studied in a range of disease models including ischemia-induced injuries, mitigatation of graft versus host disease, and inflammatory autoimmune diseases. To date, several MSC types have been clinically tested and identified to have therapeutic benefits with minimal risk of integration and

side effects, in part due to their transient survival. Furthermore, the recent emergence of mRNA-based technology through the use of chemical modifications of nucleobases has grown precipitously. Modified mRNAs (modRNAs) are synthetic messenger RNAs capable of delivering in vivo therapeutic protein levels in doses favorable to promote tissue regeneration. In addition, modRNAs are transiently expressed and have been shown to widely bypass innate immune responses when delivered into exogenous cell types. Herein, we report a method for enhancing the endogenous reparative effects of mesenchymal cells in the setting of ischemic injury, via over-expression of paracrine growth factors from a non-viral, non-integrative modified mRNA.

T-2040

GWAS VALIDATION USING PATIENT-SPECIFIC HIPSC IDENTIFIES DOXORUBICIN-INDUCED CARDIOTOXICITY PROTECTIVE DRUGS

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Doxorubicin is effective in treating a range of malignancies, but its use is limited by dose–dependent cardiotoxicity. A recent genome–wide association study (GWAS) identified a SNP (rs2229774) in retinoic acid receptor–g (RARG) as statistically associated with increased risk of doxorubicin induced cardiotoxicity (DIC). Here, we utilize human induced pluripotent stem cell derived cardiomyocytes (hiPSC CMs) to validate this deleterious SNP and determine its mechanism of action. We show that hiPSC CMs from patients with rs2229774 are more sensitive to DIC, independently confirming the accuracy of the hiPSC CM DIC model. By knockout and overexpression of RARG by CRISPR/Cas9 we confirm that RARG is the effector gene in this DIC predisposition model, which we then further substantiate by showing that correction of rs2229774 eliminates increased doxorubicin susceptibility. We go on to determine the mechanism of this RARG variant effect is mediated via suppression of TOP2B expression and activation of the cardioprotective ERK pathway. We use these patient specific hiPSC CMs as a drug discovery platform, determining that the RARG agonist CD1530 dramatically attenuates DIC, confirming that this protective effect is successful in an established in vivo mouse model of DIC. Thus, we demonstrate for the first time that hiPSC CMs can be used as a powerful precision medicine tool for GWAS validation, with simultaneous pharmacogenomics led drug discovery in a human model with direct potential for clinical translation. This study provides a strong rationale for clinical pre-chemotherapy genetic screening for rs2229774 and a foundation for the clinical use of RARG agonist treatment to protect cancer patients from DIC.

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ENDOTHELIAL CELLS AND HEMANGIOBLASTS

T-2042

NOVEL HIPSC DERIVED SYSTEM FOR HEMATOENDOTHELIAL AND MYELOID BLOOD TOXICITY SCREENS IDENTIFIES COMPOUNDS PROMOTING AND INHIBITING ENDOTHELIUM TO HEMATOPOIETIC TRANSITION IN VITRO

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Endothelium to hematopoietic transition (EHT) plays a critical role in the development of hematopoietic system during embryogenesis. Exposure to adverse factors during EHT may alter characteristics of hematopoietic stem cells causing hematologic diseases including childhood leukemia. Here we describe the results of a primary toxicity screen using a hiPSC system for generating myeloid hemogenic endothelium (HE) and blood cells by overexpression of transcriptional factors GATA2 and ETV2. Twenty chemical compounds selected from the NIH NTP Tox 21 library were applied during EHT on day 3 of differentiation (EHT toxicity), and on day 21 to myeloid progenitors growing in suspension. The toxicity screen identified compounds that inhibited hematopoietic differentiation (5-Fluorouracil, Berberine chloride, Mercuric chloride, tert-BDP, Digitonin, Benzo(a)pyrene; increase VE-cadherin/CD43 ratio FC>2, $p<0.05$, assessed by flow cytometry) and supported blood formation (Thalidomide; decrease VE-cadherin/CD43 ratio -0.22, ns). Mercuric chloride displayed strong cyto- and mitochondrial toxicity in myeloid progenitors. Inhibition of EHT by 5-Fluorouracil, Berberine chloride and Benzo(a)pyrene resulted in suppressed colony forming capacity (CFC) of differentiated cultures, decreased expression of cell surface markers CD43, CD34, CD41a ($p<0.05$) and significant downregulation of hematopoietic genes (GATA1, KLF1, CEBPA, HBE1, HBG1 etc.; $p<0.05$). In addition, gene expression analysis revealed significant upregulation of endothelial genes, which indicates switch of transcriptional program from HE to non-HE

production. Gene ontology and pathway analysis of 127 genes that are commonly downregulated by 5-Fluorouracil, Berberine chloride and Benzo(a)pyren- induced toxicity showed significant enrichment of genes involved in cell cycle regulation and G1/S checkpoints. Commonly upregulated 78 genes represent cohorts of cell adhesion molecules, mostly integrins. Thalidomide stimulated expression of erythroid factors GATA1 and GYPA, and increased Er-CFC. Therefore, TF-based differentiation system described herein effectively identified chemicals with both inhibitory and favorable effects on EHT and changes of hematopoietic marker expression, detecting mitochondrial and cytotoxicity.

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T-2044

ABCG2 EXPRESSING ENDOTHELIAL STEM CELLS IN HUMAN CORD BLOOD ECFCS AND HUVECS

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A long held question is to identify whether endothelial precursors are present within the resident vascular endothelial intima. Since both human cord blood-derived endothelial colony-forming cells (CB-ECFCs) and human umbilical vein endothelial cells (HUVECs) show similar cobblestone-like morphologies and colony-forming hierarchy, these rare circulating endothelial cells (CB-ECFCs) are thought to be produced from resident vascular endothelium (including HUVECs). This hypothesis has not been directly tested. Here, we propose that ATP binding cassette subfamily G member 2 (ABCG2) labels human resident and circulating endothelial stem cells that display clonal proliferative potential and blood vessel forming ability. We found a rare ABCG2+ EC fraction in both circulating CB CD34+CD45- cells and fresh CD34+CD45- HUVECs. The ABCG2+ ECs gave rise to significantly larger and more EC colonies than ABCG2- ECs. Single ABCG2+ HUVEC derived EC formed both capillaries and arteries in implanted collagen gels with OP9-DL1 co-transplanted cells in immunodeficient mice. After recovery of the perfused human vessels and digestion of the gels, the recovered human endothelial cells inside were able to be re-plated and making secondary ECFC colonies and these cells could be re-implanted into secondary recipient mice to generate secondary donor vasculature. We also performed CITE-seq using these two MACS-sorted populations (CD34+CD45-GPA-) from the same human subject to evaluate ABCG2 expression level as well as single cell RNA sequence (scRNAseq) analyses (data in analysis). We anticipate identification of novel markers and genes associated with self-renewal in these cell populations that will include the stem cells and their downstream progenitor progeny. We also anticipate differences in scRNAseq that may illuminate potential signaling specificities of circulating versus resident ABCG2+ endothelial stem cells.

T-2046

RESTORING INTRACELLULAR PH INDUCES FORMATION OF MATURE WEIBEL PALADE BODIES IN HUMAN IPSC-DERIVED ENDOTHELIAL CELLS

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Differentiation of human induced pluripotent stem cells (iPSCs) into vascular endothelium is of great importance to achieve organogenesis, scaffold recellularization and other tissue engineering techniques. Although differentiation of iPSCs into endothelial-like cells has been demonstrated before, it has been reported that these cells remain in an immature, fetal-like, stage. We aim to create stable iPSC-ECs which can sense and adapt to shear, are non-coagulant and can communicate with pericytes to achieve vascular supply for organoids and glomeruli of the kidney. To investigate iPSC-EC functionality we determined their ability to express von Willebrand Factor (vWF)

and formation of the elongated, cigar shaped, Weibel Palade Bodies (WPBs). We observed that stimuli, such as shear stress, KLF2 overexpression or pericyte co-culture have no additional effect on both vWF production and WPB formation. Since pro-vWF dimers need to be transported to the lower pH environment of the trans-Golgi network (TGN) for maturation, we tested iPSC-EC intracellular pH (pHi) in comparison to primary ECs. Here, we show that the higher iPSC-ECs pHi is possibly caused by decreased expression of MCT1, a member of the solute carrier family (SLC16A1), which regulates internal lactate concentration. Lowering iPSC-EC pHi with acetic acid, in turn, resulted in the formation of elongated WPBs in these cells, which is of essence for correct endothelial function.

T-2048

DIFFERENTIATION OF HUMAN ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS TOWARD ENDOTHELIAL PHENOTYPE BY IRRADIATION WITH ELECTROMAGNETIC WAVES

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A major reason medicine seek assisted regenerative cell therapy is for regeneration of lost tissue functions. Adult and stem cells reprogramming holds much promise for a variety of diseases. A general disadvantage of the presently available methods however, is the low reprogramming efficiency, which restricts their routine therapeutic application. Here we investigate the potential of terahertz irradiation (THz) to alter the phenotype of human adult adipose tissue derived mesenchymal cells (hAAMS) toward pro-endothelial cells. We used an ultra-short pulsed broadband (centered at ~10 THz) source to irradiate hAAMS cultures for 2 and 9 hours. A control hAAMS culture was placed adjacent to the irradiated sample (screened from the THz radiation). Immediately after completing the irradiation, cells were incubated for 48 hours in culture medium at 370C. Total RNA was extracted from the irradiated samples and their corresponding controls, and whole transcriptome shotgun sequencing (WTSS) was applied to reveal and compare the presence and quantity of mRNA and identify differential changes in gene expression between the irradiated samples and the respective controls. RNA-seq of mRNA extracted from the hAAMS in each of the irradiation scenarios revealed statistical evidence ($p< 0.05$) for 498 differentially expressed genes. Prolonged (9 hours) THz irradiation resulted in overexpression of 347 genes and underexpression of 151 genes. In contrast, the 2 hours prolonged irradiation, resulted in overexpression of 22 genes and suppression of 4 genes. This difference suggests that the THz influence depends on the exposure time. The RNA-seq survey reveals only 3 statistically significant differentially expressed genes after incubation of hAAMS for 48 hours at 37oC in a medium without THz irradiation. Our results show that THz irradiation of hAAMS for 9 hours causes specific changes in gene expression that are closely related to their differentiation toward pro endothelial phenotype. More than 90% of the cells are expressing exactly the group of genes that support the differentiated phenotype after 9 hours of irradiation. Importantly, cells don't die during and after the THz treatment. The expression levels of genes encoding heat shock proteins are practically unaffected.

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HEMATOPOIESIS/IMMUNOLOGY

T-2050

MOLECULAR CONTROL OF THE ENDOTHELIAL TO HEMATOPOIETIC TRANSITION

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Hematopoietic Stem and Progenitor Cells (HSPCs) are at the basis of the regulated functioning of the hematopoietic system throughout the life of the individual. In adult amniotes, HSPCs reside in the bone marrow but are produced early during development, transiently and in small numbers at the level the dorsal aorta from specialized endothelial cells (EC), termed hemogenic. These hemogenic ECs are themselves derived from non-hemogenic ECs. Hemogenic ECs, under the influence of signals yet to be defined, lose their endothelial fate and acquire a hematopoietic identity through a mechanism designated as endothelial-to-hematopoietic transition (EHT). How hemogenic ECs are specified and how EHT is fine-tuned remain unanswered questions but has major implications in regenerative medicine. We recently designed an ex vivo culture system, starting from the quail pre-somitic mesoderm, that mimics the steps occurring in the aorta to produce the first HSPCs (Yvernogeau et al., 2016; Development, 143: 1302). We have exploited this system to capture transcriptomic signatures specific for the mesoderm, ECs, hemogenic ECs and HSPCs. Using an ensemble of systems biology approaches, we have isolated gene networks specific for the different cell categories and have identified strong candidate genes, highly connected to the network, likely acting on the passage from one state to another with a particular emphasis for the specification of the hemogenic endothelium and the control of EHT. We have selected four genes that are currently under functional validation with siRNA approaches i.e., POFUT2, TESTIN, EMILIN 1&2. In addition the NOTCH and WNT pathways were also explored using small molecules. Taken together our results should help to better define key steps in the commitment towards HSPC to further produce safe and robust cells for therapeutic purposes.

T-2052

A NON-HUMAN PRIMATE CRISPR/CAS9 MODEL OF CLONAL HEMATOPOIESIS DEMONSTRATES MYELOID SKEWING, INFLAMMATORY SIGNALING THROUGH TET2-DISRUPTED CLONES

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Recent population-based genomic studies of human blood cells have identified somatic mutations associated with clonal expansions commonly arising with aging, even in the absence of cytopenias, myelodysplasia, or leukemia. Loss of function or dominant negative mutations in genes encoding epigenetic modifier enzymes such as DNMT3A, TET2, and ASXL1 are most frequently mutated in this aging-related clonal hematopoiesis (ARCH). However, the relationships between these mutations, clonal expansions, and progression to myelodysplasia or leukemia are not well-understood due to challenges modeling long-term clonal dynamics in vitro or murine models, and limited access to samples from individuals with mutations but without a hematologic diagnosis. To address this, we performed autologous transplantation of rhesus macaque hematopoietic stem and progenitor cells (HSPCs) edited with CRISPR/Cas9 to create loss-of-function mutations in DNMT3A, TET2, and/or ASXL1. Rhesus macaques (N=5) received autologous gene-edited HSPCs and engrafted promptly, and the level of indels at each target site was 2% or less. For up to 36 months post-transplantation, we have not detected clonal expansion of DNMT3A- or ASXL1-mutated HSPCs, however, there was a gradual and marked expansion of indels predicted to knock out TET2 function in blood cells. Tracking the indel signatures suggested that the expanding TET2 mutant clones originated from long-term HSPCs rather than short-term progenitors, and initially mutated clones were sufficient to initiate clonal expansions. Furthermore, hematopoietic lineage specific analyses indicated that TET2-mutated clones expanded in the stem cell compartment and preferentially accumulated in macrophages. TET2-edited bone marrow and macrophages exhibited a distinct gene expression profile, including higher expression of inflammation-related genes, such as NLRP3 or IL1β, helping to explain the increased prevalence of cardiovascular disease in ARCH. Taken together, these approaches should improve our understanding of the roles of TET2 loss of function on the development of clonal hematopoiesis in a clinically relevant setting, and allow investigation of agents to retard clonal progression.

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T-2054

ADULT ENDOTHELIAL CELL REPROGRAMMING YIELDS HAEMATOPOIETIC STEM CELLS WITH LYMPHOID IMMUNE FUNCTION IN VIVO

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During development, the hematopoietic stem cells that go on to populate the bone marrow and give rise to all blood cell lineages emerge from a specialized endothelial subpopulation. We have previously harnessed this vestigial identity to achieve the direct conversion of endothelial cells (ECs) into hematopoietic stem and progenitor cells (rEC-HSPCs); however, we have only detected functional T cells that result from the engraftment of mouse rEC-HSPCs. We reprogrammed adult human endothelium via constitutive overexpression of FOSB, GFI1, SPI1, and RUNX1 (FGRS). Reprogrammed cells maintained expression of the four transcription factors (TFs) for over 20 weeks post-transplantation into immuno-compromised mice. However, constitutive expression of Spi1 has been shown to hinder lymphoid differentiation, and mice of the NSG strain cannot educate native T or B cells to maturity. Only by turning off FGRS overexpression in mouse ECs did we obtain bona fide rEC-HSPCs that conferred transplanted congenic mice the ability to generate an adaptive immune response. We have reimagined our platform in mouse system to generate human rEC-HSPCs making use of (i) doxycycline-inducible vectors to temporarily overexpress FGRS and (ii) transgenic sub-strains of NSG mice for transplantation assays. Preliminary data show human rEC-HSPCs engraft up to 20% in the spleen or bone marrow of transplanted mice. Engrafted cells differentiate into all blood lineages, including mature T cells, in the absence of

exogenous FGRS expression in vivo. Notably, the resulting T cells undergo TCR rearrangement and clear viral particles one week after infection. This work would not only shed light on basic HSPC biology, but also translate into myriad clinical applications.

T-2056

SINGLE-CELL RNA-SEQ OF CD34+/CD45+ SORTED UNSTIMULATED HUMAN PERIPHERAL BLOOD SHOWS HEMATOPOIETIC DIFFERENTIATION HEIRARCHY AND IDENTIFIES A QUIESCENT PROGENITOR SUB-POPULATION

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Leukocytes comprise up to about one percent of human peripheral blood (PB). Within this fraction, hematopoietic stem and progenitor CD34+ cells can make up between 0.001 - 0.007 percent of the leukocyte fraction. To investigate the transcriptional profile of this population, 350*10^6 unstimulated PB mono-nuclear cells (MC) were sorted for CD34+/CD45+ expression and then single-cell RNA sequenced. After filtering, 1708 CD34+/CD45+ PBMC transcriptomes remained. The data was then visualized using SPRING, which draws a graph of cells connected to their nearest neighbours in gene expression space and projects this into two dimensions using a force-directed graph layout. This revealed the hematopoietic maturation and differentiation hierarchical tree in near entirety and visualized the continuum of states progenitor cells can occupy. Sub-population marker genes facilitated dissection of the branched tree structure revealing 1) the branch point at which megakaryoblast and erythroblasts transcriptionally diverge as marked by distinct FLT3 and GATA1 expression profiles; 2) the transcriptional similarity and differences between lymphoid and myeloid progenitor populations; and 3) identified a potentially novel sub-population of CD34+/CD45+ progenitors which appear to be transcriptionally inactive with respect to other identified populations. Differential gene expression and pathway analysis of this population reveals a signature of global down-regulated gene expression and cellular pathway inactivity, suggesting cellular quiescence. The observed hierarchical structure is validated by previously published works, thereby instilling confidence in our approach to have accurately purified and transcriptionally profiled this ultra-rare cell population from unstimulated peripheral blood. While hematopoietic stem and progenitor populations have been extensively studied in both bone marrow and mobilized peripheral blood, our data is the first to show evidence of the hematopoietic maturation and differentiation hierarchical tree in non-stimulated peripheral blood sorted CD34+/CD45+ cells. Furthermore, we identify a potentially novel, quiescent stem-cell population within this rare fraction of leukocytes, the transplantation potential of which is currently under investigation.

T-2058

RAPID AND DETERMINISTIC FORWARD PROGRAMMING OF HUMAN PLURIPOTENT STEM CELLS INTO MICROGLIA TO MODEL THEIR ROLE IN NEUROLOGICAL DISEASES

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Microglia are the resident immune cells of the central nervous system (CNS). They are derived from yolk sac macrophages that arise during the first wave of primitive haematopoiesis and populate the developing CNS during early embryonic development. The microglia population is self-maintained throughout life by continuous turnover. Currently available protocols for the generation of microglia-like cells from human pluripotent stem cells (hPSCs) rely on classical differentiation following the events of embryonic development. For the first time, they provide a tool for the scalable production of human microglia-like cells for drug discovery und disease-modelling. However, published protocols are characterised by long culture durations (up to 75 days) and the need for mechanical manipulation steps or fluorescent-activated cell sorting to enrich for intermediate progenitor populations, thus hampering their widespread application. We present a novel approach for the generation of microglia-like cells from hPSCs, which is based on transient overexpression of master reprogramming factors in hPSCs in conjunction with specific extracellular cues. Our approach yields pure populations of microglia-like cells in less than three weeks and does not rely on cell enrichment steps. The microglia-like cells are characterised by expression of typical sets of microglia markers on mRNA- and protein-level, phagocytotic activity, and physiological responses to appropriate extracellular stimuli, including changes in oxygen consumption, intracellular calcium transients, reactive oxygen species production, and cytokine secretion. The microglia-like

cells can be placed in monoculture for reductionist studies or coculture with hPSC-derived cortical neurons or 3D brain organoids for the study of cellular interactions in complex healthy or diseased environments.

T-2060

EXPANSION OF HUMAN HEMATOPOIETIC STEM-PROGENITOR CELLS IN XENO-FREE SERUM-FREE STEMPRO HSC EXPANSION MEDIUM (PROTOTYPE)

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Development of ex vivo culture systems to expand harvested human hematopoietic stem-progenitor cells (HSPCs) remains a critical translational research quest that would enhance clinical hematopoietic stem cell (HSCs) transplantation and gene therapies. To address the problem that HSCs generally die or differentiate rapidly in current ex vivo culture media, we developed a xeno-free, serum-free medium -- StemPro™ HSC Expansion Medium (Prototype) -- by extensive iterative modifications of medium constituents. Culture of primary human CD34+ cells immunopurified from healthy cord blood, mobilized peripheral blood and bone marrow in StemPro™ HSC Expansion Medium (Prototype) supplemented with FLT3L, KITL (also known as SCF), TPO, IL3, and IL6 (FKT36), resulted in massively increased numbers of immunophenotype-defined HSPCs, as compared to either uncultured day 0 cells or cells cultured in industry-standard culture media containing FKT36. For example, culture of primary human CD34+ cells from mobilized peripheral blood (mPB) for 7 days in FKT36-containing StemPro™ HSC Expansion Medium (Prototype) resulted in ~100-fold increased numbers of CD34+CD45+Lin- cells and ~2000-fold increased numbers of CD34+Lin-CD90+CD45RA- cells (an early HSPC immunophenotype), as compared to uncultured day 0 cells. The ex vivo-cultured CD34+ cells contained high frequencies of aldehyde dehydrogenase-containing cells and formed erythroid and non-erythroid hematopoietic colonies in vitro. In an ongoing in vivo hematopoietic chimera experiment, ex vivo-cultured mPB CD34+ HSPCs harbored robust in vivo-engrafting capacity at the 8-week post-transplant short-term HSC time point evaluated to date. Thus, it appears that StemPro™ HSC Expansion Medium (Prototype) supports HSPC expansion that includes self-renewal, or at least prolonged survival of short-term-HSCs. Evaluation of long-term-HSC capacity is in progress.

T-2062

THE ROLE OF RETINOIC ACID SIGNALING IN HUMAN DEFINITIVE EMBRYONIC HEMATOPOIESIS

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The directed differentiation of embryonic stem cells (ESCs) to hematopoietic stem cells (HSCs) provides an unparalleled platform to study human embryonic hematopoietic development in vitro as well as a novel source of cells for the treatment of hematological disorders. The failure to generate ESC-derived HSCs without ectopic transcription factor expression highlights the complexity of embryonic hematopoiesis and is likely due to the inappropriate specification of the putative progenitor population. Embryonic hematopoietic development consists of multiple waves of hematopoiesis, each of which has unique spatio-temporal properties and progenitor potential. Only the wave termed definitive hematopoiesis generates all hematopoietic lineages including HSCs, which support multilineage hematopoiesis in adulthood. Definitive hematopoiesis initiates at E10.5 in multiple intraembryonic sites of the mouse embryo, the best characterized being the aorta-gonad-mesonephros (AGM) region. Within the AGM, a specialized endothelial population termed hemogenic endothelium (HE) forms nascent HSCs. Former studies from our laboratory have demonstrated that ex vivo activation of retinoic acid (RA) signaling in E10.5 and E11.5 VEC+ AGM cells increased HSC potential. Additionally, we have shown that HE and the developing HSCs in the AGM can be purified based on enzymatic activity of RALDH2, the enzyme responsible for synthesizing RA from its precursor. Given its role in murine HSC development, we hypothesized that RALDH2 expression will uniquely mark HE capable of generating HSCs in human ESC-derived hematopoietic differentiations. Accordingly, we generated a human RALDH2 reporter ESC line using the CRISPR/Cas9 system. Using this line, we have identified a novel stage-specific role for RA signaling in restricting latent primitive hematopoietic potential. This more nuanced understanding of the role of RA signaling in developmental hematopoiesis will be crucial towards the long-term goal of generating human HSCs in vitro for the study and eventual treatment of hematological diseases.

Funding Source: Ontario Graduate Scholarship Award

T-2064

DEVELOPING THE NEXT GENERATION OF IPSC CELL-BASED IMMUNOTHERAPIES

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T cells are potentially curative for patients with metastatic cancer, but many patients with cancer have T cells that are 'terminally differentiated', a condition associated with treatment failure. We have observed that less differentiated T cells have a greater capacity to proliferate, persist and destroy large cancer deposits. Advances in regenerative medicine might allow the generation of rejuvenated T cells from induced pluripotent stem cells (iPSC). We have previously reported that T cells can be generated from iPSC in vitro by co-culturing them OP9 stromal cells expressing Notch-1 ligand, Delta-like-1 (OP9/DLL1). These cells have limited tumor-specificity but also exhibit unconventional and NK cell-like properties demonstrating lineage diversion into alternative lymphoid development pathways, with unknown consequences for their safety and efficacy. To generate iPSC-derived T cells with more naturalistic tumor-specific T cell programs, we sought to restore physiologic signals for selection, maturation and survival. We employed a novel 3D thymic culture system using fetal thymic tissue and generated a novel type of T cell, 'iPSC-derived thymic emigrants' (iTE). Antigen-specific CD8αβ+ iTE exhibited functional properties in vitro that were almost indistinguishable from natural naïve CD8αβ+ T cells, including vigorous expansion and robust anti-tumor activity. iPSC-derived immature T cells generated using OP9/DLL1 and 'educated' in fetal thymic organoids in a 3D culture system resembled naturally-occurring 'young' T cells, as analyzed using whole genome RNA-seq techniques. iTE recapitulated many of the transcriptional programs of naïve T cells in vivo and revealed a striking capacity for engraftment, memory formation and efficient tumor destruction. Although many milestones remain, our data show that 'Next-Gen' autologous tumor-specific T cells can realistically be generated from iPSC using 3D thymic organ tissue. Our next goal is now to employ these cells to treat patients with metastatic cancer because iPSC-derived T cells have a potentially unlimited capacity for proliferation, engraftment and anti-tumor activity.

Funding Source: This research was supported by the Intramural Research Program of the National Cancer Institute (ZIA BC010763), the Tiens Charitable Foundation and the NIH Center for Regenerative Medicine.

PANCREAS, LIVER, KIDNEY

T-2066

DERIVATION OF HUMAN HEPATOCYTE-DERIVED LIVER PROGENITOR-LIKE CELLS FOR CELL THERAPY AND DISEASE MODELLING

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Studying pathophysiological mechanisms in human liver diseases and hepatocyte-based cell therapy have been constrained by the inability to expand primary hepatocytes in vitro while maintaining proliferative capacity and metabolic function. We have previously shown that mouse mature hepatocytes can be converted to liver progenitor-like cells (LPCs) in vitro with defined chemical factors. Here we describe conditions required for reversible conversion and expansion of human hepatocytes. We isolated mature hepatocytes from surgical samples of normal human liver tissues. Culture conditions for expansion and differentiation were developed based on those for mouse hepatocytes. The reversible conversion between hepatocytes and LPCs were analyzed using transcriptome comparison and functional analyses. We developed a 3-dimensional culture of hepatocyte-derived LPCs (HepLPCs) to model host interactions with HBV and to study drug hepatotoxicity. The therapeutic effect of HepLPCs after cell transplantation was investigated in Fah-deficient mice as well as in rat carbon tetrachloride-induced cirrhotic model. Efficient conversion of human mature hepatocytes to expandable liver progenitor-like cells could be achieved through delivery of developmentally relevant cues, including NAD+-dependent deacetylase SIRT1 signaling. HepLPCs maintained many characteristics of fetal hepatocytes based on their expression of liver progenitor markers. The expanded HepLPCs can readily be converted back into metabolically functional hepatocytes in vitro and upon transplantation in vivo. Under three-dimensional culture condition, the differentiated cells regained the ability to support infection or reactivation of HBV and to analyze idiosyncratic hepatotoxicity. More importantly, a significant improvement in liver fibrosis was observed after HepLPC transplantation. Our work demonstrates the utility of the conversion between hepatocyte and liver progenitor-like cells for studying HBV biology, drug hepatotoxicity and cell therapy. These findings will facilitate the study of liver diseases and regenerative medicine.

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T-2068

FUNCTIONAL GLUCOSE RESPONSE IN HUMAN STEM CELL-DERIVED BETA CELLS IS LIMITED BY A BOTTLENECK IN GLYCOLYSIS

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Pancreatic islets regulate levels of circulating glucose by secreting the peptide hormone Insulin. Autoimmune destruction of Insulin-producing β cells within the islet is the cause of Type 1 Diabetes (T1D). This disease can be cured by transplantation of cadaveric islets into immune-suppressed patients, but immune matching and scarcity of islets available for transplantation limits the use of human cadaveric islets as a curative therapy for T1D. Stem cell-derived β (SC-β) cells offer an unlimited source of material for the curative treatment of diabetic patients. We previously reported that differentiation of SC-β cells produces transplantable endocrine organoids that secrete Insulin in response to glucose challenge in vitro. However, the magnitude and consistency of response is not as robust as observed in human islets. Studies of differentiated SC-β cells have demonstrated remarkably similar gene expression to that of human islets. The β cell response to elevated glycemic levels is a process coupled to the tightly-regulated metabolism of glucose unique to β cells. Toward a more direct measurement of glucose sensing, we have performed in-depth metabolic profiling of SC-β cells and human islets using the recently reported MIMOSA technique, combining 13-Carbon tracing and mass spectrometry analysis. In this study we have identified a glycolytic defect in SC-β cells differentiated from both hES and iPS cells. Bypassing this metabolic defect results in maximal insulin secretion in response to nutrient challenge, fully recapitulating the function of healthy cadaveric islets.

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T-2070

FUNCTIONAL ENRICHMENT FOR INSULIN PRODUCING CELLS FROM HPSCS LARGE SCALE CULTURES USING NOVEL CELL SURFACE MARKERS COMBINATION

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The limiting factor in islet transplantation treatment remains the low numbers of available islets. This has shed light on the usage of hPSCs as a potential renewable source for insulin-producing cells. By following embryonic developmental pathways, hPSCs were differentiated successfully into the pancreatic lineage and specifically to Islet-Like-Clusters (ILCs) containing insulin and other hormones needed to control glucose homeostasis. Large scale production of hPSC-derived islets are composed from heterogeneous populations that include targeted therapeutic population (~40%) along with functionally irrelevant populations that include pancreatic progenitors and poly-hormonal cells (~50%). Using cell-capture antibody array, novel antibodies to distinguish these populations were identified. Utilizing MACS technology, large scale cultures were enriched using a novel antibody combination and re-aggregated. The enriched islet-like clusters (ILCs) preparations were tested both in vitro and in vivo. In vitro, sorted ILCs exhibited an improved mature pancreatic gene expression profile, manifested by higher expression of MAFA, Insulin and NKX6.1. In addition, significantly lower expression levels of immature pancreatic genes like of AFP and CK19 was found, along with a marginal decrease in intestinal gene expression markers like CDX2. In vivo, sorted ILCs (~1,000) were macro-encapsulated using hydrogel polymer disks and transplanted under the skin of SCID-Beige mice. Already at day 7, human insulin secretion was detected at higher physiological levels (~2 fold higher) in the blood of mice implanted with sorted ILCs as compared to the non-sorted ILCs control. The secretion was highly dependent on glucose, being low in fasted animals and increased ~10 fold after IP glucose administration. Such secretion was observed weekly for over 6 weeks, implying that indeed a functional enrichment was achieved. Hence, by using the novel antibodies combination, the purity of hPSCs-derived islets can be increased by 2 fold, generating improved and more defined therapeutic cell therapy product. It is suggested that our novel cell surface markers combination strategy could be used to reduce the required cell mass loaded into an encapsulation device and potentially to decrease the device's physical dimensions.

T-2072

INSULIN NEGATIVE PANCREATIC PROGENITORS ARE DERIVED FROM THE HUMAN AND MOUSE PANCREATIC ENDODERM STAGE OF DEVELOPMENT

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Adult insulin positive (INS+) pancreatic multipotent progenitors (PMPs) are found in the adult human and mouse pancreas but their origin during embryonic development has not been fully characterized. PDX1 is expressed at mouse embryonic day 8.5 (E8.5) and marks the pancreatic endoderm stage of pancreatic development. NKX6.1 expression begins at E10.5 marking the PDX1+/NKX6.1+ pancreatic progenitor stage. The first four stages of in-vitro cytokine-induced differentiation of human embryonic stem cells (hESCs) to beta-like cells follow similar transitions of hESCs to the definitive endoderm, posterior foregut, pancreatic endoderm, and pancreatic progenitor cells, modelling early, mouse pancreatic development. Using this human in-vitro model, we report the clonal isolation of clonal sphere-forming cells at each of these early stages. Using an ins-GFP hESC line, 80% of clonal spheres generated at the pancreatic endoderm stage contain cells that are ins-GFP+, while only 10% of spheres derived from the later pancreatic progenitor stage are ins-GFP+. This percentage drop is due either to a loss of pancreatic sphere-forming cells or to an expansion of non-pancreatic sphere forming cells, at the last pancreatic progenitor stage. Flow cytometry immediately after hESCs have transitioned to each of the four stages of cytokine-induced differentiation reveals that insulin is not expressed at any of these early differentiation stages. However, the last two stages produce sphere forming cells that then give rise to ins-GFP clonal spheres. This suggests that pancreatic endoderm-derived INS+ spheres emerged from an INS- precursor cell. Using PDX1 Cre x Rosa YFP transgenic mice, clonal YFP+ spheres were formed from E10.5 pancreatic buds - a timepoint characterized as the PDX1+/NKX6.1+ pancreatic progenitor stage when insulin is not yet expressed. Pan-differentiation (non-cytokine induced-differentiation) of E10.5 pancreatic bud-derived spheres reveals that some of the progeny of PDX1+/NKX6.1+/ INS- sphere-forming precursor cells differentiated into INS+ beta-cells. These data suggest that INS- PMPs give rise to INS+ beta cells. Isolation of PMPs from embryonic development implies a novel timepoint in which to enrich for expandable lineage-restricted PMPs for cell replacement therapy in diabetes.

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T-2074

DECODING SINGLE-CELL GENE REGULATORY NETWORKS IN THE HUMAN PANCREAS AND BETA CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (iPSCs) hold tremendous potential for providing replacement insulin-producing beta (β) cells in unlimited quantities to treat diabetics worldwide. Despite considerable progress, the β-like cells derived by differentiating human iPSCs in vitro are not fully mature and fail to recapitulate the full functional properties of primary β cells isolated from cadaveric donor organs. In order to further improve the maturity of human iPSC-derived β (iPSC-β) cells, additional insight is needed into how β cell identity is established and maintained in vivo. Here, we combine single cell RNA sequencing data with machine learning algorithms to reveal gene regulatory networks active in human pancreatic cells as well as in human iPSCs- β cells. Specifically, we used single-cell regulatory network inference and clustering to identify transcription factors, regulatory states, and cell lineages based on cis-regulatory information. This approach provides critical biological insights into the mechanisms of human pancreas development and the maintenance of β cell identity. In addition, this allows for a comprehensive exploration of transcriptional and regulatory states of pancreatic cell types. Comparing in vivo β cells to those derived from human iPSCs will provide a basis to generate human iPSC-β cells functionally more equivalent to in vivo β cells. Understanding and defining the cellular mechanisms surrounding the establishment and maintenance of functionally mature β cells will have major implications for regenerative medicine in the context of diabetes treatment.

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T-2076

XENOTRANSPLANTATION OF HUMAN STEM CELL-DERIVED ENCAPSULATED LIVER TISSUE EFFECTIVELY AND SAFELY TREATS ACUTE LIVER FAILURE IN IMMUNOCOMPETENT MICE WITHOUT THE NEED OF IMMUNOSUPPRESSION

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Acute liver failure (ALF) consists in a dramatic loss of liver functions resulting in a survival rate <50%. There is an urgent need for new therapies capable of replacing liver functions in patients with ALF. We developed a human induced pluripotent stem cell (iPSC)-derived Encapsulated Liver Tissue (ELT) capable of consistently performing mature liver functions in vitro and in vivo. Thanks to the combination of complex iPSC-derived liver organoids and tailored biomaterials, the ELT performs liver-specific synthetic and metabolic functions as effectively as human hepatocytes. Here we assessed the efficacy and safety of the ELT in treating ALF in immunocompetent mice without immunosuppression. The human ELT effectively replaced liver functions and prevented death (68% survival at 5 weeks vs. 28% in controls) in immunocompetent mice with CCl4-induced ALF (xenotransplant into the peritoneal cavity without immunosuppression), with no rejection or tumor formation. Mice receiving the ELT showed lower plasma ammonia levels and less severe hepatic encephalopathy. The ELT also accelerated the host liver regeneration, which allowed, once the ELT explanted, long term survival of mice with normal liver function. The immune-isolating capacity of the biomaterial was tested by mixed lymphocyte reaction: the biomaterial isolated embedded organoids from allogeneic T cells completely. Upon implantation into immunocompetent healthy mice, the ELT did not trigger any inflammatory reaction, with no adhesions or foreign body reaction. At explant, after 4 weeks, the organoids within the ELT were alive and functional, without signs of rejection. No tumor or teratoma was observed when encapsulated highly tumorigenic cells or undifferentiated iPSCs were transplanted subcutaneously into immunodeficient NSG mice, confirming the protective capacity of the biomaterial towards tumor formation. Overall, we illustrate here the first stem cell-derived liver tissue capable of immediate, effective allogeneic/xenogeneic replacement of liver functions in immunocompetent subjects, without the risk of rejection or tumor formation. The ELT has the potential of being developed into a good-for-all, off-the-shelf, regenerative medicine product to replace liver functions in patients with ALF without the need of immunosuppression.

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T-2078

AN IN VITRO THREE DIMENSIONAL MODEL OF LIVER DEVELOPMENT FROM HUMAN PLURIPOTENT STEM CELLS

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The liver arises during embryogenesis from the liver diverticulum (LD), an out-pocketing of hepatic specified endoderm (HE) at 26 d.p.c (human). We aimed to design an in vitro model of the LD to mimic pivotal stages of liver formation. The LD is composed of a thin pseudostratified layer of HE and endothelial cells surrounded by a collagen type II-rich septum transversum mesenchyme (STM). Liver formation initiates from the coordinated interactions of the liver progenitor cells that invade the STM as finger-like projections. In our in vitro approach, human pluripotent stem cells (hPSCs) were differentiated into hepatic endoderm cells using a robust protocol involving activators of Wnt and TGFβ signaling pathways at low oxygen (5% O2) (endoderm), KGF (gut tube endoderm) and FGF2 and BMP4 (hepatic endoderm). To model the LD, in vitro derived hepatic endodermal cells, were then replated into ultralow attachment (ULA) round bottom 384 well plates (Corning) and co-cultured with endothelial cells (HUVEC) to form compact, miniature microtissues (MCTs) over a 24 hour period. These MCTs were then embedded in Matrigel to model interactions between the hepatic endoderm and the STM. These embedded MCT demonstrated radial, uniform, cord-like projections of into the matrix, similar morphologically to hepatic cords that form during liver development between E9 to E10 (mouse). These MCT grew 23% in overall diameter over a 72 hour period, and displayed enhanced gene expression consistent with migration, including epithelial-to mesenchymal transition (EMT) markers and early liver differentiation genes that was not as pronounced in controls. Immunolocalization of MCT demonstrated liver and biliary markers such as albumin, CK18, Sox9 and HNF6. Live cell permeant dyes were utilized to track individual hepatic and endothelial cell movements during the morphogenic cord development. The LD model presented here represents a novel approach to modeling hepatic cord formation, a rate limiting step during liver development. Further studies will investigate the genomic and biophysical cues that regulate such multicellular events. These studies will improve models to create 3D liver tissue in vitro for various liver regenerative medicine applications, including modeling human development, liver disease, and tissue regeneration.

Funding Source: Stem Cells in Regenerative Medicine (SCiRM) Training Program. Western New York Prosperity Fellowship

EPITHELIAL TISSUES

T-2080

CHARACTERIZATION OF THE RABBIT ORAL MUCOSA EPITHELIAL STEM CELLS FOR CAOMECS ENGINEERING

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Oral mucosa epithelial stem cells (OMECS) are commonly used to engineer epithelial cells sheets, to repair epithelium damaged tissues. Esophagus, skin and cornea are the major's targets of these cell sheets called Cultured Autologous Oral Mucosa Epithelial Cell Sheet (CAOMECS). After isolating rabbit OMECS (Animal Study 21379-01), cells of different size and shape were identified: small, medium and large. The specific identity of these cells is unknown but this heterogeneric population of cells is usually seeded together to engineer CAOMECS. We propose to seed the OMECS sorted based on their size or epithelial stem cell surface markers p75 (limbus and mucosa epithelium) to determine which cells are important for CAOMECS engineering. First, cells were sorted-based on their size (Side-scattered light). Small and medium size cells cultured together, or large cells cultured by themselves, in co-cultured with 3T3 NIH Fibroblasts mitomycin-C treated. Large cells died very quickly, when the group of small/medium cells size formed few small colonies of dozens of cells, after 9 days in culture, but they were not able to form a CAOMECS. Second, we sorted isolated OMECS using a progenitor stem cells marker p75 in 2 populations (FACS (fluorescence-activated cell sorter)): p75+ representing progenitor stem cells (10.1% of the total cell population) and p75- (89.9% of the total cell population). After sorting the cells, p75– and + cells didn't attach well on the transwell, died, and CAOMECS were not engineered. At this point, it is difficult to know if the OMECS are sensitive to the FACS/SSC sorting, or

if OMECS need to be a heterogenous population to survive and engineer CAOMECS. Further studies should be conducted to characterize the OMECS phenotype and the raw material necessary for the CAOMECS engineering.

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T-2082

NEUROENDOCRINE CELL DIFFERENTIATION IS A CONSERVED TISSUE RESPONSE TO HYPOXIA

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Neuroendocrine cells (NE cells) are specialized secretory cells found in diverse metazoan tissues. They have myriad proposed functions based in part on the peptides and amines they secrete. Aberrant NE cell differentiation and increased NE cell numbers are associated with a spectrum of lung disorders including small cell cancers. In the case of endocrine disorders like diabetes, β cell numbers are known to be regulated by physiologic parameters, such as blood glucose level. But whether NE cell numbers are generally responsive to physiologic cues is unknown. Here, we report that hypoxia stimulates neuroendocrine differentiation in multiple non-neuroendocrine cell lineages of the adult mouse trachea including basal stem cells. We show that hypoxia-dependent neuroendocrine differentiation is mediated through hypoxia-inducible factor 1 α (Hif1α). Strikingly, hypoxia-induced neuroendocrine differentiation is conserved, not only across multiple murine epithelial tissues, but also in human. These findings point to a conserved tissue response in which hypoxia leads to the generation of a cell type that can in turn secrete neuropeptides. Since most NE cells are not associated with a known function, hypoxia-induced NE cells may be useful to dissect their mechanism of action. Moreover, our findings suggest the broad conservation of a tissue level response that involves the differentiation of a specific cell type. Many molecular mechanisms have been identified within single cells to respond to various stresses: heat, nutrient limitation, and oxidative stress to name a few. Here we identify a tissue level response to a stress that actually involves the production of a unique type of cell that is poised to secrete peptides and amines in response to a hypoxic assault.

Funding Source: HHMI

T-2084

MESENCHYMALSTEM CELLS DECREASE OXIDATIVE STRESS IN THE BOWEL OF IL-10 KNOCKOUT MICE

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Inflammatory bowel disease (IBD) is an autoimmune disease characterized chronic inflammation mainly in large intestine. The interleukin-10 knockout (IL-10 KO) mouse is a well-known animal model of IBD which develops spontaneous intestinal inflammation that resembles Crohn's disease. Oxidative stress is considered a leading cause of cell and tissue damage. Reactive oxygen species (ROS) itself can cause direct cell injury and/or cause indirect cell injury by secretion of cytokines from damaged cell. In this study, human bone marrow-derived mesenchymal stem cells (MSCs) was injected to IL-10 KO mice (MSC) and oxidative stress and inflammation levels were evaluated in the large intestine and compared with those of control IL-10 KO mice (CON) and wild control mice (Wild). The levels of ROS (superoxide and hydrogen peroxidase) and secondary end product of lipid peroxidation (malondialdehyde) were significantly higher in CON but superoxide dismutase (SOD) and catalase levels were lower in MSC. Inflammation related markers (INF-γ, TNF-α, IL-4, and CD8) expression and inflammatory changes in histological analysis were much milder in MSC compare than CON. We conclude that MSCs have effects for redox balance this lead to suppression of IBD.

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T-2086

IGF1 PROMOTES PROLIFERATION OF MURINE SKIN-DERIVED PRECURSOR CELLS AND REDUCES OXIDATIVE STRESS OF THE CELLS VIA ACTIVATION OF ANTIOXIDANT REGULATORY FACTORS

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IGF1 has an important role in cell growth, differentiation and transformation of various stem cells and organogenesis during mammalian development. However, the effect of IGF1 on skin-derived precursors (SKPs) is not yet clear. The present study demonstrated the effects of IGF1 on the proliferation of murine SKPs (mSKPs). The antioxidant effect was also investigated. The proliferation was analyzed by WST-1 assay during the culture of mSKPs in IGF1-supplemented medium. To test its antioxidant effect, IGF1 was treated to the mSKPs after the induction of oxidative stress by H2O2. Western blotting, qPCR, and immunofluorescence staining were used to analyze the expression of genes related to stemness, epithelial–mesenchymal transition (EMT), antioxidant response, ageing. In results, the number, size of spheres and proliferation rate were higher in the IGF1-treatment group than those in non-treated

control group. In addition, reactive oxidative species production and oxidative stress were reduced by IGF1 treatment from 24 to 72 h of culture. The mRNA and protein level of EMT markers (Cdh2, Fn1, S100a4, Snai2, Vim and Tgfb1) and antioxidant-related markers (GPX1, HO-1 and Nrf2) were increased by IGF1 treatment. These findings show that IGF1 enhances sphere formation and cellular proliferation by stimulation of EMT genes and also reduces oxidative damage by upregulation of antioxidant enzymes. This study suggests that IGF1 can be used as the supplement for skin regeneration by its proliferation and antioxidant effects on skin cells.

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T-2088

TRANSCRIPTOME AND EPIGENOME ANALYSES FOR REGENERATED SKIN IN A RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA MOUSE MODEL

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Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic skin disease caused by mutations in COL7A1 (coding type VII collagen) and known as a one of the most severe form of EB. Loss of functional type VII collagen compromises the dermal-epidermal junction. As a result, RDEB patients suffer from the repetitive blistering and have high risk of early-onset aggressive squamous cell carcinoma. Recently, we reported that a domain of HMGB1 stimulates bone marrow stem cells, and the activated stem cells mobilized into the damaged tissue to support efficient tissue regeneration. This HMGB1 treatment model in the RDEB mouse serves as a unique opportunity to describe how the damaged skin can be reconstructed. Here, we performed single cell RNA-seq and single cell ATAC-seq to comprehensively characterized the transcriptome and epigenome of the HMGB1 treated RDEB model mouse skin. Both single cell RNA-seq and ATAC-seq analyses showed a reduction of inflammatory cells after the HMGB1 treatment. In addition, single cell RNA-seq analysis identified that the treatment increased keratinocytes marked by keratin 1/keratin 10, suggesting a functional reconstruction of the RDEB skin. Furthermore, single cell ATAC-seq analysis delineated a massive change in epigenome. These singe cell level analyses suggested that the HMGB1 treatment induced the reconstruction of the damaged skin by introducing massive changes in transcriptome and epigenome, and also serve as a foundation to design more effective RDEB treatments.

EYE AND RETINA

T-2090

OPTIMIZING A CONE RICH POPULATION FROM HUMAN PLURIPOTENT STEM CELLS

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Millions of Americas suffer from some form of retinal degeneration. While these can be the result of inherited mutations, the vast majority, such as in age-related macular degeneration (AMD) have a cause that is less well understood. As our population ages and we are seeing an increase in individuals with symptoms of AMD, there is a need for better cell based models to study retinal disease and potential therapies. The discovery of pluripotent stem cells and new methods to differentiate them into a variety of cell types and tissue makes them well suited to address these needs. Using CRISPR-Cas9 gene editing, we were able to create cell type specific fluorescent reporters that allow tracking of retinal development in both 2D and 3D cultures. Our current protocol produces 3D laminar retinal structures (organoids) containing all the respective neuronal cell types typical of the human retina. Like human retinas, these organoids are generally rod rich, and although they do contain cones, they lack a dense cone rich structure resembling the macula. Previous studies have suggested that human recombinant COCO pushes iPSCs grown in 2D towards a blue-cone specific fate through Wnt, BMP, and TGFβ inhibition. A cell was engineered to incorporate a fluorescent reporter into the SIX6 gene—an early eyefield marker—and CRX—a gene associated with the production of photoreceptors Using this dual fluorescent reporter system, we are able to track target gene expression in real-time. These cells were differentiated in both 2D and 3D cultures either in a standard medium, or a medium that had been enhanced with COCO, FGF-2, and IGF. Movement from hypoxic to normal conditions were also studied for the 2D cultures. Morphological and gene expression differences between control and treatment

groups were observed, as well as differences in how the treated groups expressed SIX6 in 2D versus 3D. Immunohistochemistry further detailed the photoreceptor makeup of the samples. The successful creation of a cone rich retinal organoid would allow for the study of an in vitro macula-like structure, which could aid in the search for treatments. This structure could also, with the use of patient derived iPSCs, provide a cone-rich section for transplant into individuals who have already suffered photoreceptor loss due to AMD.

T-2092

RETINAL ORGANOIDS REVEAL MECHANISM OF VISION LOSS IN ATF6 ACHROMATOPSIA PATIENTS

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Loss of function of the ATF6α transcription factor causes heritable photoreceptor diseases including achromatopsia and cone-rod dystrophy. These patients have severely impaired vision, loss of cone photoreceptor function, and foveal hypoplasia from infancy. The mechanism by which ATF6 defects lead to vision loss in these patients is unknown. Here, we generated 3D retinal organoids from ATF6 mutant patient iPSC cells and isogenic ATF6 knock-out human embryonic stem cells (hESCs) . We used cellular tomography, combined with OCT cryosectioned and immunohistological analysis to visualize developing rod and cone photoreceptors. We performed RNAseq analysis of retinal organoids in the presence and absence of functional ATF6, and revealed that the lack of functional ATF6 relates to a significant disruption of the ATF6 transcriptional program. We found that the loss of ATF6 signaling affects early differentiation events by suppressing loss of pluripotency and increasing the expression of early eye development markers, such as PAX6, SIX3 and RX. Microscopic analysis at day 120 and 290 confirmed the presence of rod and cone photoreceptor cells in ATF6 wild-type retinal organoids. Interestingly, retinal organoids derived from ATF6 knockout hESCs and ATF6 mutant patient iPSCs showed no development of cone photoreceptors cells at the same ages. By contrast, the development of rod photoreceptor cells appeared to be normal in all 3D retinal cups. Retinal organoid surface scanning analysis showed a similar morphology seen in the retina of ATF6 patient achromats. ATF6 mutant alleles show a selective failure to form cone photoreceptors during retinal development while rods appear to be spared. We propose that the vision defect found in ATF6 patients arise as a consequence of the development of a rod-dominant retina.

T-2094

IN VIVO RETINAL STEM CELL PROLIFERATION, EXPANSION AND RETINAL MIGRATION IS INDUCED VIA INHIBITION OF BMP AND SFRP2 IN THE ADULT MOUSE EYE

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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 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 adult mice. Intravitreal injections of the BMP antagonist Noggin or an anti-sFRP2 antibody were administered once a day for 3 days. At 1 day after the injection period, each inhibitor alone induced a ~5-fold increase in proliferating (EdU+) CE cells (Pax6+) compared to PBS injection. Noggin and anti-sFRP2 combined with the growth factors FGF2 and Insulin (FINS) induced an additive increase in EdU+/Pax6+ cells (~12-fold), while the growth factors alone showed a ~5-fold increase in EdU+/Pax6+ cells. At both 7 and 31 days after BMP or sFRP2 inhibition, clonal sphere assays revealed an over 2-fold increase in the number of sphere-forming RSCs present in the eye. Next, we employed inducible CE lineage tracing (Msx1-CreERT2;Rosa26-TdTomato mice) to investigate whether adult CE cells migrate into the retina after FINS treatment, and whether MNU-induced retinal injury could impact their migration. In uninjured eyes, we observed a ~3-fold increase in TdTomato+ cells in the retinas of FINS-treated eyes compared to PBS injection. MNU injury in PBS-treated eyes slightly increased retinal migration from the CE, whereas MNU injury did not further enhance retinal migration in FINS-treated eyes. Notably, a decreased total eye diameter was observed in 50% of the PBS+MNU eyes that was not observed in any other group, suggesting the FINS+MNU group may have been protected from this pathology. However, in eyes with normal diameter, there was a similar reduction in outer nuclear layer thickness in PBS+MNU and FINS+MNU eyes. 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 induce CE cells to migrate into the retina. Currently, we are determining if CE cells that migrate into the retina differentiate into retinal cell types, which would establish this method as a new paradigm to stimulate adult retinal neurogenesis.

Funding Source: Canadian Institutes of Health Research, Medicine by Design, National Sciences and Engineering Research Council, Foundation Fighting Blindness

T-2096

EFFECT OF CHROMOSOME 20 GENETIC ABNORMALITIES ON HUMAN EMBRYONIC STEM CELL DIFFERENTIATION INTO RETINAL PIGMENT EPITHELIUM CELLS

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The ability to maintain self-renewing human pluripotent stem cells (hPSCs) indefinitely in culture is cornerstone for research and clinical studies of pluripotent cells. However, the regenerative medicine field has come to the realisation that karyotype and sub-karyotype aberrations arise in hPSCs as a result of continuous expansion. Understanding the biological consequences of carrying common and subtle variances into a clinically relevant differentiation protocol is necessary to provide much-needed evidence of the mechanisms at play and support an educated risks/benefit assessment during regulatory evaluation. In this study, we aimed to assess the effect of frequent and commonly detected chromosome 20 (Chr20) genetic aberrations during the differentiation of hPSCs towards retinal pigment epithelium (RPE) cells, currently in Phase 1 clinical trial. Experiments were conducted on clonal lines derived from human embryonic stem cells as follows: normal karyotype; 20q11.21 copy number variation (CNV) and trisomic for the long arm and monosomic for the short arm of Chr20 (called normal, CNV and i20q, respectively). Our data show that hPSCs carrying the 20q11.21 CNV amplification undergo RPE differentiation faster and with a higher yield compared to normal cells. Moreover, both types of mutated hPSCs have a proliferative advantage over normal cells, which is also reflected in the speed of differentiation. Interestingly, the more severe aberration of the i20q clones led the population to completely abort the differentiative pathway and instead undergo apoptosis. This study illustrates that hPSCs carrying the CNV Chr20 variants can efficiently progress into RPE differentiation and outperform the normal counterparts. At the same time, variants that favour the balance towards self-renewal (i20q) fail quickly in our protocol, suggesting that the RPE differentiation culture conditions are not permissive to the survival of undifferentiated cells. Comprehensive RNA-seq examination of early stem cell fate decision regulatory networks modified by gains of Chr20 regions could explain the clonal differences in differentiation propensities and lead to the discovery of new therapeutic targets applicable to retina-associated disorders.

T-2098

DISCOVERY OF TWO STEM CELL POPULATIONS WITH DISTINCT MARKER EXPRESSION PROFILES DURING DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TOWARDS LIMBAL EPITHELIAL STEM CELLS

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Differentiation of corneal limbal epithelial stem cells (LESCs) from human pluripotent stem cells (hPSCs) represents an attractive future therapeutic option for patients suffering from bilateral limbal stem cell deficiency (LSCD). In addition, it provides new tools for studying the biological properties of LESCs in humans. Here, two genetically distinct hPSC lines were subjected to corneal differentiation in previously published conditions, with specific aim to study the hierarchy of putative limbal stem cell markers in more detail. Characterization of undifferentiated hPSC and derivative cells in several time points between 7 and 24 days was carried out using immunofluorescence labeling against a panel of selected limbal/corneal epithelial and pluripotency markers. Along the differentiation process, we observed a decrease of pluripotency markers OCT3/4 and SSEA4, accompanied by ascending expression of several widely proposed LESC-related markers PAX6, ΔNp63α, CK15 and CK14. Interestingly, ABCG2, a marker for slow-cycling limbal cells in vivo and a universal marker of stemness, was only transiently expressed at a very early phase of the differentiation. Hence, we performed further comparison of the early and latter hPSC-LESC cell populations by quantifying the protein expressions with cell counting and flow cytometry, and gene expressions with qRT-PCR. With this, two noticeably different cell populations were identified during hPSC-LESC differentiation, the earlier population expressing robustly the stemness marker ABCG2 and the latter expressing the other proposed LESC markers ΔNp63α, CK15 and CK14. Based on our results acquired with hPSC-derived LESCs, we propose a marker protein hierarchy that potentially reflects the limbal stem cell differentiation in vivo. Furthermore, novel culture conditions stabilizing the expression of ABCG2 in hPSC-LESC cultures were optimized in order to identify the exact functional role and as a continuum, the therapeutic relevance of these two identified cell populations with distinct LESC-associated marker profiles.

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T-2100

HUMAN PLURIPOTENT STEM CELL DERIVED RETINAL ORGANOIDS FOR MODELING LEBER CONGENITAL AMAUROSIS

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Modeling human retinal disease in vitro using pluripotent stem cells has opened new avenues of research in regenerative medicine. Importantly, these stem cells have the potential to differentiate into any cell type in the body, including the neural retina, and thus hold great promise for modeling human retinal disease. Leber Congenital Amaurosis (LCA) is an inherited retinal degeneration which affects vision at birth or within the first few years of life. It causes dysfunction and death of photoreceptor cells resulting in severe loss of vision and eventually leads to blindness. Though relatively rare, mutations in close to 20 genes, including the CRX (cone-rod homeobox) gene, are known to cause LCA. Using CRISPR-Cas gene -editing, a patient-specific mutation was created in the transactivating domain of the CRX gene. This mutation was introduced into iPSC bearing a Six6-Ruby3 reporter which allows for the selective enrichment of 3D retinal structures which will improve the reliability and reproducibility of differentiation experiments. We have demonstrated that these reporter organoids develop discrete laminar structures with appropriate reporter expression in progenitors and photoreceptor gene expression in photoreceptors. The similarity to human retinal development will facilitate the analysis of any retinal disease phenotype. To evaluate disease progression, organoids were collected at different time points and evaluated by immunohistochemistry and RT-qPCR to analyze gene expression. Organoids expressed ruby3 signal indicating endogenous Six6 expression and immunohistochemistry of older organoids indicated the presence of photoreceptor genes. RT-qPCR was performed to further validate the presence of genes involved in retinal development and to determine the changes involved in the mutant and control organoids. Studying human retinal degeneration in a dish allows us to understand the mechanism of disease progression and offers a reliable platform to develop assays for future drug screening to treat retinal degenerative diseases.

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T-2102

POST-TRANSPLANTATION MOUSE IPSC-DERIVED RGCS ESTABLISH NEURONAL POLARITY WITHIN NMDA-DAMAGED HOST RETINAS AND SHOW ENHANCED SURVIVAL FOLLOWING GROWTH FACTOR CO-TREATMENT

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Transplantation of iPSC-derived neurons as a strategy to replace host cells, lost in progressive neurodegenerative diseases has gained traction over the past years across the field of neuroscience. The advantage of the eye as a target organ for cell replacement therapy has been demonstrated in pre-clinical and clinical studies of retinal pigment epithelium and photoreceptor transplantation. To address visual decline associated with Glaucoma our laboratory has been working on the replacement of retinal ganglion cells (RGCs), the neuronal link between the retina and the brain. We have demonstrated that within mouse models, iPSC-derived RGCs are able to integrate within pups (83%, n=12) and adult retinas, including both healthy (57%, n=14) and diseased (67%, n=12) hosts. While the rate of transplantation success was high compared to previously published primary cell transplants, the number of surviving donor RGCs per host retina remained below 1%. To address this challenge, we have developed a co-treatment regime using known neuroprotective factors BDNF, GDNF and CNTF, delivered in a slow-release formulation. For transplantation Thy1-GFP+ cells were isolated by magnetic micro-beads at day 21 of retinal organoid culture and injected into the vitreous of the eye either alone or with co-treatment in healthy mice and mice subjected to NMDA one week prior. Electroretinography performed at 6 weeks post-transplant, showed that growth factor cotreatment partially preserved inner retina function as measured by pSTR response (32uV in co-treatment group vs 17uV in control). Furthermore, growth factor co-treatment enhanced both overall grafting success from 50% to 73% and donor cell survival. Axonal and dendritic outgrowth could be detected as early as two weeks post-transplant in healthy and NMDA treated retinas underlining the potential of iPSC-derived RGCs to establish neuronal polarity even within diseased microenvironments.

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STEM CELL NICHES

T-2104

DESIGNING A BIOLOGICAL INTERFACE COMBINING A COCHLEAR IMPLANT WITH HUMAN EMBRYONIC STEM CELLS

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Sensorineural hearing loss is the one of the most common sensory deficits worldwide. Currently, cochlear implant (CI) is the only clinically available surgical intervention to restore the sensory deficit. There are, however, limitations to current CI designs. First, a patient's pathology typically entails different degree of loss in the spiral ganglion neurons (SGNs). The loss can create an "electrode-neuron gap" that can reduce spatial selectivity. Second, current CI design in the electrode array contain 22 or less electrode channels; far less than the number of hair cells. These limitations can decrease available information that is sent to the brain. We, therefore, propose a biological interface that combines a CI technology with human embryonic stem cell (hESC) replacement therapy. By introducing human embryonic stem cell-derived otic neuronal progenitors (hESC-derived ONPs), which are neuronal precursors to human SGNs, into the scala tympani, we expect to pave electrode-neuron gaps to improve the spatial selectivity, which can further improve CI's performance. The benefits of neurotrophins such as brain-derived neurotrophic factor (BDNF) in neuronal differentiation has been shown to play a pivotal role in axonal growth and synaptic plasticity. Successful, long-term delivery of BDNF in the inner ear has yet to be established; partially because one-time administration of BDNF to the scala tympani is not biologically effective due to its short half-life. Alternatively, encapsulating BDNF in a thermoresponsive polymer-poly (polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN), which is suitable for delivery of therapeutics, could be our solution. It possesses a lower critical solution temperature (LCST) of 26℃; liquid status efficiently entraps chemokine when temperature is below 26℃ and gelation appears to slowly release chemokine as temperature reach above 26℃. The ELISA result demonstrates a steady long-term release when BDNF entrapped in PPCN, and our current goal is to assess the bioactivity of BDNF on ONP's neurite outgrowth. Furthermore, PPCN has the potential to develop in vivo cell entrapment near the CI electrode arrays, which could increase cell survival in post-transplantation to the inner ear.

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T-2106

INFUSED BONE MARROW DRIVED CELLS ARE REPAIRED FIBROSIS AND ENGULFED DAMAGED CELLS

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The 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, EpCAM, A-6, Liv8-CD44,hepatoblastmarker-Liv2,MMP9,MMP13,AK4,CXCR4,p62,CD68,TGF-beta,alfa-SMA,transcription regulator-maternal of inhibitor of differentiation -Maid). We analyzed some kind of gene by Real-TimePCR(Gene: p16,p21,p62,EpCAM,AFP,A-6,HNF4,Sirt1,2,3,6,AK-4,Hmox,Ncam,ATF,XBP1 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. In conclusion, 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.

T-2108

ELECTROMAGNETIZED GOLD NANOPARTICLES MEDIATE DIRECT LINEAGE REPROGRAMMING INTO INDUCED DOPAMIN NEURONS INVIVO FOR PARKINSON''S DISEASE THERAPY

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Electromagnetic fields (EMF) are physical energy field generated by electrically charged objects and specific ranges of EMF can influence numerous biological processes, including control of cell fate and plasticity. In this study, we show that electromagnetized goldnanoparticles (AuNPs) in the presence of specific EMF condition facilitate efficient direct lineage reprogramming to induced dopamine neurons in vitro and in vivo. Remarkably, electromagnetic stimulation leads to specific activation of the histone acetyltransferase Brd2, resulting in histone H3K27 acetylation and robust activation of neuron-specific genes. In vivo dopaminergic neuron reprogramming by EMF stimulation of AuNPs efficiently and noninvasively alleviated symptoms in the mouse Parkinson's disease models. This study provides proof of principle for EMF-based in vivo lineage conversion as a potentially viable and safe therapeutic strategy for treatment of neurodegenerative disorders.

T-2110

A TIE2-NOTCH1 SIGNALING AXIS DRIVES ENDOTHELIAL REGENERATION AND RECOVERY OF THE BONE MARROW HEMATOPOIETIC NICHE

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Regeneration of the hematopoietic niche is of critical importance to the recovery of blood after chemotherapeutic and irradiation treatment. Loss-of-function studies have determined that Notch signaling is essential for hematopoietic and endothelial development. By deleting a single allele of the Notch1 transcriptional activation domain (TAD) we generated a viable, post-natal, Notch signaling hypomorph. Heterozygous Notch1-deltaTAD (Notch1+/deltaTAD) mice appear normal and have no endothelial or hematopoietic phenotype, aside for an inherent, cell-autonomous defect in T-cell lineage development. Following chemotherapy, Notch1+/deltaTAD mice exhibited severe pancytopenia, weight loss and morbidity. This phenotype was confirmed in an endothelial-specific loss-of-function Notch1 model system. Ang1, secreted by hematopoietic progenitors after damage, activated endothelial Tie2 signaling, which in turn enhanced expression of Notch ligands and potentiated Notch1 receptor activation. In our hypomorphic model system, Notch1-deltaTAD mutant protein accumulated in endothelial cells and interfered with optimal activity of Notch1 the transcriptional complex. Failure of the Notch1-deltaTAD mutant to efficiently drive transcription of key gene targets such as Hes1 and Myc caused prolonged apoptosis and limited regeneration of the bone marrow niche. Thus, basal Notch1 signaling is sufficient for niche development, but robust Notch activity is required as the penultimate signaling event for regeneration of bone marrow endothelial niche and hematopoietic recovery.

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T-2112

TARGETING THE LEUKEMIA PROPAGATING CELLS WITHIN THERAPY-INDUCED NICHES BY CELL-BIOMIMETIC NANOPARTICLES

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Acute lymphoblastic leukemia (ALL) is one of the most common pediatric cancers. Although the treatment has achieved great success, relapse remains the major challenge. Recently we reported a therapy-induced niche (TI-niche) shielding residual leukemia cells. Precisely targeting the residual cells as well as interfering its interaction with the niche can be a promising strategy. Here, we develop a leukemia cell-biomimetic nanoparticles (NPs) and co-deliver the anti-TGFβRII to blockade

the drug-resistance signaling pathway being activated by the TI-niche. We coat NPs with leukemia cell membrane which can anchor on the leukemic niches during blood circulation. The anti-TGFβRII embedded in cell membrane will release under hypoxia-environment in the bone marrow, neutralize the TGFβRII in ALL cell membrane and sensitize the cells to chemotherapy which the NPs released. Therefor, we have developed a sequential drug delivery system which can target the therapy-resistant leukemia cells, improve the drug efficiency.

T-2114

CELL CYCLE ACTIVATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTES BY EXPOSURE TO CONDITIONED MEDIUM FROM MESENCHYMAL STEM CELLS

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Human heart has little regenerative capacity after myocardial damage due to the low proliferation potential of cardiomyocytes (CM). Hence, there is a growing interest in the production of human induced pluripotent stem cell-derived CM (hiPSC-CM) as a potentially promising strategy for regenerative therapies, and in identifying factors playing relevant roles in the regulation of the hiPSC-CMs cell cycle. Moreover, recent findings highlights the regenerative capacity of mesenchymal stem cells (MSC) and the many properties of MSC-conditioned medium and extracellular vesicles (EV) in cell culture. The aim of this study is to investigate the ability of the hiPSC-CM to re-enter cell cycle after exposure to conditioned medium from WJ-MSC. Human iPSC-CMs were obtained with a monolayer protocol. We established FUCCI-hiPSCs, a line constitutively expressing Fluorescent Ubiquitination-based Cell Cycle Indicator, which consists of system that employs red fluorescence for G1 state and green for S/G2/M. FUCCI-hiPSC-CMS were incubated with WJ-MSC-conditioned medium for 24, 48 and 72 hours. At the first 24hs, hiPSC-CM in S/G2/M increased from 14.7% to 28.7% with conditioned medium. Interestingly, while replication activity decrease over time with control medium (near 5% at 48 and 72hs), with MSC-conditioned medium maintain up to 20%. Enhanced DNA synthesis was confirmed by EdU assay and subsequent immunofluorescence. Finally, we used LC-MS/MS proteomic approach to identify proteins presents in the secretome from WJ-MSC that could explain the effect in the hiPSC-CM. Through Gene Ontology analysis we found 147 proteins involved in cell cycle. Particularly, some of them were related to the G2/M checkpoint and mTOR pathway. In conclusion, this study reveals that the exposure to MSC-contidionated medium induce proliferation of hiPSC-CMs. Our next goal is to study the differential expressed genes in the proliferative hiPSC-CM.

Funding Source: CONICET. Fundación para la Lucha contra Enfermedades Neurológicas de la Infancia.

CANCERS

T-2116

NOVEL INSIGHTS IN CANCER STEM CELL BIOLOGY: WILMS' TUMOR AND HUMAN FETAL NEPHRON PROGENITORS AS AN EXAMPLE

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Wilms' tumor (WT) accounts for 95% of renal malignancies in children and is characterized by postnatal uncontrolled proliferation of nephron progenitors (NP) without generation of functional nephrons. Little is known about WT involvement of NP in tumor progression and their relation to the microenvironment. Using our validated Smartflares technique we isolated, for the first time, NP expressing SIX2 and CITED1 (the master genes regulating nephrogenesis) from WT samples and human fetal kidneys (hFK) and compared them by RNA-seq. We established conditions for long-term culture of NP cells and studied in vitro mechanism of self-renewal vs. differentiation of NP from both WT and hFK. We also transplanted WT-NP in vivo to study tumorigenesis and targeted tumor growth and progression using neutralizing antibodies against integrins. We verified the presence of NP (SIX2+CITED1+ cells) in WT samples; RNA-Seq data confirmed their nephrogenic signature but also highlighted differences in expression of pluripotency and self-renewal related genes like OCT4, FOXO1, NANOG along with a lower expression of β-catenin compared to hFK-NP. We confirmed that both WT-NP and hFK-NP can be cultured for multiple passages in vitro and are tumorigenic in vivo. We identified for the first time a disruption in integrin expression patterns within the WT-NP that, when specifically targeted, regulates tumor stem cell self-renewal or differentiation via modulation of the β-catenin/LEF1 pathway, opening new avenues for the treatment of WT. In conclusion, this work represents the first characterization of SIX2+CITED1+ cells from WT and suggests the importance of matrix-cell interaction in development and during WT formation. These studies have the potential to increase our knowledge of human nephrogenesis and facilitate the development of new strategies aimed at halting tumor progression.

Funding Source: GOFARR Laboratory for Organ Regenerative Research and Cell Therapeutics

T-2118

MULTIPLE TREATMENT CYCLES OF NEURAL STEM CELL DELIVERED ONCOLYTIC ADENOVIRUS FOR THE TREATMENT OF GLIOBLASTOMA

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Tumor tropic neural stem cells (NSCs) can improve the anti-tumor efficacy of oncovirotherapy agents by protecting them from rapid clearance by the immune system and delivering them to multiple distant tumor sites. We recently completed a first-in-human trial assessing the safety of a single round of NSC-delivered CRAd-Survivin-pk7 (NSC.CRAd-S-pk7) in combination with radiation and chemotherapy within newly diagnosed GBM patients. While safety of NSC.CRAd-S-pk7 to tumor sites has been demonstrated, we expect the therapeutic efficacy of a single NSC.CRAd-S-pk7 treatment cycle to be suboptimal. Inspired by multiple treatment cycles of oncovirotherapy to treat peripheral tumors, here we investigate the potential therapeutic enhancements of multiple treatment cycles within the brain tumor setting. We believe that continuing the therapy after our initial dose will be a requisite for providing a regular immune system boost and update of the tumor antigen profile. Multiple treatment rounds involving adenovirus has often led to both humoral and cellular anti-AD immunity within animal models, with many studies demonstrating that repeat administration of Ad Vectors has limited efficacy over the single round treatment. In contrast, here we report an improvement in treatment efficacy when 3 rounds are administered to B6 mice with pre-established GL261 tumors. In fact, in 25% of mice, the tumor burden was reduced to occult levels after 3 rounds of treatment. Ongoing studies are investigating if differences in the vector dose, strength of the pre-existing immune response, or intratumoral administration route within tumor tropic NSCs is responsible for these favorable results. We are also monitoring the development of humoral and cellular immunity after each treatment round to establish a rationally-based treatment schedule for Pre-IND enabling studies.

Funding Source: Ben and Catherine Ivy Foundation, Alvarez Family Charitable Foundation, Rosalinde and Arthur Gilbert Foundation, Jeanne and Bruce Nordstrom.

T-2120

CELLULOCYTOSIS BETWEEN KUPFFER CELLS AND TUMOR-INITIATING STEM-LIKE CELLS PROMOTES METASTATIC COLONIZATION VIA CD47 AND FGFR2 SIGNALING

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Highly self renewing, CD 133+ liver tumor-initiating stem-like cells (TICs), are immensely invasive/metastatic cells that perpetuate secondary tumor even after eradication of primary Hepatocellular carcinoma (HCC); a process independent of propensity for lineage commitment. Here we probed into the cause for plasticity in TIC's that empowers TIC's to derail lineage commitment. We identified cellulocytosis, and define cellulocytosis as a process in which phagocytic cell (F4/80+ Kupffer cells; KC) transports an entire non-phagocytic cell (CD133+ TIC) onto itself thereby leading to a hybrid cell formation (F4/80+ CD 133+ TIC). The hybrid TIC acquire the Macrophage's tropism such that TICs gravitate to macrophage-rich metastasis sites. We performed genetic screen based on transplantation of tumor cells infected with a viral cDNA library into Mouse Livers. This identified metastatic promoting genes such as CD47, MGAT-5 (GNT-V) and Lin28. We performed Metaphase chromosome spreading analysis on co cultured cells and then FISH analysis on TIC's and Kupffer cells to identify an interaction between CD47 and SIRP. Immunohistochemistry of paraffin embedded normal, tumor and metastatic Brain tissue, revealed double positive expression of GNT-V and CD133. Flow cytometry analysis (FACS) also showed a double positive population in LPS stimulated Macrophage(F4/F80) and TICs (CD133). This proposed link between cell fusion and metastasis, not only advances our understanding of molecular mechanisms responsible for metastatic TIC generation in HCC but also represents a new target in the treatment development for metastatic HCC.

Funding Source: California Institute for Regenerative Medicine

T-2122

ACTIVATION OF ANTITUMOR IMMUNITY BY HUMAN NEURAL STEM CELL MEDIATED CPG-STAT3 ANTISENSE OLIGONUCLEOTIDES AND OX40 ANTIBODIES IN A SYNGENEIC BREAST CANCER MOUSE MODEL

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Breast cancer is the most common cancer among women in the US. Patients diagnosed at advanced stages have limited treatment options, due in part to tumor cell immune tolerance caused by Signal Transducer and Activator of Transcription 3 (STAT3). Treatment with CpG-STAT3 antisense oligonucleotides (ASOs) may inhibit STAT3 and trigger immunostimulation. The CpG moiety tethered to STAT3-ASO enables cellular uptake of the conjugate by immune cell expression of Toll-like receptor 9 (TLR9). The current study investigates the novel approach of delivering CpG-STAT3-ASOs in a clinically relevant neural stem cell (NSC) line (HB1.F3.CD21) to increase their tumor-targeted delivery and local retention at breast cancer sites. These NSCs have demonstrated tumor tropism to invasive tumors including brain, ovarian, and breast cancers. In vitro studies showed rapid uptake of CpG-STAT3 ASOs by NSCs. Following uptake, NSCs continuously released extracellular vesicles (EVs) containing the CpG-STAT3 ASOs (CpG-STAT3 ASO-NSCs) for 2–3 days. In an in vivo immunocompetent mouse model of breast cancer, in which tumors were established in left and right mammary fat pads. When NSC-delivered versus free CpG-STAT3 ASO was administered intratumorally, we observed an enhanced distribution of CpG-STAT3-ASOs in the tumor microenvironment, attracting more tumor infiltrating lymphocytes and generating a systemic anti-tumor effect. Moreover, because OX40 expression is induced on T-cells upon TLR9 activation, the addition of OX40 agonist antibodies (Abs) led to greater anti-tumor activity in CpG-STAT3 ASO-NSC treated mice. This resulted in reduced volume of both the injected and non-injected tumor, and extended long term survival. These data support further translational development of the NSC delivered CpG-STAT3-ASO and OX40 agonist Ab combination treatment, which has the potential to overcome immune tolerance and induce a systemic anti-tumor immune response in patients with advanced breast cancer.

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T-2124

MULTIPLE MYELOMA BM-MSCS INCREASE THE TUMORIGENICITY OF MM CELLS VIA TRANSFER OF VLA4 ENRICHED MICROVESICLES

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Multiple myeloma (MM) cells accumulate in the bone marrow (BM) where their interactions impede disease therapy. We have shown that microvesicles (MVs) derived from BM mesenchymal stem cells (MSCs) of MM patients promote the malignant traits via modulation of translation initiation (TI), whereas MVs from normal donors (ND) do not. Here, we observed that this phenomenon is contingent on a MVs' protein constituent, and determined correlations between the MVs from the tumor microenvironment, e.g. MM BM-MSCs, and patients' clinical characteristics. BM-MSCs MVs (ND/MM) proteomes were

assayed (mass spectrometry) and compared. Elevated integrin CD49d (X80) and CD29 (X2) was determined in MM-MSCs' MVs and correlated with patients' staging and treatment response (Free light chain, BM plasma cells count, stage, response to treatment). BM-MSCs' MVs uptake into MM cell lines was assayed (flow cytometry) with/without integrin inhibitors (RGD, Natalizumab and anti-CD29 monoclonal antibody) and recipient cells were analyzed for cell count, migration, MAPKs, TI and drug response (doxorubicin, velcade). Their inhibition, particularly together, attenuated the uptake of MM-MSCs MVs (but not ND-MSCs MVs) into MM cells and reduced MM cells' signaling, phenotype, and increased drug response. This study exposed a critical novel role for CD49d/CD29 on MM-MSCs MVs and presented a discriminate method to inhibit cancer promoting action of MM-MSCs MVs while retaining the anti-cancer function of ND-MSCs-MVs. Moreover, these findings demonstrate yet again the intricacy of the microenvironment involvement in the malignant process and highlight new therapeutic avenues to be explored.

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T-2126

KIT D816V INDUCED PLURIPOTENT STEM CELLS FOR MODELLING LEUKEMIA AND COMPOUND SCREENING

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Mutations in the stem cell factor (SCF) receptor tyrosine kinase KIT are key to leukemias confined to the mast cell compartment and over 90% of aggressive systemic mastocytosis (ASM) and mast cell leukemias (MCL) are positive for a KIT D816V mutation. The KIT D816V mutation results in ligand-independent tyrosine phosphorylation and constitutive activation of KIT leading to aberrant cell proliferation, migration and function. KIT D816V cells are resistant to the tyrosine kinase inhibitor imatinib (Gleevec/Glivec), which is successfully used for treating chronic myeloid leukemia (CML). Thus, KIT D816V patients in the terminal phase of disease are essentially left without effective targeted therapy, rendering this a fatal disease. We have generated a panel of induced pluripotent stem cells (iPS cells) of KIT D816V ASM and MCL patients, including isogenic controls without mutation. We also introduced the KIT D816V mutation into human ES cells by CRISPR/Cas to generate KIT D816V ES cells. KIT D816V iPS cells and ES cells were induced to differentiate into hematopoietic cells and further into mast cells to recapitulate KIT D816V disease in vitro. As expected KIT D816V progenitors showed characteristics of constitutive KIT signaling in biochemical assays. In addition KIT D816V hematopoietic progenitors were increased in their proliferative capacity and propensity towards mast cell differentiation. Therefore KIT D816V hematopoietic progenitors and mast cells were subjected to compound screening for identification of KIT D816V selective inhibitors and these studies are currently being performed. Our studies should pave the way towards an effective therapy of KIT D816V ASM and MCL.

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T-2128

METASTATIC POTENTIAL OF NANOG OVEREXPRESSING MELANOMA CELLS

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Cancer cell lines with different metastatic potential are important bioresources for the molecular analysis of metastasis and the development of its inhibition methods. Some studies have reported the potential involvement of differentiation-related genes in the regulation of metastatic potency. Here we have selected Nanog as such a gene and aimed at the development of Nanog gene modified melanoma cell lines. B16BL6, B16F10, and B16F1 are melanoma cell lines with different metastatic potential. Nanog overexpression vector was introduced into these 3 cell lines, respectively. Consequently, the increase of proliferation and migration potential was observed most

markedly in Nanog overexpressing B16BL6 (N-BL6). In this cell line, the expression of matrix metalo-proteinase 9 was activated that supported the increase of migration. RNA sequence revealed the increase of TGF-β expression and the decrease of the expressions of inhibitor of differentiation (ID)1 and ID3. This finding suggested that Nanog overexpression might modify melanoma cells to less differentiated state. Nanog overexpressing melanoma cells as well as the control melanoma cells were administered to 8-week-old C57BL/6 male mice from the tail vein. After breeding for 2 weeks, the number of metastatic colonies and their volume were analyzed. Typically the number of colonies on lung in N-BL6 increased to 1.2 times greater than the control. These indicate that Nanog overexpression was effective for the increase of metastatic potential of melanoma cells.

T-2130

IMMORTALIZATION OF DIFFERENT BREAST EPITHELIAL CELL TYPES RESULTS IN DISTINCT MITOCHONDRIAL MUTAGENESIS

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Different phenotypes of normal cells might influence genetic and epigenetic profiles, and tumorigenicities of their transformed derivatives. In this study, we investigated whether the whole mitochondrial genome of immortalized cells can be attributed to different phenotypes (stem versus non-stem) of their originator normal epithelial cells. To accurately determine mutations, we employed Duplex Sequencing, which exhibits the lowest error rates among currently available DNA sequencing methods. Our results indicate that the vast majority of observed mutations of the whole mitochondrial DNA occur at low-frequency. The most prevalent mutation types are C>T/G>A and A>G/T>C transitions. Frequencies and spectra of homoplasmic (high-frequency) point mutations are virtually identical between stem cell-derived immortalized (SV1) cells and non-stem cell-derived immortalized (SV22) cells. However, frequencies of rare point mutations are significantly lower in SV1 cells (5.79E-5) than in SV22 cells (1.16E-4). Additionally, the predicted pathogenicity for rare mutations in the mitochondrial tRNA genes is significantly lower (by 2.5-fold) in SV1 cells than in SV22 cells. Our findings suggest that the immortalization of normal cells with stem cell features leads to decreased mitochondrial mutagenesis, particularly in noncoding RNA regions. The identified mutation spectra and mutations specific to stem (versus non-stem) cell-derived immortalized cells have implications in characterizing heterogeneity of tumors and understanding the role of mitochondrial mutations in immortalization and transformation of human cells.

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NEURAL DEVELOPMENT AND REGENERATION

T-3002

ACCUMULATION OF 7-DEHYDROCHOLESTEROLS-DERIVED OXYSTEROLS CAUSES NEURODEVELOPMENTAL DEFECTS IN SMITH-LEMLI-OPITZ SYNDROME

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Smith-Lemli-Opitz Syndrome (SLOS) is a developmental and metabolic disorder characterized by distinct facial features, microcephaly, intellectual disability and Autism-like behaviors. SLOS is caused by a defect in cholesterol synthesis with mutations in the gene encoding 7-dehydrocholesterol reductase (DHCR7). DHCR7 catalyzes the conversion of 7-dehydrocholesterol (7DHC) to cholesterol in the final step of cholesterol biosynthesis. Defective DHCR7 resulting from the mutations leads to a greatly decreased level of cholesterol and the accumulation of 7DHC in affected individuals. 7DHC was found to be extremely reactive toward free radical oxidation, leading to the formation of several oxidative metabolites (i.e., oxysterols), many of which have been found in-vivo. Although the combination of low cholesterol level and accumulation of 7DHC-derived oxysterols likely contributes to the disease pathophysiology, it still remains unknown how these biochemical changes lead to specific neurodevelopmental defects in SLOS. Here we asked whether DHCR7 mutations cause abnormal neural development by disrupting neurogenesis in the murine cortex and SLOS patient-derived human induced pluripotent stem cells (hiPSCs). We found that 7DHC-derived oxysterols begin to accumulate at embryonic day 12.5 and continue to increase during cortical neural development by using liquid chromatography-tandem mass spectrometry. The endogenous levels of these oxysterols, such as as 3β,5α-dihydroxycholest-7-en-6-one (DHCEO), 4a-hydroxy-7-DHC, and 4b-hydroxy-7-DHC, range from sub-mM to over 10 mM. We showed that loss of DHCR7 caused decreased proliferation and self-renewal of cortical neural precursors and aberrantly increased neurogenesis in both mouse and human neural precursor cells. Importantly, treatment of mouse neural precursor cells with individual oxysterols at physiological concentrations led to a similar phenotype. Furthermore, we found that inhibition of 7DHC-derived oxysterol production by antioxidants rescued the increased neurogenesis caused by knockdown

and homozygous loss of DHCR7. These results suggest the accumulation of 7DHC-derived oxysterols is a causal factor in SLOS neural pathophysiology and cholesterol biosynthesis plays a critical role in the normal neural development.

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T-3004

IDENTIFYING THE OPTIMAL AGE OF HUMAN STEM CELL-DERIVED DOPAMINERGIC PROGENITORS FOR TRANSPLANTATION IN PARKINSON'S DISEASE

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Donor age can have a significant impact on transplantation outcomes. Surprisingly, despite the rapid advancement of human pluripotent stem cell derived dopaminergic progenitors for transplantation into Parkinson's Disease patients, little documentation exists regarding the influence/s progenitor state has on transplantation survival, composition and integration – as well as reproducibility across cell lines. To address this, we transplanted ventral midbrain progenitors at varying stages of differentiation (13, 19, 22, 25 and 30 days in vitro, DIV) into 6OHDA mice. Employment of two GFP reporter lines under the promoter PITX3 (iPSC and ESC), enabled specific tracking of graft derived dopamine neurons. Post-mortem histological analysis at 6months revealed surviving transplants at all time points for both cell lines. Graft of 13 and 29 DIV progenitors were small, whilst grafts derived from 19, 22 and 25 DIV cells were larger yet contained a higher number and proportion of non-dopaminergic cells. Cells implanted at 13DIV generated discrete grafts, containing the highest proportion of dopaminergic neurons with the least incorrectly specified cells and maintained re-innervation of developmentally relevant dopaminergic targets. IPSC grafts showed greater yield of dopaminergic neurons at 22DIV, unlike ESC grafts which generated the highest at 13DIV, and had a 3-fold increase in total DA neurons and density compared to ESC grafts, highlighting previously recognised differences in PSC lines. These findings indicate that the timing of implantation (or donor age) is directly related to the survival and outgrowth of dopaminergic neurons, and that careful assessment, on a line to line basis is required prior to translation. Ongoing analysis will determine if donor age impacts the sub-type identity (A8, A9 or A10) of transplanted dopaminergic neurons.

T-3006

UNCOVERING THE MOLECULAR IDENTITY OF CONE PHOTORECEPTOR RESTRICTED PROGENITORS IN THE DEVELOPING MOUSE RETINA

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The developing retina represents a robust and tractable model to understand how precursor cells are fate specified to produce a diversity of cell types in the central nervous system. Over the course of retinal development, seven major cell types are produced in a temporally stereotyped manner from retinal progenitor cells (RPCs). Much remains to be determined as to the extent cell intrinsic vs. extrinsic environmental factors dictate cell fate specification, and to what degree RPCs are multipotential or more lineage restricted. The retinal photoreceptor lineage illustrates both a therapeutically useful and developmentally interesting fate specification, as photoreceptors are lost in retinal degenerative diseases and produced at distinct non-overlapping timepoints in development. Here, using both adult retinal stem cells (RSCs) and RPCs isolated from the embryonic retina, we report the existence of cone photoreceptor lineage restricted progenitors that are induced by a secreted inhibitory retinal factor. Single adult RSCs proliferate in culture to produce a colony containing RPCs and pigmented epithelial progenitors. When single RSC-derived RPCs were exposed to COCO (a retinal secreted inhibitor of TGFb, BMP, and WNT signalling), they produced clones of nearly pure cone photoreceptors, indicating that COCO induces RPCs to assume a cone photoreceptor restricted lineage. Moreover, when RPCs were isolated directly from the murine retina across multiple early and late embryonic timepoints and exposed to COCO, they produced nearly pure populations of cone photoreceptor cells, indicating that the RSC cultures accurately model this developmental lineage. The ability to molecularly identify this cone restricted progenitor type would allow for in vivo analysis of lineage tracing

and ablation/expansion. Accordingly, we have performed an RNA-sequencing analysis on COCO exposed RPCs over their differentiation timecourse. This analysis suggests that Sox15 may be a novel marker of cone progenitors. This previously uncharacterized retinal gene seems to be expressed in RPCs at early E12-14 timepoints when cones are first starting to differentiate. Ongoing experiments exploring mouse Sox15 K/O and in vivo overexpression will further uncover the potential of this gene to dictate cone fate in the retina.

Funding Source: Medicine by Design. NSERC. CIHR. Vision Science Research Program. Foundation Fighting Blindness.

T-3008

SINGLE MOLECULE IMAGING OF ASCL1 DURING DIRECT REPROGRAMMING OF MOUSE EMBRYONIC FIBROBLASTS TO INDUCED NEURONAL (IN) CELLS

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The ability to directly convert differentiated somatic cell types into neuronal cells provides critical information about what defines a neuron. Elucidation of the mechanisms underlying this direct conversion has the potential to generate more comprehensive models of neurological disorders with which to study disease biology and perform drug screening, as well as possibilities for more efficient regenerative medicine methods. Ascl1, a proneural basic helix loop helix (bHLH) transcription factor, directly converts mouse embryonic fibroblasts into functional induced neuronal (iN) cells and has a unique ability among other reprogramming factors to independently locate its cognate binding sites not only in its endogenous neural context, but also in the different chromatin context of fibroblasts. However, it is unclear how precisely Ascl1 locates its targets in closed chromatin. Recent advances in single molecule imaging significantly increase the spatial and temporal resolution of tracking individual factors within the nucleus, and it is now possible to analyze their dynamic interactions with DNA. This project aims to utilize highly inclined and laminated optical sheet (HILO) microscopy to understand how Ascl1 independently activates its targets in the different epigenetic landscapes of neurons and fibroblasts. The search kinetics of Ascl1 in its physiological context will be compared to Ascl1 overexpression in reprogramming fibroblasts, as well as to the context-dependent reprogramming factor Brn2. These characteristics will also be measured in heterochromatin to determine whether Ascl1's ability to orchestrate reprogramming is due to a more efficient search pattern in closed chromatin.

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T-3010

EVALUATION OF SUBTYPE SPECIFIC ION CHANNEL FUNCTIONS ON AXONAL CONDUCTION OF CULTURED NEURONS WITH MICROFABRICATED RECORDING DEVICE

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Functional evaluation of neurons derived from human pluripotent stem cells is an important task for regenerative medicine and disease modeling. Normally, gene or protein expression analysis, and electrophysiological recording by patch clamp method are performed. However, neither method is difficult to evaluate functions from a single sample during a long term enough for functional maturation. In addition, although it is possible to evaluate the function more than one year by using a measuring device called microelectrode array, there remains a problem that it is impossible to obtain information correspond to intracellular protein expression. In this study, we developed a culture device and analysis method to evaluate propagations of action potential along individual axons with the aim of establishing a method for continuously and indirectly monitoring intracellular protein expression for a long time. As a proof of concept study, we performed three experiments: 1) continuous monitoring of developmental change, 2) pharmacological blockade of sodium ion channels, 3) subtype specific blockade. First, conduction velocity of mouse primary cultured neurons was calculated at various time points. The conduction velocity significantly increased with days, suggesting that developing neurons change their conduction properties. Second, axons were treated with various sodium ion channel blockers, TTX and lidocaine, at various concentration. The conduction velocity gradually decreased as increasing concentrations of reagents, suggesting that partial blockade of sodium ion channels decreased conduction velocity. Finally, axons were treated with subtype specific inhibitors to sodium ion channels. Addition of a subtype specific inhibitor showed that Nav 1.2 is dominant for conduction in axons of cerebral cortical neurons. Taken together, our method is suggested to be a useful tool for long term functional evaluation of cultured neurons.

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T-3012

GENERATION OF TWO DIFFERENT HUMAN MICROGLIA CELLS SEPARATELY FROM IPSC-DERIVED PRIMITIVE OR DEFINITIVE HEMATOPOIETIC PROGENITORS

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Human microglia are vital residents of the brain, where they play important roles in development, injury and disease, such as Alzheimer's (AD) disease. Arising from yolk sac primitive progenitors that colonize in the brain during embryogenesis, microglia are unique among tissue macrophages in that they are thought to remain primitive progenitors derived throughout life, without contribution from the definitive hematopoiesis. After insults such as stroke and neurodegenerative disease; however, microglia dramatically change their phenotype and are joined by infiltrating macrophages from blood. These definitive hematopoietic progenitors-derived occupants can resemble microglia in morphology and surface marker expression but appear to participate differently in disease pathogenesis, making it essential to further clarify their functions. Currently, there is no method reported to generate these two microglia cells from the same iPSCs. To understand how naive microglia and infiltrating microglia cells affect the brain in disease condition, we developed a novel protocol to efficiently produce two different microglia cells separately from primitive or definitive hematopoietic progenitors. By manipulating WNT signaling and Activin-Nodal signaling, human iPSCs were first induced to separate hematopoietic fates, primitive hematopoietic progenitors (CD43+, CD235a+) and definitive hematopoietic progenitors (CD34+, CD43-, CD235a-). Next, these two hematopoietic progenitors were grown in serum-free differentiation medium containing CSF-1 and IL-34 to differentiate into macrophages, then cocultured with our BrainXell cortical neuron and astrocyte mixed culture to induce microglia identity. We identified the different gene expression between these two microglia cells by RNA-seq profiling, and validated by qPCR, immunostaining and FACS analysis. In summary, two different microglia cells, derived separately from iPSC derived primitive or definitive hematopoietic progenitors, will provide useful models to understanding microglia function in neurological diseases like AD disease

T-3014

3D BIOPRINTING INTO TISSUE SPECIFIC HYDROGELS FOR THE STUDY OF MICROENVIRONMENTAL CONTROL OF STEM CELL FATE

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The cellular microenvironment has been shown to play a fundamental role in the regulation of cell function, stem cell fate determination, maintenance of cell potency and tissue homeostasis. Our laboratory focuses on the study of the effects of cellular microenvironment in the context of cancer and neurological models, based on the observation that a healthy environment can induce the suppression of tumorigenesis in mouse models. Insights concerning the molecular mechanisms that drive these processes are very limited, partly due to the inability of the current traditional methods of investigation, such as two-dimensional cell cultures and animal models, to accurately represent the human in vivo cellular microenvironment. Three-dimensional cell cultures allow us to overcome the structural limitations posed by monolayer cultures, and maintain the ease of experiment design, monitoring and data analysis associated with in vitro procedures. Our laboratory has established systems to overcome some of these limitations and rely on the strengths of three-dimensional culture methods to elucidate mechanisms that govern stem cell differentiation. A customized 3D extrusion-based bioprinter was developed starting from a commercially available model, allowing for precise and controlled injection of cells within three-dimensional substrates. This tool allows for design of highly controlled experiments, in which the effects of cellular microenvironment on stem cell differentiation can be studied at a single-cell resolution. For increased levels of biomimicry, tissue specific substrates are generated from extracted tissue. Collected tissue is subjected to a chemical decellularization process, followed by lyophilization, enzymatic digestion and neutralization, to generate a self-gelling product upon incubation at 37°C. Mammary and brain extracellular matrix-derived substrates have been shown to support the growth of cells of the epithelial and neuronal lineages, respectively. Here, we apply these established systems to study the effects of the environment constituted by the three-dimensional substrates on the differentiation of injected stem cells.

Funding Source: Jeffress Trust Awards Program in Interdisciplinary Research; NIH R15 GM131330

T-3016

RAPID GENERATION AND MATURATION OF CORTICAL LAYER II-III GLUTAMATERGIC NEURONS FROM HUMAN IPSCS

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Numerous neurological and psychiatric disorders involve glutamatergic (Glut) neurons specific to one or more of the six cortical layers. Death or malfunction of layer II-III specific Glut neurons underlies disease pathophysiology and disrupts higher cognitive function in Alzheimer, Schizophrenia and Autism

diseases. Study of these neurons may reveal the molecular mechanisms behind their vulnerability and enable development of more relevant disease models. Toward this goal, we developed a novel protocol to rapidly and efficiently produce layer II-III cortical Glut neurons. Human iPSCs were first induced to a neural epithelia fate, patterned to Glut neuronal progenitors (>95% SOX1+/PAX6+), and then rapidly switched to layer II-III Glut progenitors (>85% CUX1+). Using novel combinations of small molecules, the layer II-III progenitors can be expanded up to 500-fold, which allows for production of large and consistent batches of neurons. Finally, the progenitors were plated in medium containing a specialized maturation supplement that rapidly promotes morphological and functional maturation. After treating with this maturation supplement, layer II-III Glut 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. Seven days post-plating, the cultures were >90% neurons (MAP2+) with the following breakdown by layer identity: ~85% layer II-III (CUX1+/ CTIP2-/ FOXP2-), ~10% layer V (CTIP2+), and ~2% layer VI (FOXP2+). Thus, our novel differentiation protocol generates a pure neuronal culture that is highly enriched for cortical Glut neurons with a layer II-III identity. To validate whether this production protocol can be applied to other iPSC lines, we generated cortical layer II-III Glut neurons from three healthy control and three Schizophrenia iPSC lines, and we identified disease-specific phenotypes by RNA-seq profiling and MEA analysis. In conclusion, coupled with the ability to generate very large batches (>1 billion neurons) and bring about rapid maturation, layer II-III cortical Glut neurons present a highly relevant model system for disease study and drug discovery.

NEURAL DISEASE AND DEGENERATION

T-3018

HYPERPHOSPHORYLATED TAU IN HUMAN IPSC-DERIVED ASTROCYTES SHIFTS THE FOCUS FROM NEURONAL TAU PATHOLOGY TO ASTROCYTIC TAU PATHOLOGY

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Pathological accumulation of the microtubule associated protein tau is hypothesized to underlie cell death in neurodegenerative diseases such as Alzheimer's disease (AD), frontotemporal lobe dementia (FTD), Pick's disease and others. Despite numerous studies, the mechanisms that underlie aberrant tau aggregation in CNS tauopathies remain unknown. We and others have recently found that both neuronal and microglial-derived tau is sufficient to initiate tau propagation in the rodent brain. However, despite evidence that astrocytes accumulate and release pathogenic tau species, the role of astrocytic-derived tau in human tauopathies remains unknown. Therefore, with this work, we examined the expression of tau and tau phosphorylation status in human astrocytes derived from induced pluripotent stem cells (iPSCs). Our data show that overall expression of tau in cultured iPSC-astrocytes is lower than tau expression in iPSC derived neurons, however, iPSC-astrocytes display higher levels of tau phosphorylation compared to neuronal cultures. Remarkably, we found that the total level of phosphorylated tau was greater in astrocytes compared to neurons and that even in the absence of a stress-inducing stimuli, astrocytic tau was hyperphosphorylated. These results suggest that astrocytic derived tau may be more susceptible to phosphorylation than neuronal tau and that hyperphosphorylation of astrocytic tau may be an early feature of neurodegenerative disease. Future studies will examine the pathogenic potential of astrocytic-derived tau and determine the role of astrocytes in disease-associated tau propagation. In total, the work presented here provides preliminary evidence that astrocytic tau dynamics may play a role in AD-related pathologies.

Funding Source: The California Institute for Regenerative Medicine

T-3020

MODELING CALCIUM KINETICS OF NEURAL COGNITIVE DISORDERS WITH HIPSC DERIVED NEURONAL CULTURES

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Human induced pluripotent stem cells (hiPSCs), have been beneficial for modeling poorly understood diseases, specifically psychiatric disorders. By utilizing hiPSCs, animal modeling, and primary human brain material, we have generated a strongly supported hypothesis that the psychiatric disease bipolar

disease (BPD) is through the protein collapsin response mediator protein (CRMP2), that controls the form and function of dendritic spines (collapsin response mediator protein-2 (CRMP2)) and hence neuronal network activity. It is unknown how this pathway mechanistically changes these neuronal structures. If this were known, better drug targets and drugs might be devised for treating BPD. In addition, there is significant overlap in the etiology of bipolar disease and other neurodegenerative diseases such as Alzheimer's (AD). Not only is CRMP2 central to BPD, but it is also associated with AD pathophysiology(Nunes et al, 2013 Curr Alzheimer's Res). Our research focuses on understanding how abnormalities in CRMP2 underlie BPD and drug induced psychosis in conjunction with AD. Through the use of looking at neurons generated from hiPSCs and transgenic mouse models, we will attempt to discern this mechanism. The techniques will involve neurite proteomics, electrophysiological assessments of network formation (via multi-electrode array), and calcium kinetic imaging. By deriving neuronal cultures from human bipolar disorder iPSCs, we can observe how CRMP2 mediates neurological processes relevant to psychiatric disorders in conjunction with testing how stimulants known to induce psychosis impact key psychiatric genes such as CRMP2 and phenocopy psychiatric disorders at the cellular and molecular level.

T-3022

AGE RELATED AUTOPHAGY IMPAIRMENTS IN DIRECTLY REPROGRAMMED DOPAMINERGIC NEURONS IN PATIENTS WITH IDIOPATHIC PARKINSON'S DISEASE

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Understanding the pathophysiology of Parkinson's disease (PD) has been hampered by the lack of models that recapitulate all the critical factors underlying its development. Using a novel and highly efficient approach, we generated functional induced dopaminergic neurons (iDANs) that were directly reprogrammed from dermal fibroblasts of patients with idiopathic PD (n=19) as well as sex- and age-matched healthy donor (n=10) to investigate whether such cells have deficits in autophagy. We show that iDANs derived from PD patients exhibit lower basal chaperone-mediated autophagy as compared to iDANs of healthy donors. Furthermore, stress-induced autophagy resulted in an accumulation of macroautophagic structures in induced neurons (iNs) derived from PD patients, independently of the specific neuronal subtype but dependent on the age of the donor. Finally, we showed that these impairments in autophagy in the iNs derived from idiopathic PD patients lead to an increase in phosphorylated alpha-synuclein, a hallmark of PD pathology. Taken together, our results show that direct neural reprogramming provides a patient-specific model to study neuronal features relevant to idiopathic PD.

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T-3024

A SMALL MOLECULE SIRT3 AGONIST RESCUES ALS PHENOTYPES IN MOTOR NEURONS DERIVED FROM SPORADIC AND FAMILIAL ALS IPSCS

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Amyotrophic lateral sclerosis (ALS) is a progressive adult-onset motor neuron disease that affect the upper and lower motor neurons, resulting in muscular weakness and ultimately death. Majority of ALS patients (~90%) have sporadic forms of the disease where the disease etiology remains unknown. The other 10% of patients have familial ALS where mutations are commonly found in SOD1, C9ORF72, and TDP43. Despite being intrinsically heterogeneous, the disease manifestation between sporadic and familial ALS are clinically indistinguishable, suggesting a possible converging pathogenic mechanism. Motor neurons rely on mitochondrial respiration to fuel their metabolic needs and any disruption in the mitochondria function will lead to neurological disorders. Recent studies have found a causative relationship between defective energy metabolism and the survival rate of patients with ALS. However, clear mechanistic insights between altered metabolism and ALS disease manifestation is currently unknown. In this study, we investigated if defective mitochondrial respiration could be the common pathway affected in both sporadic and familial ALS. Metabolic flux analyses reveal a deficiency in mitochondrial

respiration in both familial and sporadic ALS motor neurons. This defective metabolic profile was attributed by decreased activity of SIRT3, a mitochondrial deacetylase that regulates mitochondrial function and is implicated in aging. Using a small molecule SIRT3 agonist, we were able to reverse the defective metabolic profiles in both familial and sporadic ALS motor neurons, promoting neurite regeneration and enhancing motor neuron health/survival. Comparing SIRT3 agonist with Riluzole and Edaravone, we found that the former was more effective in improving ALS phenotypes in motor neurons derived from sporadic and familial ALS iPSCs. Therefore, our study demonstrated that SIRT3 is involved in the converging pathogenic mechanism between both familial and sporadic ALS, and provides an explanation as of why age is a risk factor for ALS. In addition, we demonstrate that SIRT3 agonists can be exploited as potential therapeutics for ALS.

T-3026

TUNING THE FUNCTION OF MICROGLIA TO DEVELOP NOVEL TREATMENT OF AD

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Microglia are the neuroimmune cells that involve surveillance and phagocytosis in the central nervous system. In devastating neurodegenerative disorders such as Alzheimer's disease (AD), microglia have been shown to play major roles in internalization and degradation of amyloid-beta peptide (Aβ). Functional impairment of microglia eventually causes accumulation of Aβ, which accounts for the development of AD. It is intriguing whether tuning the function of microglia can alleviate such category of disorders. In this study, we named MDR-1, a key regulator for the development of microglia, which is downregulated in brains of aged and AD mice. We demonstrated that overexpression of MDR-1 either in culture mouse primary microglia, or in human and mouse microglial cell lines, can enhance the phagocytic uptake and degradation of Aβ. Elevated MDR-1 in microglia also cause decreased secretion of proinflammatory cytokines, such as interleukin-6 (IL-6), TNFα, and IL-1β, and increased expression of anti-inflammatory Arg1/IL-10. We thus identify a novel molecule that can potently regulates the accumulation and clearance of Aβ, as well as the inflammatory cytokine profile of microglia, which we expect promising in engineering microglia for cell transplantation and AD therapy.

T-3028

HUMAN IPSC-DERIVED GABAERGIC INTERNEURON TRANSPLANTS ATTENUATE NEUROPATHIC PAIN

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Neuropathic pain causes severe suffering and most patients are resilient to current therapies. A core element of neuropathic pain is the loss of inhibitory tone in the spinal cord. Previous studies have shown that foetal GABAergic neuron precursors can provide relief from pain. However, the source of these precursor cells and their multipotent status make them unsuitable for therapeutic use. Here we extend these findings by showing, for the first time, that spinally transplanted, terminally differentiated hiPSC-derived GABAergic (iGABAergic) neurons provide significant, long-term and safe relief from neuropathic pain induced by peripheral nerve injury in mice. Furthermore, iGABAergic Neuron transplants survive long term in the injured spinal cord and show evidence of synaptic integration. Together, this provides the proof in principle for the first viable GABAergic transplants to treat human neuropathic pain patients.

T-3030

CHARACTERIZATION OF NEUROPSYCHIATRIC DISEASE PHENOTYPES OF IPS CELL DERIVED DOPAMINERGIC NEURONS WITH 16P11.2 COPY NUMBER VARIATIONS

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Patients with 16p11.2 duplication have an increased risk of psychiatric disorders, including schizophrenia, bipolar disorder, anxiety, and depression. Patients with 16p11.2 deletion have autism spectrum disorder, including impaired social skills as well as delayed development of language and speech. Behavioral problems associated with this copy number variant (CNV) also include attention deficit hyperactivity disorder. At the moment there are no curable treatments for these neuropsychiatric disorders and often the medications cause severe side effects for the patients. In addition, the molecular and cellular deficits predisposing patients to these disorders remain to be resolved. Here, we have established a dopaminergic (DA) neuron cell culturing model to study disease phenotypes of human induced pluripotent stem cell (hiPSC) derived neurons carrying 16p11.2 duplication and 16p11.2 deletion. We have characterized the hiPSC-derived DA neurons with transcriptional gene expression profiling and this analysis showed that genes regulating synaptic development were significantly altered in the DA neurons with 16p11.2 duplication. In addition, genes regulating vesicle formation and neurotransmission were significantly altered in the DA neurons with 16p11.2 deletion compared to control cells. These genetic deficits can affect the formation of the striatal neuron networks during brain development in the patients with 16p11.2 CNVs. To understand the functional development of the DA neurons in vitro we have characterized the network development and DA signaling on the high-density multi-electrode array platform during long term culturing. We detected that the cells carrying 16p11.2 CNVs had altered activity compared to healthy control DA neurons. This data implicates that DA neuron function may be deficient in patients with these copy number variations, which may lead to different physiological and psychological symptoms. In addition, establishment of these cell culturing platforms for disease phenotyping and drug screening assays in vitro will, in the future, facilitate the design of more personalized therapies for the patients with these copy number variations.

Funding Source: The Tommy Fuss Center for Neuropsychiatric Disease Research, Translational Neuroscience Center/Boston Children's Hospital.

T-3032

REVAMPING THE CRISPR-CAS9 SYSTEM FOR TARGET SPECIFIC METHYLATION OF RISK GENES ASSOCIATED WITH ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an irreversible, neurodegenerative disease characterized by disruption of neuronal connections and cell death, leading to memory loss and cognitive decline. Pathological hallmarks of AD include inflammation, neuritic plaques, stemming from accumulation of extracellular amyloid β, and neurofibrillary tangles, arising from aggregation of intraneuronal hyperphosphorylated Tau protein. To better understand the progression of these pathological hallmarks, and identify potential therapeutic avenues, we established a model to assess DNA methylation changes in key genes associated with AD. Using induced pluripotent stem cells (iPSCs) from patients harboring a dominant, fully penetrant fAD mutation in the amyloid precursor protein gene, APP (V717I), we successfully developed an epigenetic editing tool by fusing the catalytic domain of the DNA methyltransferase, DNMT3A, to dCas9 for site specific DNA methylation of the neuronal β-secretase gene, BACE1, which plays a critical role in the processing of APP and subsequent generation of amyloid β. We generated a stable iPSC line using a third generation lentivirus system, which increased methylation of the BACE1 promoter by ~20-40%, and resulted in ~80% transcriptional repression (compared to a non-targeting gRNA). We are currently deriving additional cell lines to assess PSEN1 and PSEN2 activity, as well as additional patient cell lines. iPSC lines are currently being differentiated into mature neurons to observe changes in amyloid β production, Tau phosphorylation and other pathophysiological changes associated with AD. Our use of AD patient neurons and a clinically relevant third generation lentivirus system allows us to examine previously unrecognized effects of epigenetic modifications on key genes associated with AD, and provides a model system/tool for testing therapeutic strategies.

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T-3034

GENOME-WIDE CRISPR-CAS9 SCREENS IN ACCELERATED HUMAN STEM CELL-DERIVED NEURAL PROGENITOR CELLS IDENTIFY ZIKA VIRUS HOST FACTORS AND DRIVERS OF CELL PROLIFERATION

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Neural progenitor cells (NPCs) are essential for brain development and their dysfunction is linked to several disorders, including autism, Zika Virus (ZIKV) Congenital Syndrome, and cancer. Understanding of these conditions has been improved by advancements in stem cell-derived NPC models. However, current differentiation methods require many days or weeks to generate NPCs and show variability in efficacy among cell lines. Here, we describe human Stem cell-derived NGN2-accelerated Progenitor cells (SNaPs), which are produced in only 48 hours. SNaPs express canonical forebrain NPC protein markers, and are proliferative and multipotent. Like other human NPCs, SNaPs are susceptible to ZIKV infection and viral-mediated cell death, while also being able to support active replication of this virus. Importantly, the SNaP method his highly reproducible, having worked in 48 of 50 human stem cell lines on the first attempt. We demonstrate that SNaPs are valuable for large-scale investigations of genetic and environmental influencers of neurodevelopment by deploying them for genome-wide CRISPR-Cas9 screens. Our screens identified hundreds of new and previously characterized ZIKV host factors in a microcephaly-relevant cell type, while also detecting dozens of genetic drivers of NPC proliferation, including PTEN, NF2, and PTCH1, through a built-in fitness assay. Finally, leveraging the reproducibility of the SNaP system, we performed "population-in-a-dish" experiments in which human stem cell lines from dozens of different donors were maintained as pooled cultures of SNaPs in single flasks prior to genomic and/or transcriptomic analyses. This mixed-culture approach revealed inherent growth differences among SNaP cell lines, and located several expression quantitative trait loci (eQTLs) that are relevant to autism spectrum disorders. As a whole, this work demonstrates the utility of novel stem cell-based technologies and their application towards high-throughput investigations of human neurodevelopment.

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T-3036

INVESTIGATING THE EFFECTS OF PATHOGENIC AND PROTECTIVE GENE MUTATIONS ASSOCIATED WITH ALZHEIMER'S DISEASE (AD) OVER INJURY-INDUCED AMYLOID BETA

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Alzheimer's disease (AD) is a progressive, neurodegenerative disorder characterized by the formation of protein aggregates containing amyloid beta (Aβ) and hyperphosphorylated tau. AD is a multifactorial disease that involves a complex interaction between genetic susceptibility and environmental exposures. Mutations in PSEN1 and APP genes, such as PSEN1DE9 and APPSwedish, can lead to early onset of AD, whereas APPIcelandic mutation is reported to confer a protective effect against AD. Moreover, while traumatic brain injury (TBI) also increases the risk of developing AD, the effects of TBI in AD-associated outcomes in individuals carrying these mutations are still unknown. To start addressing these questions, here we used neurons derived from TALEN/CRISPR-engineered human induced pluripotent stem cell (hiPSC) lines harboring either APPSwedish, PSEN1DE9, or APPIcelandic mutations. Neurons were rapidly stretched using a novel microfluidic device to simulate mild injury. As expected, among non-injured neurons expressing PSEN1DE9 and APPSwedish, but not APPIcelandic, presented higher levels of extra- and intracellular A^2 than control neurons. Twenty-four hours after injury, control neurons exhibited increased extracellular levels of A^2 when compared to non-stretched control group. Intriguingly, A^2 levels in neurons carrying AD-pathogenic or -protective mutation were not affected following mechanical trauma. These results suggest that, consistent with its protective potentials against AD pathologies, APPIcelandic mutation also demonstrates neuroprotective effects against mechanical injury. Strikingly, despite the increased levels of A^2 at steady state, mechanical stretch did not enhance amyloidogenesis in neurons expressing familial AD (FAD) pathogenic mutants, implying they reached saturated amounts of A^2 prior to the injury, thus, they are impervious to the mechanical damage. In future experiments, we will investigate whether A^2 generation in FAD mutants can be further upregulated using pharmacological compounds expected to enhance A^2.

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T-3040

EFFECTS OF LIPOPOLYSACCHARIDE ON THE SELF-RENEWAL AND NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS AND NEURAL STEM CELLS

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Prenatal infections in early brain development can engender adverse neurological outcomes in children. We hypothesized that prenatal infections may affect the neural differentiation of embryonic stem cells (ESCs) and further interfere with neural development and differentiation. To mimic the most common scenario of prenatal infection, mouse ESCs and ESC-derived neural stem cells (NSCs) were treated with lipopolysaccharide (LPS). In the ESC stage, 65% ± 1.3% of toll-like receptor 2 (TLR2)-positive cells and 1.6% ± 0.5% of TLR4-positive cells in ESCs were detected by flow cytometry. Notably, the percentage of TLR2(+) cells significantly decreased to 1.6% ± 0.4% and that of TLR4(+) cells significantly increased to 7.0% ± 0.9% after the ESC-to-NSC transition at days in vitro (DIV) 7. Furthermore, LPS did not affect the viability of mouse ESCs but significantly increased the number of ESC-derived neurospheres during the ESC-to-NSC transition. When LPS was administered during the ESC-to-NSC transition, we further observed that LPS significantly upregulated the mRNA expression levels of neuronal markers (Tuj1, Map2), astrocytic marker (Gfap), and oligodendrocyte marker (O4 and Oliog2) in the differentiated neural cells at DIV14, by using quantitative reverse transcription polymerase chain reaction analyses. Whole transcriptome analysis further revealed that significant upregulation of membrane dynamics of plasma, lysosomal and vacuole membranes and intracellular protein activities are involved. Notably, administration of LPS during the NSC-to-neural differentiation resulted in the downregulation of the above neural markers through significant upregulation of cell death pathways, such as p53 signalling pathway, and ferroptosis. In conclusion, this study revealed the dynamic expression profiles of TLRs during early neural development and that the timing of prenatal infection could be crucial for determining the types and severity of neural disorders developed in adulthood. Our findings may facilitate the development of preventative and therapeutic strategies for the adverse neurological side effects of prenatal infections.

ORGANOIDS

T-3042

DEVELOPMENT OF COLLAGEN BASED 3D MATRIX FOR GASTROINTESTINAL TRACT-DERIVED ORGANOID CULTURES

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An organoid is a cell organization grown in a three-dimensional (3D) culture system which represents all characteristics of its origin tissue. This organ-like structure requires a supporting matrix to maintain its characteristics and functions. Matrigel is mainly used as a matrix for 3D culture systems; however, it may not be used for clinical applications, as it is created from mouse sarcoma and contains unidentified components. This study attempts to develop a new, collagen-based matrix that may serve as a substitute for Matrigel in the organoid culture method. A collagen-based matrix was made, using type 1 collagen, Ham's F12 nutrient mixture, and bicarbonate. Characteristics of mouse colonoids were analyzed by morphology and quantitative messenger RNA (mRNA) expression, revealing that the mouse colonoids grown in the collagen-based matrix and in Matrigel had quite similar morphology, specific markers, and proliferative rates. Mouse small intestine–derived enteroids, stomach-derived gastroids, and human colon–derived colonoids were also cultured, all of which were successfully grown in the collagen-based matrix and had similar properties to those cultured in Matrigel. This suggests that the collagen-based matrix could be used for gastrointestinal tract–derived organoid culture as an extracellular matrix instead of Matrigel. This technology will render organoid cultivation inexpensive and clinically applicable, which may facilitate various therapeutic clinical trials of gastrointestinal tract–derived organoids.

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T-3044

NEURAL TISSUE ENGINEERING WITH IPSC-DERIVED NEURAL STEM CELLS USING A HYPER-CROSSLINKED CARBOHYDRATE POLYMER

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Spinal cord regeneration is at the fingertips of developing tissue engineering efforts, but complete functional recovery of spinal cord injury is still out of reach. Scaffolds provide a physical substrate for cells to adhere in three dimensions, thus enabling de novo reconstruction of gross tissue architecture by loaded neural stem and progenitor cells or through immigration of native cells. Some scaffolds have been shown to facilitate partial functional recovery in spinal cord injury animal models, but the gold standard is not yet developed. Scaffolds can also be used to grow neural organoids in vitro, providing a scalable model for neural tissue in high-throughput neurotoxicity screens or developmental experiments. Another major advantage in this approach is the ability to use iPSC-derived stem and progenitor cells, allowing the study and use of autologous cells. Scaffold

design is important to the successful growth of native-like tissue for several reasons. We present a novel hyper-crosslinked carbohydrate polymer (HCCP) for neural tissue engineering for the first time. HCCP exhibits key requirements of an ideal tissue engineering scaffold, including absence of host immune response, high porosity, bioresorbability, biocompatibility, and biomechanical properties similar to native tissues. HCCP has been approved by the U.S. FDA for clinical utilization for bone repair and regeneration and also shown to promote the generation of cancer, cardiac, and renal organoids. In this study, HCCP is seeded with iPSC-derived neural stem cells and used to grow large scale neural organoids in vitro and screen for drug neurotoxicity. HCCP is also used to regenerate the spinal cord in a hemi-section spinal cord injury model in vivo. The polymer is seeded with neural stem cells prior to implantation and gait analysis is used to evaluate functional recovery during regeneration. The favorable characteristics of HCCP provide an attractive scaffold for tissue engineering and stem cell biology in developing organoid models. The unique properties of HCCP also provide an effective tool for screening drugs, which targets the central nervous system, and investigating their effects on human brain during development.

T-3046

THE DEVELOPMENT STUDY APPLICATION OF BRAIN ORGANOIDS BY HUMAN PLURIPOTENT CELLS

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Around 2010 year, several researchers had report floating 3D culture method as like serum-free culture of embryoid body-like aggregates (SFEB) that is source for brain organoid generation. First, we were isolated human primary somatic cell from human teeth and human brain tumor patient's tissue. Second, obtained somatic cells were reprogramming using mRNA tools and characterized. Third, reprogrammed induced pluripotent stem cells (iPSCs) were generated cerebral organoid for development study as follows. Human primary somatic cells were isolated using enzymatic method and cell banking. And then these cells were reprogrammed using mRNAs (OSKMNL) mixed with evasion mRNAs (EKB) for 4 days. Human iPSCs like colonies were pick up around 2 weeks after transfection. Human iPSCs were stained with Alkaline Phosphatase as a stemness marker and characterized by immunocytochemistry to detection of pluripotency-associated genes as like TRA-1-60 and TRA-1-81 on live. Reprogrammed colonies were characterized and aggregated to generate cerebral organoid which organoid were cultured 3D floating method. Human iPSCs were collected and aggregated for embryonic body (EB) formation during germ layer differentiation, 9,000 cell/well for 6 days. And then EBs were induced of neural differentiation step to use neural induction media, contained with inducer of neural morphogen that differentiated neural ectoderm. And then neuroepithelial like tissues were transfer to matrigel droplets for the expansion of neuroepithelial buds and expansion culture until one month and two months. During organoid generation, each step was measured on bright filed and organoid like 3D cell were immunostained with neural marker as follow, Tuj1, PAX6, and Sox2. Reprogramming of human somatic cell is good source to regeneration study and Organoids may be valuable in the investigating developmental biology study. In the further study, generated brain organoids will be required to investigate their availability of the interaction of neural cell-to-cell signaling.

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T-3048

DEVELOPING HUMAN VASCULARIZED BRAIN ORGANOIDS

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Increasing evidence suggests that the neurovasculature is compromised throughout the initiation and development of neurodegenerative diseases. While clinical observations and rodent models have provided invaluable insight into these processes, generating an experimental human model facilitates a more mutable system with scalable potential for therapeutic screening. Human brain organoids derived from induced pluripotent stem cells (iPSCs) provide significant advantages with regards to cellular complexity and organization compared to traditional 2-dimensional cell culture methods. However, current organoid models are deficient in vascular tissues that likely play a significant role in brain development. Using iPSCs, we simultaneously generated brain microvascular endothelial cells, pericytes derived from the neural crest, and cortical brain organoids by directed differentiation. In conjunction with the neurogenesis phase, we embedded organoids in a droplet of extracellular matrix containing a single-cell suspension of endothelial cells and pericytes, generating a self-organizing vascularized cortical organoid (vCO). Over 30 days of culture,

vCOs develop endothelial cell networks exhibiting extensive interactions specifically with a subset of S100β+ radial glia. Moreover, we also observed regions of PDGFRβ+ pericyte-endothelial cell interactions. One of the hallmarks of the blood-brain barrier is the presence of contiguous tight junctions. In vCOs, endothelial cell networks exhibited cell borders delineated by the claudin-5, suggesting that the local microenvironment promotes the development of barrier function. Vascular networks within the organoid can be maintained over 100 days, at which point endothelial cell networks are encapsulated by S100β+ and GFAP+ astrocytes. Morphological features were comparable across vCOs and were replicated in 2 iPSC lines. Collectively, these data represent the first in vitro model of a human brain organoid containing elements of a functional blood-brain barrier, and may provide insights into neurovascular development, as well as neurodegenerative and neurovascular disease. In particular, this model enables an unprecedented view into the purely cellular aspects of these pathologies in the absence of the circulatory system.

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T-3050

GENERATION OF 3D RETINAL ORGANOIDS FROM HUMAN NAÏVE PLURIPOTENT STEM CELLS ELIMINATES INTERLINE VARIABILITY OF RETINAL DIFFERENTIATION

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Retinal Organoid (RO) development from human pluripotent stem cells (hPSCs) has thus far been optimized only in selected 'permissive' conventional hPSC lines with efficient neural differentiation capacities. Reproducible generation of ROs with photoreceptor progenitors from any hPSC line without interline variability would facilitate therapeutic regeneration in ophthalmologic disorders in a universal, defined manner. We have established that conventional, lineage-primed hPSC can be stably-reverted to naïve hPSC (N-hPSC) with enhanced multi-lineage differentiation potential following chemical inhibition with LIF-2i and the tankyrase inhibitor XAV939 (LIF-3i). To test whether LIF-3i reversion improves RO development amongst 'non-permissive' hPSC lines with poor neural potential, isogenic primed vs N-hPSC were differentiated with an established 3D retinal protocol. Differentiated neural 'horse-shoe' (HS) shaped domains were cultured in suspension and matured into retinal cups (RC) with laminated neural layers. 3 hESC and 3 hiPSC lines were differentiated into ROs +/- LIF-3i reversion. 2 of 6 non-permissive conventional hPSC lines incapable of HS development differentiated into RC organoids following LIF-3i reversion with efficiencies comparable to "permissive" hPSC lines (e.g. H9, IMR90-4). Eye field-specific gene expression at 2 weeks and photoreceptor lineage markers at 8-20 weeks in N-hPSC were robustly detected in RCs generated via qRT-PCR and immunofluorescent confocal microscopy, including Rhodopsin+ CRX+ Recoverin+ photoreceptor progenitors, HuC/D+ ganglion/amacrine cells, PROX1+ horizontal cells, and MITF+ pigmented epithelium. N-hPSC-derived RO displayed improved long-term maturation of in vitro Rhodopsin+ photoreceptors with proper histo-architecture. 13 week-old RCs were dissected into neural retinal sheets and transplanted into the subretinal space of ischemia reperfusion-injured NOG-SCID mice eyes, and human cell specification and engraftment was evaluated at 3-6 months post-transplant. We propose that improved RO development from N-hPSC will facilitate the clinical development of autologous or universal donor-based ocular cell therapies and enhance the engraftment and long-term survival of retinal cell lineages following cellular transplantation.

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T-3052

VASCULARIZATION AND MATURATION OF HUMAN IPSC-DERIVED KIDNEY ORGANOIDS THROUGH TRANSPLANTATION IN THE COELOMIC CAVITY OF CHICKEN EMBRYOS INCREASES GLOMERULAR MODELLING

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Human induced pluripotent stem cell (hiPSC) derived kidney organoids closely resemble in vivo kidney tissue, offering unprecedented possibilities for modelling disease and development. Despite recent advances in kidney organoid generation, currently available protocols still yield organoids that lack a functional vasculature. As kidneys are highly vascularized organs that depend on the presence of vascular endothelial cells and blood flow for their correct development and functionality, it is not surprising that the organoids remain immature. Unfortunately, this limits their applicability. Particularly problematic is the modelling of glomerular diseases, since all three components of the glomerular filtration barrier, namely endothelial cells, glomerular basement membrane (GBM) and podocytes, are either absent or immature in in vitro kidney organoids. Recently, we have shown that transplantation of hiPSC-derived kidney organoids under the renal capsule of mice leads to functional vascularization and maturation of the organoids. However, this model is labor-intensive and time consuming, requiring 28 days for optimal maturation. Here, we describe a new model where we transplanted kidney organoids inside the coelomic cavity of early chicken embryos. We observed rapid vascularization by host vasculature as well as reorganization of organoid-derived human endothelial cells. Through intravascular injection of fluorescently-labeled lectins, we show functionality of and anastomosis between the chicken- and human- derived blood vessels. Upon 8 days of transplantation we did not observe misdirected differentiation to stromal tissue. Furthermore, unbiased wide field transmission electron microscopy analysis of these organoids demonstrated glomerular maturation with the formation of podocyte foot processes, slit diaphragms and a GBM. Tubular structures also showed signs of maturation, with the appearance of cell junctions and microvilli. These data demonstrate that intracoelomic transplantation inside chicken embryos is an efficient method to vascularize and mature kidney organoids. This highly accessible model could be employed to rapidly mature organoid glomerular structures in order to improve the modelling of glomerular kidney disease.

T-3054

A SERUM-FREE DEFINED CULTURE SYSTEM FOR STUDYING THE DIFFERENTIATION AND FUNCTION OF STROMAL PROGENITOR CELLS IN KIDNEY ORGANOIDS

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The adult kidney is a complex organ consists of more than 20 different types of cells, but it develops from a rather simple structure, i.e., metanephros, consists of three types of progenitor cells: ureteric bud (UB), cap mesenchymal (CM), and stromal (SM) cells. Recent studies have reported that aggregates made of dispersed cells from mouse metanephroi, or hiPSC-derived CM and UB cells, can self-organize and differentiate to create nephron-like structures. These kidney organoids can thus serve as a model system for disease modeling and drug screening. However, most of these studies have focused mainly on CM and UB cells without detailed insights on how SM progenitor cells differentiate and function in kidney organoids.Here we present a serum free defined culture system suitable for studying the differentiation and function of SM progenitor cells in kidney organoids. For this purpose, cells derived from E13.5 Foxd1-EGFP mouse metanephroi were used to prepare either the conventional aggregates that contain SM progenitor cells (AG+SM) or aggregates devoid of SM progenitor cells (AG-SM), where Foxd1-EGFP+ SM progenitor cells were excluded by FACS sorting. Aggregates were cultured on membranes placed at air-liquid interface for up to 7 days. When aggregates were cultured in 10% FBS containing medium, self-organization and differentiation of UB and CM cells were observed in both AG+SM and AG-SM organoids, but no differentiated SM structures emerged in either organoids. In contrast, when AG+SM were cultured in a serum free defined medium, containing a cocktail of inhibitors and growth factors, structures stained positive for differentiated SM markers, such as Renin, Pdgfrb, and alpha smooth muscle Actin, were detected in AG+SM but not in AG-SM. We also noticed prominent growth of CM cells in AG-SM as compared to AG+SM, consistent with the function of SM progenitor cells to induce the differentiation of CM cells. In conclusion, we report here a novel culture system which is suitable for studying the differentiation and function of SM progenitor cells in kidney organoids. This culture system may serve as a useful platform to study the role of SM cells in kidney development, pathology and regeneration.

T-3056

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL TO BRANCHING LUNG BUD ORGANOIDS and MATURATION INTO ALVEOLAR LUNG ORGANOIDS

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We describe a stepwise protocol to efficiently differentiate human iPSCs to branching lung bud organoids and then further matured into alveolar lung organoids. Branching lung bud organoids (LBOs) are formed by day 20 and exhibit airway-like structures with morphological and functional characteristics similar to developing lungs in vivo. LBOs express SFTPB, a pulmonary-associated surfactant protein B that is secreted by alveolar lung cells, pulmonary endoderm markers SOX9, NKX2.1 and EPCAM, the mesenchymal marker VIMENTIN, and MUC5AC, a marker for airway goblet cells. By days 30-40, large branching structures with rounded expansion at the distal tips resembling alveolospheres could be observed. To enable high-throughput (HTP) screens, large numbers of lung organoids would be required. We show that branching lung organoids can be maintained for an extended period and moreover can be cryopreserved. Upon thawing, cryopreserved lung organoids can recover their branching morphologies upon encapsulation in Matrigel and can be further differentiated into spatially organized alveolar lung organoids. The ability to culture and cryopreserve lung bud organoids that retained the ability to differentiate into alveolar lung organoids is a significant step towards widespread adoption of LBOs for disease modeling and HTP screens.

T-3058

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO COLONIC ORGANOIDS AND THEIR APPLICATIONS IN TOXICOLOGY SCREENS

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In recent years, 3D organoids have emerged as a new tool for disease modeling and for pre-clinical toxicity screening applications. Organoids are self-organizing three-dimensional structures derived from either pluripotent stem cells or from primary tissues with the ability to recapitulate some of the spatial architecture and in vivo function of organs. Here, we describe a stepwise protocol in serum-free medium to efficiently differentiate human pluripotent stem cells to colonic organoids. Two human iPS cell lines were used; one derived from PBMCs and the other from human foreskin fibroblasts. Colonic organoids generated from both hiPS lines could be propagated and expanded in long-term culture and expressed the appropriate colonic markers. In comparison to previous 2D in vitro cell culture models, organoids resemble very closely the intestinal epithelial layer in vivo. They can be used in applications including high throughput screenings for agents that target the intestinal epithelium and for the identification of potential toxicity of new compounds. Human iPS derived colon organoids could be cryopreserved and upon thawing were highly viable and could be further expanded and re-developed into 3D colon organoids. Efforts are underway to further characterize the functional attributes of the colon organoids and validate their use in a low to mid-throughput toxicology screen.

TISSUE ENGINEERING

T-3060

POSITIVE EFFECTS OF PRE-COATING DECELLULARIZED LIVERS WITH HEPATIC CELLS CONDITIONED MEDIUM

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Currently, liver transplantation from compatible donors main alternative therapy for patients with irreversible hepatic injuries. Hepatic tissue engineering is an important approach for the development of new organs aiming future human transplantation. Bioengineering technologies have been developed to produce decellularized liver scaffolds which could be recellularized with human cells. However, high quality liver recellularization methods are still under investigation. In order to enhance the recellularization process, the aim of this work was to improve the hepatic matrices recellularization process by pre-coating the extra cellular matrix (ECM) with human hepatic cells-conditioned medium (CM). Furthermore, we are investigating the potential of human liver cells to adhere on several ECM proteins as well as CM components. Livers from male Wistar rats were collected and decellularized by perfusion of 1% Triton-X solution with 0.05% NaOH. The samples were analyzed by histology, immunohistochemistry, scanning electronic microscopy and

DNA-content analysis. Decellularized livers were coated with HepG2-CM, recellularized up to 5 weeks with hepatoblasts, mesenchymal stem cells (MSC), both differentiated from hiPSCs and human aortic endothelial cells (HAEC). iPSCs and differentiated cells were characterized by RT-qPCR, flow citometry and immunofluorescence. The organization and integrity of the hepatic ECM was maintained after decellularization. The recellularization steps were successfully improved on pre-coated liver ECM as compared with non-coated liver ECM. Absence of nuclei and cellular residues were confirmed by optical and electronic microscopy. Residual DNA-content was below the immunogenic limits (10ng/mg; $p< 0.001$). Cell adhesion assay showed high capability of hepatic adhesion on collagen I + CM, fibronectin + CM, and laminin + CM but not only on CM. Thus, recellularization of pre-coated livers-ECM was significantly improved showing the positive effects of the association of liver ECM with CM components.

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T-3062

BMAL1 FUNCTIONALIZED 3D RECONSTRUCTED EPIDERMIS: HUMAN MODELS RECAPITULATING CHARACTERISTICS OF DEREGULATED CIRCADIAN RHYTHM SKIN PRECURSORS

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In mammals, desynchronized circadian rhythm leads to various biological symptoms. In skin and hair follicle, human epidermal stem cells function in vitro is regulated by circadian oscillations, thus contributing to tissue aging when deregulated. The impact of circadian oscillations, through a feedback loop involving Clock pathway on hair and skin stem cells function in vitro is well described in mice. We recently demonstrated that the in vivo deregulation of the Clock pathway affects regenerative properties of human skin and hair precursor cells. The present study aimed at developing functionalized 3D reconstructed skin models mimicking the premature aging features of regenerative defect observed in human Epidermal precursor cells (hEpi) after a long term deregulated circadian rhythm in vivo. Using primary culture of human epidermal keratinocytes two modified primary lines of hEpi were generated: hEpi overexpressing BMAL1 (constitutive overexpression of BMAL1) and hEpi shBMAL1 (knockdown for BMAL1). Cell cultures, measurement of colony area, count of clones and 3D reconstructed skin were carried out using these two lines. Our results demonstrate that BMAL1 overexpression and BMAL1 knockdown affect hEpi keratinocytes clone-forming efficiency as well as the reconstructed epidermis process. In this study, two BMAL1 functionalized 3D reconstructed skin models were developed that recapitulate the observations previously described in human precursor cells that were isolated from donors with a long term in vivo deregulated circadian rhythm. These models will allow i) the Clock pathway deregulation mechanisms leading to an alteration of the skin precursors properties to being better understood and ii) compounds apt at rescuing the regenerative potential of keratinocyte precursors to being screened.

T-3064

B2M AND CIITA DEFICIENT HUMAN EMBRYONIC STEM CELLS LACK HLA CLASS I AND II SURFACE PRESENTATION

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For clinical translation of an allogenic stem cell-derived treatment, matching of HLA molecules or extensive immunosuppression will be essential to avoid graft rejection. In order to minimize this burden, we generated human embryonic stem cells (hESC) lacking human leukocyte antigen (HLA)-I and -II molecules . Since HLA genes are highly polymorphic, we edited B2-microglobulin (B2M) to prevent HLA-I proteins anchoring in the cell membrane, and class II major histocompatibility complex transactivator (CIITA) to hinder activation of HLA-II genes. Using CRISPR/Cas9 technology, we first created an hESC-B2M-knockout (hESC-B2M-KO) line and evaluated pluripotency and HLA-I gene and protein expression in several single-cell clones. Next, to assess the differentiation competence of the edited clones we differentiated them into retinal pigment epithelium (RPE) cells and further characterized them. Results demonstrated loss of B2M on transcriptional and protein levels. Intracellular HLA-I was still detected but the cells failed to present HLA-I on the cell surface. Mature hESC-RPE-B2M-KO also exhibited proper cobblestone morphology as well as upregulation of RPE-related genes, comparable to wildtype cells. We selected one of the hESC-B2M-KO clones to edit CIITA aiming to generate a double-knockout hESC-B2M/CIITA-KO line. HLA-II transcript levels were undetected in hESC-B2M/CIITA-KO , while CIITA expression was comparable to wildtype. Following differentiation of the double-knockout line into RPE cells, we demonstrated that these cells retained cobblestone morphology, pigmentation and expression of RPE-related genes but lacked surface expression of HLA-II proteins. In conclusion, we demonstrate that hESC-B2M/CIITA-KO can be generated

using CRISPR/Cas and that they can be differentiated into clinically relevant cells such as RPE cells. These edited hESCs may provide a valuable cell source for regenerative medicine and cell replacement therapies.

T-3066

MODELLING THE CONGENITAL HEART CONDITION OF TETRALOGY OF FALLOT WITH HUMAN INDUCED PLURIPOTENT STEM CELLS AND ENGINEERED CARDIAC TISSUES

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Congenital heart disease (CHD) is the most common congenital malformation with a prevalence of 8 to 10 per 1000 live births. Tetralogy of Fallot (TOF), as characterized by the presence of ventricular septal defect, pulmonary stenosis, hypertrophic right ventricle and an overriding aorta, is the most common cyanotic CHD. Its pathogenesis is, however, unclear. Indeed, primary defects of TOF are difficult to be discerned from other defects secondary to anatomical changes. Our study aim to employ human induced pluripotent stem cell-derived ventricular cardiomyocytes (hiPSC-vCMs) differentiated from normal individuals and TOF patients together with state-of-the-art cardiac tissue engineering to examine the intrinsic abnormalities of TOF, while single-cell-RNA-sequencing further provides their molecular bases. As a first step, single-cell transcriptomes from TOF and control constructs were compared across different engineered tissue configurations: hiPSCs, hiPSC-vCMs at day 30 after initiation of cardiac differentiation (hiPSC-vCMs), human ventricular cardiac anisotropic sheets (hvCAS) and tissue strips (hvCTS) fabricated from hiPSC-vCMs. Cardiac genes including TNNI3 and TAGLN were downregulated in TOF-hiPSC-vCMs. Comparisons between normal and TOF hvCAS further revealed the pathway involved in myocardial contraction as the top under-represented pathway in the TOF group, with MYH7, MYL7, MYL2, TNNI3 and TPM2 being significantly downregulated. By contrast, the pathway involved in glycolytic process was found to be the top over-represented pathway in the same tissue construct with ALDOA, ENO1, ENO2, GPI, GAPDH, LDHA, PFKP and PGK1 being significantly upregulated. Taken together, our findings provide evidence of possible intrinsic impairment of contractility of cardiomyocytes in TOF independent of the secondary effects of the structural cardiac malformation. Further studies are underway to determine the force generation in hvCTS generated from control- and TOF-hiPSC-vCMs.

T-3068

HARNESSING THE POTENTIAL OF 3D TOPOGRAPHY AND GRAPHENE FOR EFFICIENT DIFFERENTIATION OF MESENCHYMAL STEM CELLS INTO NEURONS AND CARDIOMYOCYTES

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Neurons and cardiomyocytes are terminally differentiated post-mitotic cells with very limited regenerative potential. Hence, injuries or degeneration to these cells might incapacitate normal body functions. The present study aims at developing PCL scaffolds with nano-graphene fillers to differentiate human Mesenchymal Stem Cells (MSCs) into functional neurons and cardiomyocytes. Graphene may provide appropriate microenvironment with topographical and electrical cues for MSCs differentiation owing to its excellent electrical and mechanical properties. Scaffolds with 15% of PCL and 0.01, 0.05 and 0.1 wt% of graphene as filler in the polymer matrices were prepared by electrospinning. For biological studies, sterile scaffolds were seeded with human Mesenchymal Stem Cells. For neuronal differentiation, induction medium was supplemented with EGF, FGF2 and Oxysterol while for cardiac differentiation; Oxytocin was used as an inducer. Physical characterization of the scaffolds showed highly porous mimicking the extracellular matrix of native tissue. Live and dead staining and proliferation studies showed that all scaffolds were biocompatible and support cell attachment and proliferation respectively. Cell differentiation over PCL 15% G0.05 scaffolds revealed better neuronal and cardiac differentiation as evaluated by morphological analysis , alignment and expression of proteins specific to neurons (MAP2 and TH) and cardiomyocytes(MLC and CTnI). This study indicates the role of graphene nanocomposite scaffold in enhanced differentiation of MSCs into DA neurons and cardiomyocytes. Presence of graphene in the scaffolds not only improved MSC adhesion, morphology but also upregulated

the cell membrane calcium ion efflux, thereby indicating better functionality of differentiated cells. Thus, we envisage that low amount of graphene nanofiber based scaffold can serve as a potential graft for developing future therapies for regeneration of degenerated neuronal and cardiac cells. These in vitro studies will further be validated in in vivo animal models.

Funding Source: The work done in this study was generously supported by Department of Biotechnology, India.

T-3070

GENERATION OF LUNG ORGANS FROM MOUSE EMBRYONIC STEM CELLS VIA BLASTOCYST COMPLEMENTATION IN MICE

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Generation of the pancreas and kidney from stem cells via blastocyst complementation has been reported, but to our knowledge, there has been no report regarding lung generation. Fibroblast growth factor 10 (Fgf10) is a gene involved in the development of limbs and lungs in mice. Fgf10-/- mice present phenotypes indicating limb and lung deficiencies. We attempted to generate lung organs from embryonic stem cells via blastocyst complementation in this study. Previously, we developed Fgf10-/- mice by the CRISPR/Cas system. Because Fgf10-/- mice die immediately after birth, Fgf10+/- genotype mice are maintained in Fgf10 Exon1-/+ and Fgf10 Exon3-/+. Four hundred and seven compound heterozygous mutant embryos (Exon1-/+ × Exon3 -/+) in total were prepared and then enhanced green fluorescent protein (EGFP)-positive murine ES cells were microinjected into 8-cell-stage embryos. Three hundred sixty microinjected embryos were transferred into pseudopregnant mice and 91 littermates (25.3%) were obtained. Fifty-two littermates (57.1%) were EGFP positive (chimeric) and 19 littermates of these died at birth, while 11 littermates of these were able to survive until they have been weaning. Nine out of 91 littermates (9.9%) showed limb defects and died at the time of birth. No EGFP expression were detected in these littermates and genomic analysis using surveyor mutation test and DNA sequence analysis indicated a compound heterozygous genotype (Fgf10 Ex1 -/+ and Fgf10 Ex3 -/+). Other 20 out of 91 littermates (22%) showed ventral hernias and/or opened eyes with EGFP expression and a wild genotype of Fgf10. Two out of 11 weaned chimeric mice were indicated deriving from compound heterozygous embryo (Fgf10 Ex1 -/+ and Fgf10 Ex3 -/+). Histological analysis exposed that the lung tissues of the two weaned compound heterozygous mice were well developed and showing positive expression of the EGFP. This study suggests the possibility of lung organ generation from ES cells via blastocyst complementation in a lung aplastic mouse model.

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T-3072

MULTIPARAMETRIC STUDY OF HUMAN HEPATOTOXICITY ON A NOVEL MICROLIVER DEVICE

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Hepatotoxicity remains one of the biggest reasons for the attrition of candidate drugs during the later stages of drug development. The cell lines and animal models due to species differences provide limited physiological relevant information and have resulted in ‘silent’ hepatotoxic drugs being introduced into clinical trials, garnering huge financial

losses for drug companies through withdrawals and late-stage clinical failures. Therefore, interest in new systems to identify human drug toxicity are high, particularly at preclinical phase. We have developed a novel 'MicroLiver' device that provides bioinspired microfluidic environments to allow spatiotemporal control of various chemical and physical culture conditions that are unavailable with other methods, which in turn allows for manufacturing of more physiologically relevant drug screening platforms. Our microengineering approach using microfluidic technology is designed to simulate liver structure and function. The 'MicroLiver' system is integrated with Hepatocyte-like cells (HLCs) derived from patient-specific, and genomically diverse human induced pluripotent stem cells (iPSCs) and are a promising alternative to animal studies to enable greater precision, safer and personalised targeting for the drug development process. Human somatic cells reprogrammed into iPSCs utilising the viral-free delivery system to introduce (Yamanaka) reprogramming factors (OCT3/4, KLF4, SOX2, MYC). The iPSCs were characterised for pluripotency and then differentiation into HLCs for 18 -30 days. The HLCs evaluated through assessment of cytochrome (CYP) P450 (CYP1A2, CYP3A4 and CYP2D6) activity and the expression of liver-specific genes were integrated onto the microfluidic devices, and the toxicity of selected chemicals evaluated. ATP assays assessed the cell viability and proliferation after drug exposure. The 'MicroLiver' system to study hepatotoxicity allows spatiotemporal control of various chemical and physical culture conditions that are unavailable with other methods. Our approach through the use of functional and stable patient-specific iPSC-derived HLCs counteracts the disadvantages associated with utilising primary hepatocytes, cell lines and animal models while reducing animal research and improving drug safety.

Funding Source: The study is co-funded by Innovate UK

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

T-3076

INFORMED CONSENT FOR IMPLANTATION OF HUMAN GENOME-EDITED EMBRYOS INTENDED TO RESULT IN LIVE BIRTHS

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We now know that the consent form and actual consent process that were employed in Dr. He Jiankui's infamous and secret "CRISPR baby" clinical trial were misleading at best and deceptive at worst. The language was over-technical, benefits were exaggerated, stated risks were underplayed, the most serious risks were omitted, and responsibility for untoward effects was shifted to the vulnerable population of research subjects recruited to the clinical trial. In that off-target editing did occur, the effects on the children are unknown, and monitoring into the future is unclear. While it is arguable whether implantation of genome-edited human embryos should not be done at all, because it has already been attempted, it is realistic to expect that it will happen again. Thus, in this poster we present consent form language that would meet the regulatory requirements for informed consent, while sensitively and accurately explaining the benefits and all of the risks of human embryo genome-editing to would-be recipients of these embryos, and to the offspring themselves. Additionally, we discuss potential appropriate parent populations to enroll in these studies, and factors to consider to avoid coercion in recruitment. Because informed consent is a process, we present points to consider for who should and who should not administer informed consent, how the consent process might be conducted, and how long-term participant oversight and protection should be ensured before, during, and following the birth of any offspring for this very sensitive research.

T-3078

AN INTERACTIVE LEARNING APPROACH TO ADVANCE STEM CELL SCIENCE EDUCATION IN THE MIDDLE AND HIGH SCHOOL CLASSROOM: A NOVEL STUDENT-CENTERED TEACHING MODEL

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The California Institute for Regenerative Medicine (CIRM)-funded program: Bridges to Stem Cell Research at Pasadena City College was designed to include an outreach component to advance stem cell education and awareness amongst diverse student populations at local participating high schools. While initial outreach efforts included high school visits by CIRM Bridges interns to discuss stem cell-related projects and a follow-up Stem Cell Training Workshop at Pasadena City College (PCC), the workshop limited the number of students that could participate. To extend outreach activities to the classrooms of participating teachers and allow more students to learn about stem cells in an interactive way, a portable Stem Cell Demonstration Laboratory for high school students and accompanying teaching module were developed. The Stem Cell Demonstration Laboratory includes a mini laminar flow hood, a small CO2 incubator, and a microscope with imaging capabilities which allows the students to perform many of the techniques offered in the workshop in their classrooms. The Stem Cell Demonstration Laboratory and module have been offered since 2012 and has reached out to over 734 high school sophomores, juniors, and seniors. Within the last two years, the Stem Cell Demonstration Laboratory for high school students was extended to the middle school classroom, reaching over 278 seventh grade students. The teaching module has recently been developed into a short course to include lectures and facilitated group discussions on stem cell science and regenerative medicine, as well as complementary laboratory activities.

The preliminary descriptive data suggests that the Stem Cell Demonstration Laboratory and its complementary teaching module may be an appropriate student-centered teaching model for the middle school science classrooms as well as high school biology or biotechnology classes. In addition, providing hands-on stem cell training activities directly in the classroom may motivate students to eventually pursue stem cell research, biological sciences or related disciplines at the post-secondary level to build the STEM pipeline and increase diversity in STEM-fields to mirror that of California.

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

T-3082

CLINICAL-GRADE HLA HOMOZYGOUS IPS CELL STOCK AT CIRA

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Human induced pluripotent stem cell (iPSC) repositories from human leukocyte antigen (HLA) haplotype homozygous donors have been called iPSC haplobank. The haplobank is aimed for minimizing the influence of immune rejection and considered important to a future clinical strategy for the allogeneic cell transplantation. Since 2013, we have built an iPSC stock which is a clinical-grade HLA homozygous iPSC haplobank optimized for Japanese population and provided the stock to research institutions and companies for developing iPSC-based regenerative medicine. Our iPSC stock is generated from peripheral blood mononuclear cells and umbilical cord blood cells. For the healthy HLA homozygous donor recruiting, we are collaborating with Japanese Red Cross Society, the Japan Marrow Donor Program and several cord blood banks which have already performed HLA typing for huge numbers of people. As of January 2019, we have already established five donor-derived 21 iPSC lines with three types of HLA haplotypes, and provided to 13 institutions and 12 projects. The three haplotypes cover approximately 32% of the Japanese population in terms of HLA matching. In the manufacturing of our iPSC stock, we have developed a feeder-free and xeno-free culture system for complying with the regulatory requirements. In order to assuring the quality, a comprehensive quality testing is performed, including microbiological sterility, morphology, vector clearance, viability, marker expression and genomic analysis. Here, we share our efforts and experience of establishing clinical-grade iPSC stock.

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T-3084

IMMUNOMODULATORY EXOSOMES DERIVED FROM HUMAN BONE MARROW MESENCHYMAL STEM CELLS FOR TREATMENT OF ACUTE RESPIRATORY DISTRESS SYNDROME

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Acute respiratory distress syndrome (ARDS) is an inflammatory disease that leads to impaired gas exchange and respiratory failure. Despite decades of research, there is no specific therapy for this condition and mesenchymal stem cells (MSCs) are currently in clinical trials for ARDS. The protective effects of MSCs appear to be largely mediated by paracrine mechanisms, which includes release of small extracellular vesicles, such as exosomes. This pilot study aims to provide an estimate of therapeutic efficacy for MSC-derived exosomes (MEX) in a lipopolysaccharide (LPS) induced mouse model of acute lung injury (ALI) to be used in the design of a definitive preclinical study. Human bone marrow-derived MSCs were cultured with serum-free media and MEX were isolated by ultracentrifugation. MEX displayed a mean size of 72nm using nanoparticle tracking analysis, cup-shaped morphology by electron microscopy, and enrichment of surface markers CD63 and CD81 by Western Blot analysis. In an in vitro wound healing assay, MEX markedly increased the rate of endothelial cell migration compared to fibroblast exosomes as a control, at 12h and 24h ($P<0.05$), which was dose-dependent. An ALI time-course study was conducted to determine temporal changes in local inflammatory response. Total cell count and IL-1beta in BALF peaked at 24h post-LPS, whereas IL-6 was highest 10h post-LPS. In a separate study, ALI mice (N=4-9 per group) were randomized to treatment with saline, MSCs, MSC conditioned media, or MEX given 30 mins post-LPS. Bronchoalveolar lavage fluid (BALF) and plasma were collected 72h later for analysis of inflammatory cytokines and total cell count. In ALI mice, MSC conditioned media significantly attenuated the increase in IL-1beta levels in BALF, whereas MSCs and MSC-exosomes resulted in a trend towards decreased levels of IL-1b and total cell count. This preliminary study suggests that MSC-derived exosomes can improve endothelial cell migration in vitro and potentially reduce the inflammatory response to ALI in vivo. Power calculations based on this data will determine the sample size necessary

for a definitive preclinical study. Based on findings from the time-course study, delivery of MSC-exosomes at a time of elevated inflammation (10h post-LPS) may augment lung tissue accumulation of MEX in future experiments.

T-3086

SAFETY AND EFFICACY OF HUMAN EMBRYONIC STEM CELLS DERIVED ASTROCYTES FOLLOWING INTRATHECAL TRANSPLANTATION IN SOD1G93A AND NSG ANIMAL MODELS

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ALS is a Motor Neuron (MN) disease characterized by the loss of MNs in the central nervous systems. As MNs die, patients progressively lose their ability to control voluntary movements, become paralyzed and eventually die from respiratory/deglutition failure. Despite the selective MN death in ALS, there is growing evidence that malfunctional astrocyte play a crucial role in disease progression. Thus, transplantation of healthy astrocytes may compensate for the diseased astrocytes. we developed a GMP-grade protocol for generation of astrocytes from human embryonic stem cells (hESC). The first stage of our protocol is derivation of astrocyte progenitor cells (APC) from hESCs. These APC can be expanded in large quantities and stored frozen as cell banks. Further differentiation of the APC yields an enriched population of astrocytes with more than 90% GFAP expression (AstroRx). In vitro, these cells possess the activities of functional healthy astrocytes, including glutamate uptake, promotion of axon outgrowth and protection of MNs from oxidative stress. A secretome analysis shows that AstroRx cells secrete also several inhibitors of metalloproteases as well as variety of neuroprotective factors (e.g. TIMP-1&2, OPN, MIF and Midkine). In ALS animal models, hSOD1G93A high-copy number transgenic mice and rats, intrathecal injections of the AstroRx significantly delayed disease onset and improved motor performance compared to sham-injected animals. A nine-month safety study conducted in immunedeficient NSG animal model under GLP conditions showed that intrathecal transplantation of AstroRx to the cerebrospinal fluid is safe. Transplanted AstroRx attached to the meninges along the neuroaxis and survived for the entire duration of the study without formation of tumors or teratomas. Cell-injected mice gained similar body weight as the sham injected group and did not exhibit clinical signs that could be related to the treatment. No differences from the vehicle control was observed in hematological parameters or blood chemistry. These findings demonstrate the feasibility, safety and potential efficacy of intrathecal injections of AstroRx for the treatment of ALS and allowed the initiation of the first in human phase I/IIa clinical trial in ALS patients (clinicaltrials.gov identifier: NCT03482050).

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GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-3088

ACCURATE ANNOTATION OF ACCESSIBLE CHROMATIN IN MOUSE AND HUMAN PRIMORDIAL GERM CELLS

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Extensive and accurate chromatin remodeling is essential during primordial germ cell (PGC) development for the perpetuation of genetic information across generations. Here, we report that distal cis-regulatory elements (CREs) marked by DNase I-hypersensitive sites (DHSs) show temporally restricted activities during mouse and human PGC development. Using

DHS maps as proxy, we accurately locate the genome-wide binding sites of pluripotency transcription factors in mouse PGCs. Unexpectedly, we found that mouse female meiotic recombination hotspots can be captured by DHSs, and for the first time, we identified 12,211 recombination hotspots in mouse female PGCs. In contrast to that of meiotic female PGCs, the chromatin of mitotic-arrested male PGCs is permissive through nuclear transcription factor Y (NFY) binding in the distal regulatory regions. Furthermore, we examined the evolutionary pressure on PGC CREs, and comparative genomic analysis revealed that mouse and human PGC CREs are evolutionarily conserved and show strong conservation across the vertebrate tree outside the mammals. Therefore, our results reveal unique, temporally accessible chromatin configurations during mouse and human PGC development.

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T-3090

HYPERBARIC OXYGEN EXPOSURE INCREASED CDX2 AND DECREASED OCT4 EXPRESSION IN MOUSE BLASTOCYSTS VIA UPREGULATING NRF2-NOTCH1 AND DOWNREGULATING NF2-HIPPO SIGNALING

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It has been reported that exposure of pregnant female rats or hamsters in the early-to-mid stages of gestation to 100% oxygen under 3–4 atm for over 90 min/day led to reduced rat fetal weight and increased placental weight as well as malformed hamster fetuses. To further elucidate the etiology and molecular mechanisms underlying the hyperbaric oxygen (HBO)-induced maldevelopment of the placenta and embryo, we conducted HBO exposure to female mice in the preimplantation stages of pregnancy. C57BL/6 female mice post coitum were exposed to 100% O2 under 3.0 atm for 90 min/day for totally four days, followed by removal of the uterus and collection of E4.0 blastocysts. In comparison with the blastocysts derived from normoxia-exposed female mice, immunofluorescence analyses in the blastocysts derived from HBO-exposed mothers revealed significantly increased CellROX® oxidative stress signals and nuclear Nrf2 staining whereas significantly decreased membranous Nf2 staining throughout the whole embryo, while significantly decreased Oct4 expression, increased nuclear NF-κB and active caspase-3 signals as well as ectopic nuclear staining of Cdx2 and Yap and the Notch1 intracellular domain (N1ICD) were detected in the inner cell mass (ICM) of the HBO-exposed blastocysts. Interestingly, we found that microinjection of Nf2 overexpressor cDNA into the zygotes followed by oviduct transfer before HBO exposure significantly decreased the nuclear levels of Yap, NF-κB and active caspase-3 without affecting ectopic Cdx2 and decreased Oct4 expression in the ICM of the HBO-exposed blastocysts. On the other hand, microinjection of Nrf2 siRNA into the zygotes significantly decreased ectopic Cdx2 and N1ICD expression without increasing Oct4 expression or decreasing Yap, NF-κB and active caspase-3 signals in the ICM of the HBO-exposed blastocysts. Only co-microinjection of both Nf2 cDNA and Nrf2 siRNA into the zygotes was capable of preventing aberrant expression of Cdx2, Yap, N1ICD, NF-κB and Oct4 in the blastocysts following HBO exposure. Therefore, our study demonstrates for the first time that the Nrf2-Notch1 and Nf2-Hippo signaling pathways synergistically regulate normal expression of Cdx2 in the trophectoderm and Oct4 in the ICM at the blastocyst stage.

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T-3092

SELF-ORGANIZATION OF THE IN VITRO ATTACHED NON-HUMAN PRIMATE EMBRYO

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Implantation of the blastocyst is a developmental milestone in mammalian embryonic development. Before implantation of the embryos into the uterus, the mammalian embryo establishes three founding populations of cells, epiblast, hypoblast and trophoblast. The epiblast gives rise to the fetus, while hypoblast and trophoblast form vital extraembryonic tissues, such as the placenta. Furthermore, embryonic stem cells (ESCs), extraembryonic endoderm (XEN) and trophoblast stem cells (TSCs) have been successfully derived from rodent epiblast, hypoblast and trophoblast and these stem cells have been good models to understand embryonic development in mouse. However, human and non-human primate early development radically diverges from the rodent paradigm. Although, gene expression analysis revealed that primates use a variety of different factors to regulate early embryonic development, ESCs, XEN and TSCs that captured biologic features completely of epiblast, hypoblast and trophoblast have not been established in primate. Therefore molecular mechanisms of early primate development are poorly understood. Recently established

in vitro implantation platforms in the human have expanded the knowledge of post-implantation development however, human embryos cannot extensively use because of ethical reasons. Common marmoset (Callithrix jacchus, marmoset) is an excellent model to study primate embryogenesis, because they allow access to naturally conceived embryos. In this study, we established in vitro post implantation culture in marmoset embryos. As the result, all embryos successfully attached to in vitro implantation platform. In these embryos, 4 out of 10 (40%) embryos successfully developed and their morphologies were similar to human Carnegie stage 5 embryos which is consist of inner cell mass, bilaminar embryo, trophoblast and hypoblast. These embryos expressed Oct4 in the epiblast and Gata6 in the hypoblast. This in vitro post implantation embryo culture system in marmoset would provide unprecedented insights into human development, with far-reaching implications for stem cell research, placental research and treatments for implantation failure.

T-3094

CHEMICAL REVERSION OF HUMAN PLURIPOTENT STEM CELLS TO A NAÏVE EPIGENOMIC STATE WITH HIGH FUNCTIONAL PLURIPOTENCY REDUCES THE DEVELOPMENTAL BARRIERS TO HUMAN-MURINE CHIMERISM

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Naïve human pluripotent stem cells (N-hPSC) may allow development of human tissues in interspecific chimeras for human disease modeling. However, human chimerism following injection of N-hPSC into animal embryos has thus far proven inefficient, ephemeral, and restricted to early fetal stages. Efficient human chimerism faces barriers that minimally includes: discordant interspecific developmental kinetics, N-hPSC methods producing unstable epigenomes, and inefficient interspecific cell-cell adhesion. Herein, we evaluated these barrier variables utilizing N-hPSC derived via supplementing LIF-2i with the tankyrase inhibitor XAV939. N-hPSC upregulated protein expressions (CD77, NANOG, KLF2, DNMT3L), and signalling pathways (pSTAT3) of the human preimplantation epiblast. N-hPSC transcriptional signatures clustered with E6 human epiblast, and global CpG methylation decreased by ~50%. N-hPSC maintained normal karyotypes, and PD0325901 inhibition of pERK1/2 did not destabilize DNMT1 or CpG methylation at imprinted loci. Improved multilineage differentiation correlated with reduced lineage-primed gene expression, CpG demethylation of PRC2 targets, JARID2 downregulation, diminished H3K27me3 bivalency, and global H3K9 methylation loss. To test interspecific chimerism potency, we injected single N-hPSC cells expressing GFP/puromycin transgenes into murine blastocysts. Robust human cell contribution was confirmed in >10% of E9.5-E11.5 fetuses using fluorescent reporters (GFP), human-specific antigens (HNA, STEM121), PCR amplification of human mitochondrial/GAPDH sequences, and expansion of puromycin-resistant human cell lineages. Human chimerism was ~25% at E7.5, 1% at E9.5, and lost by E12.5. Chemical blastocyst complementation dramatically improved human cell contribution at E9.5, but disrupted normal morphogenesis. N-hPSC expressing murine-E-cadherin and injected into morulae robustly contributed human cells to CDX2+ trophectoderm of hatching blastocysts and E11.5 murine embryos and placenta, but not thereafter. These data demonstrate that XAV939-regulated N-hPSC with naïve epigenomes and high functional pluripotency efficiently contribute to early fetal murine organogenesis; unknown barriers may impair further human chimerism at later murine fetal stages.

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CHROMATIN AND EPIGENETICS

T-3096

XIST MEDIATES BOTH X-CHROMOSOME DAMPENING AND X- CHROMOSOME INACTIVATION IN HUMAN DEVELOPMENT

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Female placental mammals silence one of the two X-chromosomes through X-chromosome inactivation (XCI). This essential process is mediated by the lncRNA XIST that spreads along the X to mediate gene silencing. During female human pre-implantation development, a unique mechanism for dosage compensation takes place, where instead of silencing of one of the two X-chromosomes as in XCI, gene expression is reduced from both X-chromosomes in a process called X-chromosome dampening (XCD). Thus, X-linked gene dosage in humans is regulated first by XCD and upon implantation by XCI. Moreover, XIST accumulates on both dampened X chromosomes at this stage, but unable to induce complete gene silencing. Thus, X-linked gene dosage in humans is regulated first by XCD and upon implantation by XCI. To understand the unique mechanism of dosage compensation that takes place during human pre-implantation development, we are addressing the fundamental questions of how XCD is achieved mechanistically and why XIST is not inducing silencing during XCD. To study these questions, we are taking advantage of naïve human embryonic stem cells (hESCs) and somatic cells, which capture the XCD and XCI state, respectively. To this end, we have explored XIST localization in female naïve hESCs and somatic cells and discovered that XIST spreads over the entire X, but displays striking differences in enrichment along the X between XCD and XCI, which are explained by differences in the 3D folding of the X. We also made the remarkable observation that XIST spreads to autosomal regions in naïve hESCs but not in somatic cells, consistent with XIST dispersal observed by imaging. Importantly, in naïve hESCs and blastocysts, X-linked and autosomal genes enriched for XIST are downregulated, demonstrating that XIST mediates XCD as well as autosomal repression. Additional data demonstrate that XIST fulfills its function in XCD and autosomal repression through SPEN, similar to its mechanism of action in XCI. We propose that XIST concentration is lower within the dampened X-chromosome compared to the inactive X, which prevents complete gene silencing in naïve hESCs. Taken together, our data show that XIST mediates both XCD and XCI, uncover an unprecedented function of XIST in autosomes, and define new principles that govern the regulation of gene expression by lncRNAs.

T-3098

CRISPR-CAS9 BASED SCREENINGS TO INTERROGATE THE MOLECULAR BASIS OF DEVELOPMENTAL COMPETENCE IN ESC LINEAGE SPECIFICATION

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A central question in developmental biology is to understand how cell-lineage intermediates interpret and execute inductive cues from their environment during embryo differentiation. It is believed cell-intermediates acquire two types of competences at defined developmental windows, a signaling competence to decode inductive signals from their environment and an epigenetic competence to undertake the chromatin modifications required to promote gene expression. However, the molecular mechanisms that sustain epigenetic competence are poorly understood. Previous approaches to discover chromatin regulatory regions (CRR) are mostly based on descriptive techniques that led to inferred correlations but are not well suited to decipher causal relationships between chromatin modifications and gene expression. In contrast, the CRISPR system has made possible to unbiasedly discover and functionally interrogate CRR. In this study, we used the specification axis from human ESC to the pancreatic lineage as a proof of principle to study how CRR are established through development. We combined the analysis of chromatin conformation, histone modification and transcription factor (TF) binding dynamics with CRISPR genomic and epigenomic screenings to identify and characterize the CRR with the potential to regulate PDX1 expression. Using a PDX1 reporter line and a tiled gRNA library targeting 100kb around PDX1, we interrogated the presence of CRR using three CRISPR-based screenings. Cas9 and dCas9-KRAB screenings identified a set of CRR necessary to activate PDX1 expression along pancreatic differentiation, discovering some previously unannotated regions both upstream and downstream the PDX1 gene body. In parallel, we performed a dCas9-VP64-based screening, as well as functional interrogation assays to uncover the CRR that are sufficient to promote PDX1 expression at different developmental stages. The combined analysis of TF binding and chromatin features revealed the molecular determinants that characterize the potential of each CRR to promote PDX1 expression along differentiation. Collectively, we will establish an unbiased approach to dissect the dynamics of CRR along development and determine the basis to discover the molecular mechanisms that support epigenetic competence.

T-3100

H3 K-TO-M MUTATIONS REVEAL CRUCIAL ROLES FOR H3K36 AND H3K9 METHYLATION IN DIFFERENTIATION, BOTH IN VITRO AND IN VIVO

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Development and differentiation are associated with profound changes to histone modifications. However, the direct biological consequences of individual modifications remain incompletely understood due to the paucity of selective tools. Here, we describe a transgenic strategy to globally but specifically suppress the methylation of target histone residues in pluripotent stem cells and mice. Our system takes advantage of lysine (K) to methionine (M) mutants of histone H3, which function as dominant negative inhibitors of methylation at their respective sites. When expressed in embryonic stem cells, these mutants induced widespread changes to the chromatin landscape and a corresponding block in differentiation. Upon induction of H3K9M or H3K36M in adult mice, we observed potent differentiation defects in a variety of stem/progenitor cells and regenerative tissues, including intestine, testes, and blood-related organs. Focusing on hematopoiesis, H3K36M expression led to severe anemia and rapid lethality due to a block in erythropoiesis. H3K36M mice also exhibited aberrant lymphoid, megakaryocyte, and hematopoietic stem cell defects. By contrast, mice expressing H3K9M survived long term and exhibited distinct hematopoietic phenotypes including an expansion of multipotent progenitors. H3K36M and H3K9M induction led to genome-wide reduction of H3K36 and H3K9 trimethylation patterns, respectively. We observed corresponding changes in chromatin accessibility and gene expression landscapes, providing a mechanistic explanation for the observed phenotypes. Strikingly, we find that discontinuation of mutant histone expression largely restores differentiation programs, suggesting that the effects of methylation loss are reversible. Collectively, our work provides direct evidence that individual chromatin modifications are required at multiple stages of differentiation and offers powerful tools to interrogate the physiological consequences of these modifications in vivo.

T-3102

REGULATION OF 3D GENOME REORGANIZATION DURING CELL FATE TRANSITION

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3D genome reorganizes when normal cells switch fate, respond to stimuli, undergo senescence etc. Yet, real time visualization of genome reorganization has remained a challenge. We used Neural Crest Cell (NCC) to Ectomesenchymal Cell (EMC) transition as a model to study the genome organization changes that happen during differentiation. When induced to differentiate rapidly proliferating NCCs transiently exit the cell cycle and homogenously differentiate to ectomesenchymal cells over a period of 96hr. After 96hr of differentiation the genome of NCCs gets organized into DAPI dense and less dense regions and H3K9Me3 foci begin to appear. Nuclei of NCCs undergo a 3-5 times volume expansion within the first 20hr of differentiation during which massive chromatin redistribution occurs, followed by formation of multi lobed nuclei which are rapidly resolved in the final stages of the transition to reform the spherical nucleus. This massive chromatin redistribution occurs without an increase in transposon accessible DNA sites. Insitu HiC analysis has revealed the progressive nature of reorganization. We have also identified CHD7, a chromatin remodeling protein to be necessary for this large scale genomic restructuring. Knockdown experiments with shRNA where we can get 75% knockdown of CHD7 show that the knockdown completely inhibits the nuclear expansion and the subsequent ectomesenchymal transition. NCCs derived from CHARGE patient cells with a haploinsufficieny for CHD7 show a similar defect in the nuclear expansion stage followed by inability to upregulate key mesenchymal genes as shown by RNAseq analysis of the patient cells compared to the mutation corrected cells. We for the first time, have identified a cellular transition during normal development where 3D genome reorganization occurs and have also discovered that a chromatin remodeler is necessary for this 3D genome reorganization.

PLURIPOTENCY

T-3104

HIGH-DENSITY DYNAMIC SUSPENSION CULTURE SYSTEM OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN MINIATURIZED MULTICOMPARTMENT DIALYSIS DEVICE

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Human induced pluripotent stem cells (hiPSCs) is a cell type which has decent self-renewal and exhibit capability to regenerate a broad type of organ. In order to realize their clinical and industrial application, a large number of cells were required. The three-dimensional dynamic suspension culture of the hiPSCs spheroid is a promising method for improving their expansion. However, some problem such as the requirement of expensive growth factor usage and difficulties to preserve the pluripotency has still remained difficult. In this study, we would like to improve and evaluate the advantage of high density (HD) hiPSCs culture using our proposed miniaturized dialysis device. To maintain the small molecule nutrient and waste product exchange, the HD hiPSCs expansion was performed in miniaturized multicompartment culture system separated by 12 kDa MWCO dialysis membrane. The gellan gum was also administrated in culture medium to reduce the shear-induced effect caused by rotational culture. The results showed that the increase of hiPSCs expansion efficiency was affected by the accumulation of their native secreted macromolecular substance in well-maintained culture environment. Several important growth factors such as bFGF, TGFβ-1, and Nodal were successfully recycled and accumulated in culture compartment during the expansion which achieved in minimum growth factor supplementation. As a result, the hiPSCs can be expanded at a very high density up to 32 millons cells/ml with 8 times efficiency in well-preserved pluripotency indicated by the higher gene expression level of OCT4, SOX2, and Nanog in comparison with conventional suspension culture system. Resulted hiPSCs also showing better differentiation potential into organoid-like spheroid consisted cell type from three germ layer confirmed by higher expression of trilineage differentiation marker. This novel miniaturized medium refinement system represent the potential utilization of the native cellular interaction in high-density suspension culture to enhance the cost-effective production of hiPSCs for various application.

T-3106

IDENTIFICATION AND CHARACTERIZATION OF SURFACE ANTIGENS RECOGNIZED BY NAÏVE HPSC-SPECIFIC MONOCLONAL ANTIBODIES

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Pluripotent stem cells exist in two distinct states in mammals: (1) naïve pluripotency that represents several molecular characteristics in pre-implantation epiblast and (2) primed pluripotency that corresponds to cells poised for differentiation in post-implantation epiblast. The different pluripotent states in hPSCs can be interconvertible by growth conditions with 2iL (PD0325901, CHIR99021 and LIF) and various chemicals for inhibitors of signaling pathways. However, the conversion conditions for naïve hPSCs are still controversial. Therefore, the generation of human naive pluripotency in vitro requires specific culture conditions and signalling pathways that differ from naive mESCs. To identify and characterize the molecules that are critical for the maintenance of naïve pluripotency of hPSCs, we generated a panel of murine monoclonal antibodies (MAbs) specific to the naïve H9 hPSCs cultured in (1) 2iL-F/A (2iL, Forskolin and Ascorbic acid), (2) 2iL-X/F/P (2iL, XAV939, Purmorphamine) or (3) LCDM (hLIF, CHIR99021, (S)-(+)-Dimethundene maleate, Minocycline hydrochloride, IWR-1-endo, Y-27632) medium by decoy immunization. We finally selected 56 surface or 107 intracelluar MAbs which bind to the naïve H9 cells, but only weakly or not at all to primed H9 and MEFs by flow cytometry analysis. To identify the cell surface antigens of the MAbs, biotinylated naïve H9 or various cancer cells were subjected to immunoprecipitation. Of these, MAb N1-A4, N15-F8, N16-F2(c1/c2), N25-G12, N32-B6, N38-B2 and N49-A7 immunoprecipitated approximately 60, 30, 45, 63, 50, 80 or 80KDa, respectively. LC-MS/MS identified that N1-A4 recognized heat shock protein 60 (HSP60). HSP60 is identified in the natural environment of undifferentiated hPSCs, namely, the blastocoel fluid, which is in contact with all the cells in the blastocyst. HSPs containing HSP60 are also essential for successful preimplantation development. N16-F2(c1/c2) recognized membrane associated carbonic anhydrase 14 (CA14) which is a zinc-binding metalloproteinase that catalyzes reversible hydration of carbon dioxide. The molecular function of HSP60 or CA14 in naïve hPSCs and the identification and characterization of the target antigens of other naïve hPSC-specific MAbs is under investigation.

T-3108

PRECISE LEVELS OF SNF5 ARE REQUIRED FOR HUMAN PLURIPOTENT STEM CELLS DEVELOPMENTAL FATE REGULATION

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SNF5 is one of the core subunits of the SWI/SNF chromatin-remodeling complex. Therefore, loss of function (LOF) or gain of function (GOF) of this protein might have significant effects on the epigenetic state of the cells and on their phenotype. Indeed, SNF5 LOF is the solely genetic lesion in Rhabdoid tumor of the kidney and in AT/RT tumor in the brain. SNF5 is also required for early embryonic development as it was shown that SNF5-/- mouse blastocysts lose their hatching capacity and that SNF5 regulates the expression of Nanog during the differentiation of mouse embryonic stem cells. Here we aimed to study the effect of SNF5 LOF and SNF5 GOF on human pluripotent stem cells (hPSCs). For this purpose, we introduced a conditional SNF5 over-expressing cassette into the AAVS1 locus and targeted the endogenous SNF5 gene. Using this system, we show that both SNF5 GOF and SNF5 LOF lead to rapid changes in cell fate, however in a different manner. SNF5 GOF leads to down-regulation of pluripotency markers such as Oct4 and to spontaneous differentiation of the cells mainly into neuronal lineage. By contrast, SNF5 LOF significantly affects the morphology of the cell colonies, which start to grow as 3D structures, but does not appear to directly affect the expression of pluripotency markers. These morphological changes suggest that SNF5 LOF affects the interactions between the cells and the extra cellular matrix. Indeed, global gene expression analysis reveals significant down-regulation in pathways related to the extra cellular matrix upon SNF5 LOF. To further study the effect of SNF5 LOF on the pluripotency of the cells, we evaluated their in-vitro differentiation capacity toward embryonic bodies (EBs). Notably, the LOF cells show compromised capability to differentiate into EBs compared to normal cells. Taken together, our results reveal the crucial role of SNF5 in human pluripotent stem cell biology and show that SNF5 has to be strictly regulated to maintain the self-renewal and differentiation capacity of the cells.

T-3110

TRANSPOSABLE ELEMENTS ARE REGULATED BY CONTEXT- SPECIFIC PATTERNS OF CHROMATIN MARKS IN MOUSE EMBRYONIC STEM CELLS

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The majority of mammalian genomes are devoted to transposable elements (TEs). Whilst TEs are increasingly recognized for their important biological functions, they are a potential danger to genomic stability and are carefully regulated by the epigenetic system. However, the full complexity of this regulatory system is not understood. Here, using mouse embryonic stem cells, we show that TEs are suppressed by heterochromatic marks like H3K9me3, and are also labelled by all major types of chromatin modification in complex patterns, including bivalent activatory and repressive marks. We identified 29 epigenetic modifiers that sig- nificantly deregulated at least one type of TE. The loss of Setdb1, Ncor2, Rnf2, Kat5, Prmt5, Uhrf1, and Rrp8 caused widespread changes in TE expression and chromatin accessibility. These effects were context-specific, with different chromatin modifiers regulating the expression and chromatin accessibility of specific subsets of TEs. Our work reveals the complex patterns of epigenetic regulation of TEs.

T-3112

ALIGNING HUMAN NAIVE AND PRIMED STATES OF PLURIPOTENCY WITH HUMAN PRE- AND POST-IMPLANTATION USING SINGLE-CELL TRANSCRIPTIONAL ANALYSIS

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Pluripotent stem cells are categorized into two distinct culture condition states that represent progressing developmental physiologies: naive and primed. While pluripotency itself describes an intrinsic potential for self-renewal and a definitive capacity for differentiation into all germ lineages, the molecular signaling required for maintenance of this property significantly changes during endogenous developmental progression. As pluripotency arises in the developing epiblast cells during pre-implantation blastocyst expansion, it is then maintained exclusively in the post-implantation epiblast lineage. Based on functional in-vivo mouse studies, naïve stem cells represent pre-implantation pluripotency, while primed stem cells represent a

post-implantation stage. These states are characterized by their differences in cell morphology, metabolism, epigenetic signature, transposable element signature, surface protein composition and transcriptional profile. Pluripotent states have been classified based on their resemblance to endogenous cells, although because mouse has been the canonical model for mammalian pre-implantation and post-implantation studies, conserved terminology has yielded classifications in human self-renewing states. Advancements in single-cell transcriptomics have allowed us to compare and contrast transcriptional circuitry between in-vitro pluripotent states and cells picked from human preimplantation stages (day 3-7) and in-post-implantation model (day 10). Our analysis resolves that human naïve hPSCs transcriptionally are more representative of an early peri/post-implantation epiblast cell, than a pre-implantation epiblast cell. E10 epiblast maintains expression of naïve specific transcripts, while also co-expressing several primed specific genes.

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T-3114

GENETIC VARIATION INFLUENCES PLURIPOTENT GROUND STATE STABILITY IN MOUSE EMBRYONIC STEM CELLS THROUGH A HIERARCHY OF MOLECULAR PHENOTYPES

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Mouse embryonic stem cells (mESCs) occupy a ground state where pluripotency-associated transcriptional and epigenetic circuitry are highly active. However, we observed variability in expression of core pluripotency genes in genetically diverse mESCs grown in ground state conditions. To dissect the genetic basis of this variability, we profiled gene expression and chromatin accessibility in 185 mESC lines derived from genetically heterogeneous Diversity Outbred mice, and grown in the absence of ERK1/2 inhibition. We mapped thousands of loci that affect chromatin accessibility (caQTL) and/or transcript abundance (eQTL). We found eleven instances where distant QTL co-localize in clusters or hotspots, suggesting a common regulator. These hotspots include one on Chr. 10 that influences the expression of the rare 2C-like state and another on Chr. 15 that influences the expression of 254 genes including many known pluripotency-related genes. We applied causal mediation analysis and identified Lifr (leukemia inhibitory factor receptor) transcript abundance as the causal intermediate. Moreover, a joint mediation analysis of gene expression with chromatin accessibility yielded a single peak upstream of Lifr containing one SNP. Orthogonal functional assays and CRISPR allele swap experiments verified that this SNP strongly influences Lifr expression and pluripotency. Thus, we detected a causal chain of molecular events: a single SNP modulates regulatory element accessibility, which affects Lifr expression, leading to large-scale transcriptional shifts including known and novel pluripotency-associated genes. These results reveal that genetic variation controls mESC ground state through interacting gene expression networks, and these differences in ground state stability are likely to influence differentiation propensity. It is now widely recognized that genetic variation influences differentiation propensity of human pluripotent stem cell lines. Our study highlights the power of mouse genetic reference populations for dissecting these molecular regulatory networks using relatively small panels of cells.

T-3116

VARIABILITY AND MULTIPOTENCY EVALUATION OF MESENCHYMAL STEM CELLS DERIVED FROM EQUINE MUSCLES BEFORE AND AFTER DRUG LOADING: THE EXAMPLE OF CURCUMIN

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A new therapeutic perspective of stem cells (MSCs) concerns their potential use for drug delivery purposes. However, when considering MSCs as delivery agents, it is important to consider that incorporation of the therapeutic agent may possibly affect their biology and particularly their mesenchymal stem cell properties. A minimally-invasive process to obtain MSCs from muscles of different species has been previously

developed (WO2015091210). These muscle's derived MSCs are easy to sample but their characterization remains more limited than bone-marrow derived MSCs. In our study, we aim to evaluate i) variability of MSCs sampled from different horses and overtime in culture and ii) whether drug loading affects their mesenchymal stem cell properties. For this purpose, we started first with a well-tolerated and widely used medicinal polyphenol, i.e. curcumin whose pharmacokinetic properties are not favourable for use as a drug. The preliminary analyses of the biochemical signature of MSCs in culture by means of Fourier transformed infrared spectroscopic microscopy (FTIR) suggest high degree of similarity among MSCs from 5 different donors, a feature that seems to remain till passage 8. Those cells efficiently uptake a hydrosoluble curcumin salt complexed with cyclodextrin (NDS27; WO2009144220A1). Importantly, we have found that this drug loading does not alter their viability, cellular proliferation or their multipotency characteristics, at least in terms of expression of CD29, CD44, CD73, CD90, CD105 and OCT4 markers as evaluated by means of qRT-PCR. Finally, the immunomodulation potential of the MSCs on T lymphocytes is also not modified according to their proliferation in co-cultures. Those preliminary results encourage further investigations of muscle-derived MSCs as therapeutic delivery agents for various purposes including inflammatory diseases.

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PLURIPOTENT STEM CELL DIFFERENTIATION

T-3120

WHEN DOES EMBRYONIC STEM CELLS BEGIN TO SHOW THYROID HORMONE MARKERS DURING DEVELOPMENT?

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The key role of thyroid hormone (TH) during development is well recognized for its effect on cell lineage determination, tissue and organ maturation, morphogenesis and adult phenotype establishment. On the contrary, very little is know on the embryo sensitivity to thyroid hormone during early differentiation, at blastocyte stage and until implantation. In this study, we investigated the expression level of molecular machinery involved in the TH cellular signalling and determining the intracellular content of active TH (triiodothyronine, T3) in the rat embryonic stem cells at different very early stages, cultured as single cells, clusters and embryonic bodies (EBs), which are formed in 7 days. The different differentiation stages were characterized by the expression of pluripotency and early differentiation markers Oct4, Stella, AFP. While AFP and Stella increases in EBs compared to single cells, Oct4 expression level and immunoreactivity decreases. We then analysed the expression level of the membrane transporters MTC8 and MTC 10, the TH activating enzymes deiodinase 1 and 2, the TH inactivating enzymes deiodinase 3, the nuclear receptors TRα and TRβ by RT-PCR and immunocytochemistry. In the EBs, we observed a strong up-regulation (up-to 9 times) of the TH activating enzyme D1 and D2 compared to single cells, such as the up regulation of the membrane transporters (up-to 4 times) and all the forms of thyroid hormone nuclear receptors (up-to 4 times). This preliminary results indicate that in rat embryonic stem cells the TH molecular machinery appears very early during development, in experimental conditions mimicking the pre-implant phase of blastocytes. The functional significance of this data remain to be establish, but, notably, in conventional culture protocols embryonic stem cells are grown in the presence of serum, that contains thyroid hormones.

T-3122

DEVELOPING A VISUAL MODEL AND GENOMIC MAP FOR CARDIOMYOCYTE DIFFERENTIATION

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The Allen Institute for Cell Science is creating a visual model of cell organization and behavior from pluripotency through cardiomyocyte differentiation using human induced pluripotent stem cells (hiPSCs). We have used gene edited iPSC-derived cardiomyocytes and live imaging to study the organization of the major cellular structures in cardiomyocytes and are coupling live imaging with single cell transcriptomics to identify and follow the cell states and transitions that accompany the differentiation of hiPSCs into cardiomyocytes. Using the WTC hiPSC line and CRISPR/Cas9, we have fluorescently tagged ~40 loci representing key cellular organelles, sub-structures, and signaling molecules. We have also developed a scarless gene editing strategy to endogenously tag transcriptionally silent loci and used this methodology to tag five sarcomeric proteins (troponin I1, alpha actinin 2, myosin light chain 2a, myosin light chain 2v, and titin). Following genetic, cell biological, and stem cell QC, these clonal hiPS cell lines are differentiated and replated onto glass using optimized protocols for live cell imaging, allowing us to study the organization of the endogenously tagged structure within the cardiomyocytes. We are additionally using single cell RNA-seq and RNA FISH to profile the transcriptional heterogeneity at various stages of differentiation. Using SPLiT-seq, we have generated transcriptomic data for 4 timepoints from pluripotency through day 90 of cardiomyocyte differentiation and identified sub-populations of cells based on gene expression profiles. We observed changes in expression of genes encoding for structural proteins, transcription factors, metabolic switches and calcium handling and confirmed these findings with RNA FISH in cardiomyocyte populations. We are also conjoining high-resolution live cell imaging and parallel transcriptomic profiling

using RNA FISH. Here, we present our optimized methods for silent gene editing and cardiomyocyte differentiation, live cell images of various organelles and sarcomeric structure in the edited cardiomyocytes, and transcriptomic analysis and validation using RNA FISH of various cardiomyocyte populations to explore cell states and transitions.

T-3124

NFIA AND NFIB ARE JOINTLY REQUIRED FOR MOUSE NEURAL STEM CELL SELF-RENEWAL AND DIFFERENTIATION

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Understanding postnatal neural stem/progenitor cells (pNSPC) self-renewal and lineage specification is key to future neural stem cell therapies. Here we assess the effects of loss of single or multiple Nfi genes on murine pNSPC self-renewal and differentiation in vitro. We showed previously that germline loss of either Nfia or Nfib reduces astrogenesis in cortex and spinal cord and results in prenatal dysgenesis of the corpus callosum. Conversely, germline loss of Nfix has minor effects on astrogenesis but promotes oligodendrogenesis. To assess their role in pNSPC self-renewal and differentiation in vitro we generated floxed alleles of Nfia, Nfib and Nfix. Mice homozygous for these alleles and carrying R26CreERT2 are viable and pNSPCs were cultured from the subventricular zone (SVZ). pNSPCs were cultured in the presence of EGF and FGF (proliferation (prolif.) conditions) then placed into medium lacking these growth factors (differentiation (diff.) conditions). Transcript levels of markers of self-renewal and differentiation were assessed by qPCR from RNA of cells cultured without (WT) or with 4OHT (NFI-deleted) during both prolif. and diff. conditions. Treatment with 4OHT during prolif. efficiently deleted all floxed alleles with >99% loss of transcripts within 3 days. Single deletion of Nfia or Nfib resulted in no obvious changes in self-renewal or neuronal differentiation but reduced the expression of astrocyte markers upon differentiation, consistent with our previous studies on loss of Nfia or Nfib in vivo. Surprisingly, simultaneous deletion of Nfia and Nfib resulted in a major reduction in self-renewal as seen by reduced PCNA and Nestin expression and the loss of colony forming ability. In addition, increases in the neuroblast marker DCX and oligodendrocyte marker MBP were seen under prolif. conditions. Upon differentiation, there were increases in DCX but relatively reduced expression of astrocyte and oligodendrocyte markers. This loss of self-renewal appears specific for the combined loss of Nfia and Nfib as combined loss of Nfia and Nfix or Nfib and Nfix does not. We are currently assessing the molecular mechanisms that influence these changes in self-renewal and lineage-specification by RNA-seq analysis of prolif. WT and NFI-deleted pNSPCs and quantification of the cell types formed upon differentiation.

Funding Source: NYSTEM

T-3126

ELASTIN-SECRETING VASCULAR SMOOTH MUSCLE CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Elastin (ELN) plays an essential role in providing recoil capacity to elastic tissues, including the skin and blood vessels. Abnormalities in elastin expression and deposition cause vasculopathy throughout the body that contributes to significant morbidity and mortality in infancy and childhood. Even though prescribed blood pressure medications could reduce vascular stiffness in patients with elastin deficiency, such treatment had no effect on vessel remodeling in elastin deficient mice, instead led to functionally decreased vessel diameter and reduced end organ blood flow. Therefore, it would be of benefit to identify drugs that could increase elastin deposition and improve the biomechanical properties of vessels. We have developed induced pluripotent stem cell (iPS) lines from elastin-deficiency patients and control fibroblasts. Currently, we are working to generate iPS-derived vascular smooth muscle cells (iVSMC) from these lines using chemically defined and serum free media. The differentiated cells remain relatively immature and express SMA, SM22a, SMEMb, and calponin, but do not express additional maturity markers like MYH11. As in human and mouse tissues, elastin is expressed by our cell lines for only a brief period, with initial elastin mRNA expression on day 2 and peaking on day 3 of differentiation and disappears by day 5. Single cell RNA-seq study further confirms the transition of TBXT+ mesoderm cells to SM22a+ iVSMC from day 2 to day 3 of differentiation. More than 90% of elastin-expressing cells co-express SM22a at day 3 (ELN+/SM22a+) revealing they are of smooth muscle origin. Gene enrichment analysis demonstrates an increased level of genes involved in signal-recognition particle (SRP)-dependent protein-membrane targeting as well as extracellular matrix generation in ELN+/SM22a+ cells. Elastin protein is detectable in cells by day 5 and in the matrix in robust quantities by day 9. Additionally the protein appears to remain within the cell for a prolonged time, not entering the matrix until other elastic fiber assembly genes such as FBLN4, FBLN5 and LOX are up-regulated. Our study first demonstrated that iPS-derived vascular smooth muscle cells expressed ELN in early phase of differentiation, while ELN deposition machinery was initiated by the presence of other elastic fiber assembly genes.

T-3128

IDENTIFYING SMALL MOLECULE MODULATORS OF OTIC PROGENITOR CELL PROLIFERATION AND DIFFERENTIATION FOR THE TREATMENT OF HEARING LOSS

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Hearing loss is statistically suggested to affect 5% of the global population. This is most often caused by damage to the hair cells of the cochlea. While these cells, re-enter the cell cycle to replace lost hair cells in birds and other animals, in adult mammals, this process is inactive. But gene therapeutic approaches have suggested that this process could be possible in adult mammals. Previous studies established and characterized a LGR5+ progenitor cell population in the cochlea, that have the capacity to undergo proliferation and asymmetric division in a manner that leads to the generation of new support cells and hair cells. This preliminary research has generated a technique to culture otic organoids and primary otospheres that are positive for LGR5, ATOH1, and MYOVII markers. To date, high throughput screening (HTS), for hearing loss therapies are limited, in part, by the difficulties of isolating and purifying these cell types of interest. We have observed that specific otic progenitor markers can be expressed from hiPSC cultures in a dish. We aim to establish a high-throughput phenotypic-based screening in small molecules, that modulate LGR5+ progenitor cells for hair cell regeneration. First, we used design-of-experiment based optimization to establish a system for generating hiPSC-derived LGR5+ progenitor cells into hair cells in vitro. We then designed an assay to identify compounds which selectively proliferate and differentiate LGR5+ progenitor cell populations. The objective of this research is to identify small molecule drug candidates that will mitigate damaged-induced hearing loss in humans.

Funding Source: California Institute for Regenerative Medicine (CIRM), The Scripps Research Institute

T-3130

EARLY PREDICTION OF THE DIFFERENTIATION TENDENCY CONTRIBUTES TO GENERATION OF FUNCTIONAL MELANOCYTES DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) provide a promising source for cellular therapy; however, distinct iPSC lines present different differentiation capacities even in the same condition, which is always a relatively complicated and time-consuming process. To predict the differentiation tendency of iPSCs in the early stage, we compared four iPSC lines in the capacity of embryonic bodies (EBs) formation and maintenance, the expression of specific germ layer markers as well as in the melanocyte differentiation capability. Furthermore, to confirm whether the prediction was correct or not, in vivo experiments were performed and induced melanocytes (iMels) were transplanted into immunodeficient mice using a modified hair follicle reconstitution assay. As a result, we found that iPSCs which could form EBs with regular and smooth morphology and express higher levels of ectoderm marker genes (SALL3, EDNRB and SOX1) and lower levels of mesoderm and endoderm marker genes (MEIS2, GATA4, MEF2C, FOXA2 and HOXA1), had a better differentiation capability into melanocytes. After transplantation, these human-origin iMels were detected in the mouse hair bulb and epidermis up to 7 weeks, showing DOPA positive staining and expressing melanocytic markers, MITF, PAX3 and TYRP1. They also produced melanin which were localized in the hair bulb, epidermis and hair shaft. In addition, PAX3+MITF+TYRP1- melanocyte stem/progenitor cells were co-localized with hair follicle stem cells marked with KRT15 and ITGA6 in the bulge region, not only in the anagen hair follicles but also in the catagen/telogen hair. In conclusion, our data demonstrate that the differentiation tendency of iPSCs might be predicted using parameters in EBs stage, specifically, the formation and maintenance of optimal EBs and the expression level of germ layer-specific markers. In addition, it is the first time to show the long-term survival and function maintenance of iPSC-derived melanocytes in vivo and to demonstrate their hair follicle and epidermis reconstitution capacity. The bulge region with existing hair follicle stem cells functions as a niche for melanocytic stem cells and should support and provide the long-term maintaining of functional iMels.

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T-3132

MOLECULAR MECHANISMS UNDERLYING THE BLASTULA STAGE SPECIFICATION OF NEURAL CREST FROM A PLURIPOTENT STEM CELL STATE

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Neural crest (NC) is a multipotent stem cell population that gives rise to multiple derivatives in vertebrate embryos. The specification of NC has been a topic of great discourse over the past two decades. Studies in chick, rabbit, and Xenopus embryos have eluded to NC specification during early gastrulation. However, molecular mechanisms involved in the earliest specification of NC from a pluripotent stem cell state remain unknown. Here we present for the first time the earliest NC cell fate specification in amniotes using avian and human NC (hNC) models. In avian embryos, specification assays and fate map analysis identified a population of specified NC in the intermediate epiblast region of the blastula embryo, and this specification is independent of mesodermal and neural contributions. The specification of human NC (hNC) was assessed using a robust 5-day model of hNC development based on Wnt-signaling activation in human embryonic stem cells (hESC). Functional analysis of prospective NC (pNC) using mesendoderm differentiation assays suggest a restricted stem cell potential of pNC unlike hESC. Further molecular characterization of pNC at high temporal resolution identified stoichiometric changes in the expression of pluripotency genes, accompanied by a distinct expression profile of pNC and NC genes within 6 hours of NC induction from hESCs. Perturbation of pluripotency genes during the first 48 hours of NC induction drastically affects hNC formation, suggesting a role for these pluripotency factors during hNC specification. This study demonstrates for the first time the restriction in NC cell fate in avian and human NC that diverges from the epiblast/ESC fate at unprecedented early facets of development, and provides novel insight into the role of pluripotency genes during cell fate specification from a pluripotent stem cell state.

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T-3134

PARTIAL REPROGRAMMING: A HARD-TO-FIND CAUSE OF HUMAN INDUCED PLURIPOTENT STEM CELLS' REDUCED PROPENSITY FOR ENDODERM DIFFERENTIATION

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The propensity for hepatic differentiation varies significantly among induced pluripotent stem cell (iPSC) clones. What determines the susceptibility of a population to differentiate into definitive endoderm (DE) and its derivatives remains to be determined. Here we investigate the causes of such a variability and assess the role of the state of pluripotency and reprogramming stage. We compared three TRA-1-60-positive, well characterized human iPSC populations with good (P01 and P02), and suboptimal (P03) propensity for hepatic differentiation in terms of pluripotency markers, self-renewal capability, methylation and gene expression profiles, metabolism. We demonstrate that P03 population proliferates more actively, shows lower expression of genes characterizing "primed" embryonic stem cells (ESC), and responds with delayed dynamics upon differentiation into DE. While P01 and P02 use anaerobic glycolysis, P03 preferentially uses oxidative phosphorylation. This has a direct effect on the overall state of DNA methylation, with P03 characterized by global DNA hypomethylation compared to other iPSCs. Such data, together with MDB2-dependent repression of Nanog and a characteristic microRNAs expression profile, suggest that P03 is partially reprogrammed. We identified a peptide hormone capable of promoting full reprogramming of iPSC clones by enhancing expression of key pluripotency markers (TET1, NANOG, LIN28, TRIM71, CDH1) and consequently switching the cells from a state more evocative of "naïve" ESCs to a "primed" state. A short treatment with such a growth factor can, at least partially, restore the propensity of P03 for DE differentiation. Overall, our findings suggest that iPSC populations that might be mistakenly considered well reprogrammed according to standard characterization parameters are indeed partial reprogrammed. Such a status is responsible for their reduced propensity to differentiate into endoderm derivatives, propensity that can be restored with a targeted intervention.

T-3136

SINGLE CELL ANALYSIS ELUCIDATES THE MECHANISM OF CARDIAC AND HEMATOPOIETIC DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Clinical application of human induced pluripotent stem cells (hiPSCs) such as disease modeling requires efficient differentiation of the desired populations. Signals that specify the fates of mesodermal cells to the Primitive and Definitive hematopoiesis, and the Ventricular and Atrium cardiomyocytes

have been extensively investigated. However, transcriptional control within each cell and the mechanisms of cell fate decisions toward different lineages including cardiac and hematopoietic cells have not been elucidated sufficiently. Here we conducted a comprehensive study of mesodermal differentiation mechanism at a single cell level, using two methods of single-cell analysis, single cell qPCR and single cell RNA seq. We performed single cell qPCR analysis of 93 genes from 1,725 cells in total from each hematopoietic and cardiac differentiation from hiPSCs, which revealed heterogeneity of differentiating mesodermal cell at day2-4. Knockdown of transcription factors such as EOMES and FOXH1, which were expressed at the bifurcation of each lineage significantly increased the hematopoietic differentiation efficiency while they decreased the cardiac differentiation efficiency, affecting the downstream genes like GATA6 and CDX2. These results suggest that these transcriptional factors specify the fate of each cell promoting one fate while suppressing others. To examine a novel mechanism involved in the early stage specification of the mesoderm, we conducted single-cell RNA sequencing from 71 cells at day 0-3 of differentiation. Pseudo-time analysis that evaluates temporal changes of expression revealed that specific pathways are temporarily activated in addition to known WNT signaling and TGFβ signaling. To investigate the transcriptional regulation, we performed the single-cell regulatory network inference and clustering (SCENIC) analysis. SCENIC revealed that upstream genes such as SALL3 regulated the downstream genes involved in the epithelial-mesenchymal transition, suggesting that these genes were involved in the differentiation of mesoderm. Taken together, these findings provide new understandings of cardiac and hematopoietic differentiation, which applies to yield the desired population efficiently for clinical application.

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T-3138

METHOD FOR 3D HEPATIC DIFFERENTIATION IN STIRRED BIOREACTORS OF HUMAN PLURIPOTENT STEM CELLS IN CHEMICALLY DEFINED, ANIMAL-ORIGIN-FREE CULTURE MEDIA, STEMFIT

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Human pluripotent stem cells (hPSCs) are promising cell sources for regenerative therapy, drug discovery, because of their potential to expand long-term in vitro and ability to differentiate into various types of somatic cells. Currently, differentiation protocols of hPSCs are often based on 2D manner and a limited number of 3D differentiation methods is available. 3D cell culture platforms are reported to better mimic in vivo conditions and have advantages such as scalability and automatability. For applications such as cell therapy, a large number of cells are required, on scales that will require 3D differentiation systems for large scale manufacturing. In this study, we demonstrate an efficient 3D differentiation system of hPSCs using clinical-grade (Japan), defined media. First, hPSC spheroids were formed from single cell suspensions for uniform and controllable spheroid size using “StemFit Basic03”, an animal component-free and chemically defined hPSC maintenance medium. Next, hPSC spheroids in the spinner flask were directly differentiated in dynamic suspension into definitive endoderm (~90% CXCR4+), immature hepatocytes, and then mature hepatocytes using “StemFit AS400”, which is animal component-free and chemically defined nutrient supplement for hPSC differentiation. Obtained hepatic spheroids highly expressed markers such as albumin, AFP, and OTC. Our results indicate that the combination of hPSC spheroid formation with “StemFit Basic03” and differentiation with “StemFit AS400” nutrient supplement will enable high density, scalable differentiation of hPSCs under chemically defined, animal-origin-free conditions.

T-3140

SINGLE-CELL TRANSCRIPTOMICS REVEALS A CD31+CD105- SUBPOPULATION OF EARLY ENDOTHELIAL CELLS WITH ELEVATED HEMATOPOIETIC POTENTIAL FROM HUMAN PLURIPOTENT STEM CELLS

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Accumulating evidence shows that endothelial cells are the origin of blood cells both in vitro and in vivo. However, the heterogeneous nature of endothelial cells and the mechanism regulating their derivation remain largely elusive. In this study, we found by using single-cell transcriptomics analysis that CD31+ endothelial cells are highly heterogeneous even at its emergence from human pluripotent stem cells (hPSCs). Among them, a CD31+CD105- subpopulation of cells are highly potent for hematopoietic differentiation. Interestingly, genetic deletion of CD105 promotes the hematopoietic differentiation of hPSCs. While inhibition of TGFβ signaling facilitates the generation of CD31+CD105- subpopulation of cells, overexpression ETS1 suppresses its derivation. Our findings indicate that CD105 can be used as a reliable surface marker to enrich hematopoietic potent endothelial cells. The discovery of the underlying mechanism should also benefit the derivation of functional blood cells from hPSCs for translational medicine.

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T-3142

ANALYSIS OF EXTRACELLULAR RNAS DURING PANCREATIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Ever since human embryonic stem cells (hESCs) were first successfully cultured, it was recognized that hESCs had the potential to be used for cell replacement therapies to treat traumatic injury, developmental disorders, and degenerative diseases. hESC-derived pancreatic ß cells are of particular interest for the treatment of Type 1 Diabetes Mellitus. Multiple methods involving sequential addition of growth factors and small molecules to direct in vitro pancreatic differentiation of hESCs have been developed, but existing protocols have failed to produce a pure population of mature and functional ß cells. Extracellular RNAs (exRNAs) have been shown to mediate cell-cell communication in several biological contexts, but their potential roles as autocrine or paracrine effectors in hESC differentiation and maturation are not well understood. In this study, we aim to use small RNA sequencing to generate expression profiles of exRNAs isolated from conditioned hESC culture media at each stage of in vitro pancreatic differentiation up to the pancreatic progenitor stage. To discern differences in exRNA cargo among carrier subclasses (CSs), including extracellular vesicles and ribonucleoprotein complexes, immunoaffinity separation is performed with magnetic beads conjugated to antibodies raised against CS markers, such as CD63, CD81, CD9 and AGO2. ExRNAs purified from the purified CSs are sequenced and the data are compared to those from the source cells and unfractionated total supernatant. Identification of exRNAs released at each stage of differentiation will provide information necessary for characterizing their roles in intercellular communication and regulation of differentiation in vitro. This will lay the foundation for further investigations into the roles of exRNAs in pancreatic differentiation.

T-3144

MOUSE ZSCAN5B DEFICIENCY IMPAIRS DNA DAMAGE RESPONSE AND CAUSES CHROMOSOME ABERRATIONS DURING MITOSIS

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Zygotic genome activation (ZGA) begins after fertilization and is essential for establishing pluripotency and genome stability. However, it is unclear how ZGA genes prevent mitotic errors. Here we show that knockout of the ZGA gene Zscan5b, which encodes a SCAN domain with C2H2 zinc fingers, causes a high incidence of chromosomal abnormalities in embryonic stem cells (ESCs), and leads to the development of early stage cancers. After irradiation, Zscan5b-deficient ESCs displayed significantly increased levels of γ-H2AX despite elevated expression of the DNA repair genes Rad51l3 and Bard. To determine whether DNA damage repair is dependent on Zscan5b, a piggyBac vector carrying wild-type Zscan5b-GFP driven by a CAG promoter for long-term stable expression was transfected into Zscan5b-deficient ESCs. We found a significant increase in DNA damage after irradiation in Zscan5b deficient irradiated ESCs. However, a lower frequency of γH2AX foci was present in transfected Zscan5b deficient cells, suggesting that restoration of Zscan5b expression in these cells was sufficient to restore the genome stability. To test whether Zscan5b binds either directly or indirectly to chromosomes, and helps to form nucleosome structures, we performed a co-immunoprecipitation analysis using protein extracted from ESCs transfected with 3XFLAG-Zscan5b-EGFP. The components of bands from 3XFLAG-Zscan5b-EGFP combined proteins were analyzed and the histone H1 family, H1.1, H1.2 and H1.4, was specifically detected in bands. We cross- validated these results by transfection and by an immunoprecipitation assay using the cells transfected with a pcDNA3.1+C-eGFP plasmid carrying mouse Zscan5b cDNA and three pcDNA3.1-MYC-HIS A plasmids carrying mouse Histone 1,1, 1.2, and 1.4 cDNAs. The sizes of these bands correspondeds to the molecular sizes of histones H1 family, H1.1, H1.2 and H1.4 of in the antibody-added protein sample. These results supports that ZSCAN5B binds to the linker histone H1, and may protect chromosomal architecture. Our report demonstrates that the ZGA gene Zscan5b is involved in genomic integrity and acts to promote DNA damage repair and regulate chromatin dynamics during mitosis.

T-3146

HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES SHOW STRONGER CORRELATION AND HIGHER SIMILARITY TO HUMAN ADULT LIVER THAN TO HUMAN FETAL LIVER

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Human pluripotent stem cell derived hepatocytes (hPSC-HEP) display many properties of mature hepatocytes, including expression of many important genes of the drug metabolizing machinery, glycogen storage, and production of multiple serum proteins. However, hPSC-HEP have not yet been shown to fully recapitulate the complete functionality of in vivo mature hepatocytes, and they typically express some fetal hepatocyte markers. In this study, we applied the COMBAT algorithm to two transcriptomics data sets generated applying different microarray platforms, to obtain one data set containing samples from different developmental stages during hPSC-HEP differentiation, human fetal liver tissues (FL) from different gestational weeks and human adult liver tissues (AL). We performed Similarity and correlation analysis on this data set. In addition, we performed functional analysis including cytochrome P450 activities, albumin secretion and urea production on hPSC-HEP from cells generated by our group. Our results showed that the transcriptional correlation of hPSC-HEP to adult liver tissues was higher than to fetal liver tissues (0.83 and 0.70, respectively). Moreover, hPSC-HEP showed higher similarity to AL than to FL (0.75 and 0.56 respectively). Functional analysis showed cytochrome P450 activities of hPSC-HEP and albumin secretion that are comparable to cryoplateable human primary hepatocytes. The results showed also the ability of hPSC-HEP to produce urea. To conclude, our results show for the first-time

higher similarity of hPSC-HEP to AL both on the transcriptional and the functional levels. These results demonstrate substantial improvement from previous studies categorizing hPSC-HEP as immature and resembling fetal hepatocytes.

T-3148

TIME-COURSE AND DOSE-DEPENDENT TRANSCRIPTOME PROFILING REVEAL KEY REGULATORS FOR NEURAL CONVERSION OF HUMAN IPSCS UNDER CHEMICALLY DEFINED CONDITIONS

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The systematic study of cell differentiation provides deeper insights into developmental pathways and how they control complex genetic programs. Neural induction of human pluripotent cells can be used as a model system to investigate the interplay between pathway manipulation and gene activation/silencing in chemically defined E6 medium. Small molecule based inhibition of bone morphogenetic protein (BMP) and transforming growth factor-beta (TGF-β) pathways (dual SMAD inhibition) is a widely used approach to convert pluripotent cells into neuroectoderm and neural crest. While previous studies characterized human neural induction in bulk cultures, high-resolution analysis should capture the dynamic molecular changes more comprehensively. Here, we performed seven day time-course single-cell sequencing (scRNA-Seq) to reconstruct differentiation trajectories induced by blocking BMP and TGF-β pathways separately and in combination. Using dual SMAD inhibition and single concentrations of small molecules (0.1 uM LDN 193189, 2 uM A83-01), we identified distinct transition stages characterized by transiently expressed genes (e.g. SIX3, HESX1, and LMO1), which led to expression of PAX6, DLK1, TPBG, TMSB15A, HES4, IGFBP5, FOXG1, SOX11. Next, to systematically investigate gene expression dynamics, we used RASL-Seq for gene expression profiling upon BMP inhibition and TGF-β inhibition alone or in combination across seven different small molecule concentrations. During BMP inhibition, FOXG1, SOX1, FZD5, ZIC4, HEXS1, SIX3, PTN, HES4 were strongly upregulated while PAX3, FOXD3, SOX10, SNAI2, S100B were antagonized in a dose-dependent manner. Conversely, TGF-β inhibition alone positively regulated PAX3 and SOX10, suggesting that this strategy favors the induction of neural crest. In summary, modulation of cell signaling pathways via high-throughput gene expression profiling and small molecule titration can control expression of transcription factors determining cell specification. This strategy should help to optimize cell differentiation protocols using precisely calibrated small molecule combinations to produce functional phenotypes for clinical therapies.

T-3150

POTENTIAL ROLE OF TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 CHANNELS IN MOUSE EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

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Transient receptor potential (TRP) channels are broadly expressed in a variety of tissues and cell types. They are able to respond to a wide range of stimuli in the cellular environment, which makes them act as cellular vanguard sensors involved in nociception, taste perception, temperature and osmolarity sensation. Among TRP channels, TRP ankyrin 1 (TPRA1) channel was initially described as a cold-sensitive non-selective cation channel expressed in neuron. Recently, emerging evidence indicates that TRPA1 is expressed in various cell types including cardiomyocytes (CMs) and plays an important role in CM contractile function. Mouse embryonic stem cells (mESCs) are able to self-renew and maintain pluripotency to differentiate into all cell lineages including CMs. In CMs, mitochondria can not only supply energy to cells but also has a key role in the regulation of calcium homeostasis and cell contraction. The function of mitochondrion is tightly related to its morphology, which is determined by continuous fission and fusion, called mitochondrial dynamics. Up till now, there is limited knowledge on how TRPA1 regulates intracellular calcium ([Ca2+]i) and action potential in CMs and whether TRPA1 exerts an effect on mitochondria function. Our preliminary results indicated that mESC-derived CMs (mESC-CMs) expressed TRPA1. In addition, we found that the activities of TRPA1 are positively associated with the Ca2+ transients (CaTs) in mESC-CMs. Moreover, TRPA1 activator increased mitochondrial fusion while TRPA1 blocker increased mitochondrial fission. However, the exact mechanism is still under investigation. In the future, our research will focus on elucidating how the TRPA1 activity influences mitochondrial dynamics and their effects on the function of mESC-CMs.

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T-3152

ISOLATION AND ENRICHMENT OF HUMAN STEM CELL DERIVED PROXIMAL LUNG SUBPOPULATIONS

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The human lung is a complex organ composed of over 40 cell types, including epithelial, mesenchymal, immune and endothelial populations. Directed differentiation from human embryonic (hESC) and induced pluripotent stem cells (iPSC) into respiratory epithelial cells using small signaling molecules and growth factors can be used as a robust platform to study human lung development and disease in vitro. However, even with an optimized proximal lung differentiation protocol, the resulting cell culture is a mix of various types of lung cells, which makes studying cell specific molecular pathways difficult. By identifying cell surface markers of specific proximal lung cells, desired subtypes can be isolated and enriched via FACS to enhance lung development and disease modeling research. We aim to screen hESC derived proximal lung cells with a library of CD antigens using a SOX2-GFP reporter cell line by sorting cells positive for both GFP and the target CD antigen. Proximal cell types of the sorted cells will be verified using gene expression profiles and immunocytochemistry. We have performed an investigative bioinformatic screen of 350 CD antigens using Ingenuity Pathway Analysis (IPA) to predict likely surface markers of proximal lung subpopulations including basal, secretory, goblet, and ciliated cells. We then generated proximal lung cells in a 2D culture system from a H9 hESC line with a SOX2-GFP reporter and confirmed the presence of proximal markers such as P63, SCGB3A2, MUC5A and FOXJ1 via immunostaining. Lastly, we performed FACS on the hESC derived proximal lung cells by selecting for cells with both endogenous GFP expression as well as the CD antigen of interest. In summary, we have narrowed down the 350 CD antigens to our top 10 hits using IPA as well as FACS analysis of SOX2+ proximal lung cells. We aim to determine the cell types that these CD antigens correlate with through gene expression profiles and immunostaining of proximal markers.

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T-3154

DEFINING THE TRANSCRIPTIONAL NETWORK THAT GOVERNS PERIPHERAL GLIA SPECIFICATION BY USING HUMAN PLURIPOTENT STEM CELLS

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The neural crest (NC) is a transient and multipotent embryonic stem cell population that migrates throughout the embryo to populate numerous derivatives. It generates peripheral neurons and glia, smooth muscle cells of the outflow track of the heart, craniofacial bone and cartilage, and skin melanocytes. Thus, human neural crest stem cells (hNCSCs) offer the possibility to study cell-fate decisions during embryonic development. The peripheral glia or Schwann cell lineage not only plays an essential role in nerve repair and regeneration, but is also responsible for several demyelination disorders, such as the Charcot-Marie-Tooth disease. Despite its importance, the transcriptional program that regulates hNCSC specification into human Schwann cells (hSCs) remains unknown. Moreover, recent findings in different stem cell types suggest that fundamental metabolic processes are actively implicated in stem cell maintenance and lineage specification, although in NC this remains to be elucidated. Therefore, the overall goal of this project is to define gene regulatory networks, translational landscapes and metabolic profiles that distinguish hNCSCs from hSCs. To accomplish this aim, we take advantage of the human embryonic stem cell (hESC) system. We efficiently differentiated hESCs into hNCSCs using a combined TGF-β and GSK-3β inhibition. Afterwards, hNCSCs were treated with neuregulin-1 and forskolin to induce the specification into the glial lineage. To characterize the specification process, we analyzed the expression of various hNCSC and hSC markers, such as Sox10 and p75, during the course of differentiation. We observed that Sox10+ p75+ cells acquire a bipolar morphology at the end of the treatment, resembling Schwann cell precursors. With this strategy we plan to recapitulate the neural crest specification into peripheral glia by using hESCs, and to unravel which are the main drivers for this fate decision. These findings will allow a better understanding of hSC development, and consequently of its role in nerve repair and neuropathies.

T-3156

SINGLE-CELL TRANSCRIPTOME ANALYSIS OF HESC PANCREATIC DIFFERENTIATION REVEALS PRODUCTIVE AND NON-PRODUCTIVE PATHS OF DIFFERENTIATION

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HESCs differentiated to specific cell types have tremendous potential for use in cell replacement therapy for a variety of conditions. Among the most sought after target cell types are pancreatic beta cells for treatment of Type 1 diabetes mellitus. However, the efficacy and safety of such therapies remain suboptimal. We performed single-cell RNAseq on hESCs at sequential stages of pancreatic differentiation, as well as human fetal pancreas tissue and adult islets, to determine the heterogeneity at each stage of differentiation. We applied a network permutation analysis to the key transcriptional networks characterizing component cell subpopulations. Our results indicate that the overall trajectory of differentiation for the bulk of the population of hESCs travels smoothly from the undifferentiated state toward the developmentally relevant states occupied by fetal pancreas and adult islet cells, with the exception of the pancreatic progenitor stage of differentiation, which deviates from the expected direct path between the preceding posterior foregut and following endocrine pancreas stages. In addition, our approach provides evidence of some individual cells transitioning between cell fates. We found a subpopulation of cells positive for both POU5F1 and the SOX17 mediator CER1. Consistent with other studies, these results suggest that POU5F1 might be required during early differentiation to transition out of the pluripotent state, and may partner with SOX17 for endodermal specification. This subpopulation of POU5F1/CER1 double positive cells may be evidence of cells that have not yet achieved a definitive endoderm-like state. These results point to complex transcriptional networks that may be activated or repressed during the in vitro differentiation process, and will be applied to future studies aimed at optimizing this process to enable production of mature functional pancreatic beta cells derived from hESCs or other types of human pluripotent stem cells.

PLURIPOTENT STEM CELL: DISEASE MODELING

T-3160

MRI TRACKING OF IPS CELLS-INDUCED NEURAL STEM CELLS IN TRAUMATIC BRAIN INJURY RATS

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Induced pluripotent stem cells (iPS cells) are promising cell source for stem cell replacement strategy applied to brain injury caused by traumatic brain injury (TBI) or stroke. Neural stem cell (NSCs), derived from iPS cells could aid the reconstruction of brain tissue and the restoration of brain function. However, how to trace the fate of iPS cells in host brain is still a challenge. In our study, iPS cells were derived from skin fibroblasts using the four classic factors Oct4, Sox2, Myc and Klf4. Then these iPS cells were induced to differentiate into NSCs, which were incubated with superparamagnetic iron oxides (SPIOs) in vitro. Next, 30 TBI rat models were prepared and divided into 3 groups (n=10). One week after brain injury, group A&B rats received NSCs (labeled with SPIOs) implantation, while group C rats received non-labeled NSCs implantation. After cell implantation, all the rats were performed T2*-weighted magnetic resonance imaging (MRI) scan at day 1, and 1 week to 4 weeks, to track NSCs distribution in rats' brains. One month after cell implantation, all the rats were performed manganese-enhanced MRI (ME-MRI) scan. In group B, diltiazem was infused during the ME-MRI scan period. Thus, (1) iPS cells were successfully derived from skin fibroblasts using the four classic factors Oct4, Sox2, Myc and Klf4, expressing the typical antigens including SSEA4, Oct4, Sox2 and Nanog. (2) iPS cells were induced to differentiate into NSCs, which could express Nestin and differentiate into neural cells and glia cells. (3) NSCs were incubated with SPIOs overnight, and prussian blue staining showed intra-cellular particles. (4) After cell implantation, T2*-weighted MRI scan showed these implanted NSCs could migrate to the injury area in chronological order. (5) The subsequent ME-MRI scan detected NSCs function, which could be blocked by diltiazem. In conclusion, using in vivo MRI tracking technique to trace the fate of iPS cells-induced NSCs in host brain is feasible.

T-3162

EXOSOMES DERIVED FROM MESENCHYMAL STEM CELLS ALLEVIATED ILC2-DOMINANTE ALLERGIC AIRWAY INFLAMMATION

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Type 2 innate lymphoid cells (ILC2s) were reported to be involved in the pathogenesis of allergic diseases. The cost of allergic airway inflammation is a significant burden to the society, promoting us to look for better treatment. We previously reported the immunomodulation of mesenchymal stem cells (MSCs) on suppressing airway inflammation. Extracellular vesicles (EVs) or exosomes are one of the key secretory products of MSCs involved in their immunomodulation. In this study, we aimed to investigate the effects of exosomes from MSCs on allergic airway inflammation and the possible mechanisms. We isolated exosomes from human induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) media, and determined their characteristics. We induced mouse ILC2 dominant airway inflammation model by intratracheal instillation of IL-33. Compared with the model group, the intravenous injection with MSC-derived exosomes (MSCexo) significantly suppressed the level of IL-5/13 Th2 cytokines in bronchoalveolar lavage fluid (BALF) and reduced the proportion of ILC2s in lung. Additionally, we co-cultured the peripheral blood mononuclear cells (PBMCs) from patients with allergic rhinitis with MSCexo, and MSCexo significantly inhibited the IL-5 production in response to IL-33. However, fibroblast-derived exosomes (Fbexo) did not exhibit the similar effects. We further identified that MSCexo had higher levels of miR-146a-5p compared to Fbexo. After inhibiting the expression of miR-146a-5p in MSCs, the inhibitory effect of exosomes on inflammation was significantly reversed both in mouse model and in vitro. Our data suggest that exosomes from MSCs suppress the ILC2 dominant allergic airway inflammation, which is at least partly mediated via transfer of miR-146a.

T-3164

GENOME-WIDE MICROHOMOLOGIES ENABLE PRECISE TEMPLATE-FREE EDITING OF PATHOGENIC DELETION MUTATIONS

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To investigate the function of a gene or to create disease models, researchers often aim to generate gene “knockouts” using designer nucleases such as CRISPR/Cas9. This type of gene editing faces two challenges, the predictability of mutations created at the target loci and of their phenotypic outcome. Recently published manuscripts report predictable mutations using CRISPR/Cas9-induced template-free non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ) DNA repair at random genetic loci or in artificial sequence libraries. However, these published tools give limited insight into probable phenotypic outcomes. In order to combine predictable gene editing outcomes with existing knowledge on phenotypes, we devised a methodology relying on precise deletions generated by MMEJ to re-create already known mutations. MMEJ is mediated by microhomologies (μHs) flanking the DNA double strand break. Here we introduce a tool called MHcut that identified μHs flanking the majority of all naturally occurring deletion mutations across the human genome. In total, 11 million deletions are flanked by μHs, covering 88% of protein-coding genes. Notably, over 99% of these mutations are as of yet unexplored. Using CRISPR/Cas9 in human induced pluripotent stem cells (hiPSCs) and embryonic stem cells (hESCs), we precisely recreated target μH-flanked pathogenic deletion mutations by MMEJ. In addition, we demonstrated both gain- and loss-of-function phenotypes in three hiPSC disease models. We anticipate this precise gene editing methodology and the data set of over 11 million μH-flanked deletions to enable not only functional genetic studies and drug screening, but to also yield potential targets for gene therapy.

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T-3166

GENERATION OF A SERIES OF ISOGENIC HUMAN PLURIPOTENT STEM CELL LINES AS MODELS OF AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder that affects 1 in 59 children, but lacks effective medical treatment. Although several highly penetrant genetic variants have been linked to ASD, researchers have a limited understanding of the causal gene networks, disease-relevant cell types, druggable targets, and biological signatures associated with ASD. Induced pluripotent stem cells (iPSCs) preserve the patient's genome and serve as valuable tools to dissect the cellular basis of disease pathology, but their comparisons to unaffected controls can be hampered by differences in genetic background, reprogramming approaches, cell culture methods, or quality standards. To overcome these limitations, we used CRISPR-mediated genome engineering to introduce ASD-linked genetic variation into a well-characterized iPSC line from a healthy neurotypical adult. Specifically, we prioritized modeling 13 syndromic forms of ASD: five are monogenic- MECP2 y/- (Rett), SHANK3 +/- (Phelan-McDermid), TSC2 +/- (tuberous sclerosis), FMR1 y/- (fragile X), and SLC9A6/NHE6 y/- (Christianson), and eight are copy number variants- reciprocal deletions and duplications at 7q11.23 (Williams-Beuren), 15q11-13 (Angelman/Prader-Willi), 17p11.2 (Smith-Magenis) and 16p11.2. All lines are derived from single-cell clonal isolates and have been rigorously validated by digital and G-banding karyotyping. While the monogenic models are currently available through the NIMH Stem Cell Center at RUCDR, additional characterization of copy number variation lines is underway prior to widespread distribution to the research community. We anticipate that our comprehensive resource of monogenic and copy number variant ASD stem cell models built on the same genetic background will be invaluable for uncovering convergent and divergent molecular and cellular mechanisms in ASD.

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T-3168

ROS DEPENDENT INCREASED AUTOPHAGOSOME FORMATION MEDIATES SYNAPTIC DYSFUNCTION IN A PATIENT DERIVED MODEL FOR KOOLEN-DE VRIES SYNDROME

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Koolen-de Vries syndrome (KdVS) is a heterogeneous multisystem disorder characterized by developmental delay, intellectual disability, facial dysmorphisms, epilepsy, and congenital malformations in multiple organ systems. It is caused by heterozygous loss of KANSL1. KANSL1 is a scaffold protein of the nonspecific lethal complex that contains the histone acetyltransferase MOF, which acetylates histone H4 on lysine 16 (H4K16ac) to facilitate transcriptional activation. Our recent studies in mice have shown that heterozygous loss of Kansl1 leads to changes in gene expression related to synaptic transmission and to a decrease in basal synaptic transmission and plasticity, but the underlying cellular mechanisms remain unknown. H4K16ac is known to be essential for the regulation of autophagy, a process controlling degradation and recycling of proteins and shown to play a role in synapse development and function. Here, we made use of KdVs patient- and control-derived induced pluripotent stem cells (iPSCs) and derived neurons thereof (iNeurons) to link heterozygous loss of KANSL1 to deregulated autophagy and subsequent synaptic dysfunction. Using several KdVS patient cell lines we found that, under basal conditions, the number of autophagosomes is increased in both, KANSL1 deficient iPSCs and iNeurons. By investigating gene expression profiles that are associated with H4K16 acetylation, we found that SOD1 gene expression was down-regulated. Reduced expression of this antioxidant enzyme is leading to a consecutive increase in oxidative stress and autophagy. Furthermore, in maturing KdVS iNeurons, increased autophagosome formation at the synapse resulted in reduced synaptic density and network activity. We could partially rescue the observed autophagy phenotype as well as the synaptic deficits by treating KANSL1 deficient neurons with antioxidants to lower the amount of reactive oxygen species (ROS). Taken together these results identify increased oxidative stress as a cause for increased autophagosome formation and subsequent synapse loss in KdVs, providing a promising starting point for the development of a more targeted therapy.

T-3170

ELECTROPHYSIOLOGICAL RECORDINGS OF NEURONS DERIVED FROM SLEEP BRUXISM PATIENT-SPECIFIC IPSCS

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Sleep bruxism (SB) is classified as a sleep-related movement disorder characterized by involuntary jaw-closing muscle activity. Mechanical stress of SB is shown to be responsible for poor prognosis of dental treatment and seriously compromises patients' quality of life. However, little is known about the etiology of SB. We previously found that a single nucleotide polymorphism (SNP) in serotonin 2A receptor (5-HT2A) gene, rs6313 C>T, increases the risk of SB. 5-HT2A receptors are widely expressed in central nervous system including GABAergic premotor neurons projecting to the trigeminal motor nucleus, which play a central role in inhibitory regulation of masticatory muscle activity during sleep. The aim of this study is to elucidate the functional difference of these GABAergic neurons between C and T allele carriers using electrophysiological recording of iPSC-derived neurons. First of all, human iPSCs from SB and healthy controls were differentiated into neurons with the characteristics of the ventral hindbrain, where 5-HT2A positive neurons are enriched. Regional identity of neurospheres were confirmed by qRT-PCR with 5-HT2A and VGAT gene-specific primers. Neurons derived from neurospheres were immunostained with anti-5-HT2A receptor and anti-GABA antibodies. Whole-cell patch-clamp recordings were performed on these neurons with current-clamp method. Then, voltage-clamp method was utilized to record currents following the administration of selective 5-HT2A receptor agonist (TCB-2). We confirmed that iPSCs were successfully differentiated into GABAergic and 5-HT2A receptor positive neurons by using qRT-PCR and immunostaining. Furthermore, these differentiated neurons generated repetitive action potentials in response to current injections and TCB-2 evoked inward currents. These functional observations suggested that neurons differentiated from patient-specific iPSCs contained both GABAergic and 5-HT2A receptor positive neurons. In conclusion, the successful electrophysiological recording of iPSC-derived neurons allows us to examine the effects of SB-associated genetic variation, which might elucidate the etiology and underlying mechanism of SB.

T-3172

RAPID REVERSAL OF DIABETES AFTER TRANSPLANTATION OF HUMAN BETA CELLS FROM PATIENT IPS CELLS WITH CRISPR CORRECTION

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Stem cell-derived β (SC-β) cells are a powerful tool for disease modeling, drug screening, and cell therapy. Patient induced pluripotent stem (iPS) cells allows for the study of diabetes by providing an unlimited cell source and has potential in autologous cell therapy, removing the need for immunosuppression. Monogenetic forms of diabetes, including Wolfram Syndrome (WS), are an immediate application of SC-β cell technology, as β cells from WS patients are rarely available for study and mouse models do not faithfully recapitulate the disease. WS is caused by a pathogenic variance in the WFS1 gene, inducing endoplasmic reticulum (ER) stress. To enable disease modeling and determine the cell therapy potential of autologous SC-β cells, we reprogrammed WS patient fibroblasts into iPS cells and corrected the disease-causing sequence with CRISPR/Cas9. After differentiation of patient-matched unedited and corrected WS iPS cells with our 6-stage protocol to produce SC-β cells, single-cell RNA sequencing was performed on approximately 10,000 cells. Unsupervised clustering using Seurat identified α-, β-, and δ-like cell populations, with corrected β-like cells having increased β cell and decreased stress markers. Surprisingly, cluster analysis identified multiple off-target non-pancreatic cell types produced with the unedited iPS line, including neural, epithelial, and muscle tissues, while the corrected line produced predominately pancreatic endocrine. Corrected WS SC-β cells achieved robust dynamic glucose-stimulated insulin secretion (GSIS) similar to primary islets and to SC-β cells generated from unrelated non-patient donors, demonstrating their maturity and projected cell therapy success in vivo, while unedited WS SC-β cells had greatly reduced GSIS and insulin content. Transplantation of corrected WS SC-β cells rapidly alleviated diabetes within 10 days in mice with pre-existing diabetes that had been treated with streptozotocin. However, the unedited WS SC-β cells were unable to regulate blood glucose in mice even after 10 weeks. Human insulin was detected in all transplanted mice although was significantly less for unedited WS SC-β cell transplants. This technological platform allows for the study of human diabetes pathology and is enabling for drug screening and cell replacement therapy.

Funding Source: NIH (1R01DK114233-01 and 5T32DK10842-02), JDRF (Career Development Award), WashU Center of Regenerative Medicine, Diabetes Research Center

T-3174

NOVEL INSIGHTS INTO THE MOLECULAR MECHANISMS UNDERLYING X-LINKED DYSTONIA-PARKINSONISM BY HARNESSING X-CHROMOSOME INACTIVATION

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X-linked Dystonia-Parkinsonism (XDP) is an adult-onset Mendelian neurodegenerative disease endemic to the island of Panay, Philippines. Conventional genetics analysis identified a founder haplotype consisting of: five single-nucleotide variants (disease-specific single-nucleotide changes, DSC-1, -2, -3, -10, and -12), a 48-bp deletion, and a 2627 bp SINE-VNTR-Alu (SVA)-type retrotransposon insertion. XDP haplotype boundaries mapped on Xq13.1, with the main disease-associated mutations lying within the non-coding region of TAF1 gene. TAF1 (TATA-binding-protein (TBP)-associated factor 1), being a subunit of the TFIID complex, mediates the transcription by RNA polymerase II (RNAP II). Therefore, it has been proposed that XDP pathogenesis might be dependent on TAF1 dosage insufficiency or aberrant transcription around the SVA region. For X-linked human diseases, such as XDP, the natural process of X-chromosome inactivation (XCI) provides an efficient way to generate genetically matched isogenic female induced pluripotent stem cell (iPSC) lines. However, XCI status in human iPS cell models is highly variable. In this study, we characterize the status of XCI in iPSC lines derived from multiple XDP female carriers. In particular, we use these iPS cell lines to uncover the mechanisms underlying XDP etiology. As such, cellular and molecular phenotypes associated with XDP are here identified by delineating the transcriptome profiles of a pair of isogenic female iPSC lines where either the wild-type or the mutant X chromosome are active. Overall, this initial analysis allowed us to harness the X-chromosome inactivation to understand the molecular features underlying XDP pathology, opening further possibilities to deepen into other XDP etiology-associated factors, such as brain regions, TAF1 isoform, as well as retrotransposon-derived transcripts and their regulators.

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T-3176

DOWN SYNDROME-IPSC NEUROGENESIS CONNECTS DIFFERENTIAL METHYLATION TO DYSREGULATED GENES

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Down syndrome (DS) is caused by trisomy 21 (T21) in humans and affects approximately one in 700 live births. Impaired cognition is a major disabling feature in DS and despite major efforts the molecular mechanisms leading to the morphological and functional brain abnormalities associated with T21 remain unknown. To clarify the role of differential methylation on transcriptional dysregulation and neurodevelopment in T21, we established an induced pluripotent stem cell (iPSC) derived neural cell model showing a transcriptional profile comparable to the early-mid gestational period. The DNA methylation pattern was analysed using Illumina HumanMethylation 450k BeadChip and paralleled by RNA sequencing. We first assessed the genome wide methylation pattern in T21 and euploid iPSC neural derivatives and identified 500 differentially methylated positions (DMPs). The DMPs in T21 neural lines were distributed across the genome with enrichment of hypomethylated sites on chromosomes 2, 8, 19, 21 and 22. Approximately half of DMPs (281 of 500) could be annotated to a total of 202 genes. Gene Ontology (GO) analysis of these 202 genes revealed enrichment of neurotransmitter transporters (GO:0006836). Integrated analysis of methylation and transcriptome data sets revealed altered expression in 77 out of the 202 genes in T21 neural lines. Furthermore, the most profound methylation changes (>9DMPs/gene) associated with differential expression in T21 neural lines was observed for a cluster of genes (ZNF441,

ZNF69, ZNF700 and ZNF763) on chromosome 19 encoding zink finger transcription factors. These ZNF genes with yet unknown functions are highly expressed during normal embryonic brain development. Taken together, our data suggest that differential methylation contributes to transcriptional dysregulation in T21 neural cells and our data highlights a set of candidate ZNF transcription factor genes that now require further investigations.

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T-3178

PATIENT-SPECIFIC TISSUE CHIP TECHNOLOGY TO STUDY RARE DISEASES OF THE BRAIN

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Here we describe patient-specific brain-on-a chip technology to model Tuberous Sclerosis Complex (TSC), a rare neurodevelopmental disorder manifesting itself with cortical malformations ("tubers"), subependymal giant cell astrocytomas, and epilepsy, but also affecting other organs. TSC is caused by loss-of-function mutations in the TSC1 or TSC2 gene that result in aberrant mTOR signaling. Our patient-specific brain-on-a-chip model, the NeuroVascular Unit (NVU), consists of a vascular chamber populated with hiPSC-derived brain microvascular endothelial cells (BMEC) seeded on a supporting membrane, with astrocytes seeded on the opposite side of this membrane that defines a neuronal compartment containing hiPSC-derived neurons and astrocytes embedded into hydrogel. We compared cell viability in the neuronal chamber using a live/dead stain and found twice as much cell death in TSC- than control neuronal compartments (p=0.01, N=10). In addition, we found that TSC vascular compartments are significantly more leaky than their control counterparts as assessed by measuring FITC-dextran diffusion across the BMEC cell layer (p=.001, N=10). Interestingly, the replacement of TSC astrocytes seeded onto the opposing side of the BMEC-containing vascular chamber with control astrocytes in otherwise TSC-cell populated NVUs restored viability in the neuronal compartment and BMEC barrier function in the vascular compartment to control levels. The present model for TSC pathogenesis suggests a key role for increased mTORC1 signaling. Rapamycin, which is therapeutically used in some TSC patients, inhibits mTORC1 (but not mTORC2) activity. Continuous perfusion of rapamycin (0.2 nM) through the vascular chamber significantly improved cell viability in the neuronal compartment and barrier function of the vascular chamber (p=0.02, N=10). We are presently assessing mTOR signaling by quantifying the phosphorylation of downstream targets such as S6-Kinase, S6 ribosomal protein and 4E-BP1 in the neuronal and vascular compartments of control and TSC NVUs. In summary, we have developed a patient-specific brain-on-a-chip model to study TSC pathogenesis by determining TSC cellular and biochemical phenotypes and to assess effects of presently used and prospective therapeutics.

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T-3180

INTRINSINC HUMAN GLIAL PROGENITOR CELLS TRANSCRIPTOME DYSREGULATION IN VANISHING WHITE MATTER DISEASE

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Vanishing White Matter (VWM) disease is one of the most common congenital disorders of myelin. It is caused by mutations in the eukaryotic translation initiation factor subunit genes (EIF2B1-4), the pathogenic effects of which manifest almost exclusively in the CNS. VWM is unique in its susceptibility to stress events, such as fever or head trauma, which typically precede a rapidly progressive demyelination and clinical deterioration. Despite its association with white matter loss, the cellular basis of VWM has remained unclear. Since both astrocytes and oligodendrocytes have been implicated in this disorder, we asked if bipotential human glial progenitor cells (hGPCs) might manifest pathology in the setting of EIF2B1-4 mutations. To that end, we engineered a human embryonic stem cell line (GENEA19) with homozygous single point missense mutations associated with distinct VWM phenotypes (a severe form, EIF2B5 cG584A>R195H; a classic form, EIF2B4 cC728T>P243L; and a milder form, EIF2B5 cG338A>R113H). Cells were instructed to hGPCs fate and isolated by CD140a-based FACS for RNA-sequence analysis. Functional analysis of VWM GPCs expressed higher levels of pro-apoptotic genes (TP53I3, BAK1, BAX, FAS), and exhibited dysregulation of genes involved in cell proliferation (CCNA1, CDKN2D, CDKN1B, SIX3). VWM-derived GPCs upregulated genes involved in inflammatory response (ANXA1, ANXA2. HLA-A, HLA-B, HLA-C, IFITM2, IFITM3, TRIM5, TRIM22) as well as in extracellular matrix (ECM) remodeling (CD44, MMP2; the integrins ITGB1, ITGA3, ITGA7; and the laminins LAMA5, LAMB1). Although the most severe line manifested the larger number of dysregulated genes, a subset of genes commonly dysregulated were identified as possible key denominators of VWM pathology, including CCNA1, SIX3, AQP4, TP53I3, BRINP1, ANXA1, NELL2 and ARHGEF28. VWM-derived hGPCs thus manifest intrinsic pathology at a stage preceding their differentiation as astrocytes or oligodendrocytes. They express

pro-apoptotic transcripts at baseline, suggesting vulnerability to cell stress, and upregulate genes involved in inflammation and ECM remodeling, suggesting the potential for aberrant intercellular signaling patterns among glial cells within VWM white matter.

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T-3182

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% O2 or 20% O2 condition. Consistent with previous report, 5% O2 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. In addition, we have established Keratin 14-EGFP reporter iPSCs using Crispr/Cas9 system in order to monitor the differentiation status into keratinocytes. This study is expected to be a first step in the investigation of the underlying mechanism and a novel therapeutic development of Kindler syndrome.

T-3184

INSULIN RESPONSE IN HUMAN STEM CELL-DERIVED METABOLIC TISSUES

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We are in the midst of a worldwide epidemic of type 2 diabetes (T2D) and associated obesity. These disorders represent a complex interaction between genes and environment. In T2D there is a well-defined progressive pathogenesis, beginning with insulin resistance in peripheral tissues such as muscle, fat and liver. This is initially compensated for by increased insulin secretion, but eventually beta cells exhaust and insulin level gradually declines leading to clinical hyperglycemia. While some alterations in insulin action leading to insulin resistance have been defined and genome wide association studies have identified many genes associated with risk of T2D, the primary defect(s) in peripheral tissues leading to insulin resistance remains unclear. To elucidate the molecular basis of insulin resistance, we first need to accurately define the acute insulin response. Omics data on human insulin response is sparse, especially in a time-lapse fashion, as there are severe practical restrictions on performing these experiments. We show transcriptional response to insulin stimulation in a human in vitro model system, and we are able to characterize this acute response in several metabolic tissues derived from human pluripotent stem cells (hPSCs). The tissues we have investigated are adipocytes, hepatocytes, skeletal muscle, endothelial cells and vascular smooth muscle. We see a response in gene expression as early as 5 minutes after stimulation and can monitor this signal over time. These types of experiments are impossible to conduct in humans, demonstrating a clear advantage of our system. Several crucial transcription factors, such as MLXIPL and SREBF1, have a conserved response in multiple tissues. Both of these proteins are well known players in glucose and lipid metabolism. These early responders will turn on consecutive waves of transcription, from which we can gain an understanding of the cell's exact reaction to insulin stimulation. In this setting we then induce insulin resistance, through genome editing or chemical means, and comprehensively survey the changes in insulin response. Ultimately, we aim to illuminate novel and druggable targets in the insulin signaling pathway that would be of value in understanding and treating insulin resistance in the setting of human metabolic disease.

T-3186

USING PATIENT-DERIVED HIPSCS TO MODEL THE ROLE OF GLIA IN AUTISM SPECTRUM DISORDER

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Autism Spectrum Disorder (ASD) is defined as a group of neurodevelopmental disorders associated with impaired social communication, repetitive behaviors, and intellectual deficits. However, early diagnosis of ASD remains challenging due to its complexity and heterogeneity. In some subtypes of ASD, an increase in brain size precedes the first clinical signs, suggesting that understanding the mechanisms leading to brain overgrowth could provide important insights in disease onset. Here, we use human induced pluripotent stem cell (hiPSC) technology to model ASD associated with disproportionate megalencephaly (ASD-DM) and investigate the cellular and molecular mechanisms involved. While an overall enlargement in brain size has been shown through brain imaging of autistic children with megalencephaly, the iPSC models to date have primarily focused on modeling neurons in ASD. In this study, we investigate changes in glial cells by differentiating hiPSCs to brain-derived glia from control subjects and ASD-DM subjects. Changes in morphology, including the cell body size and the number of processes, and proliferation rates were compared across control and patient-derived hiPSCs. Preliminary data shows that the ASD-DM-derived glia have more processes. Also, increases in proliferation could be observed in glia from ASD-DM compared to the controls. We also investigate and compare changes in gene expression associated with the cell cycle. Overall, these data indicate that ASD with megalencephaly is possibly a result of enhanced proliferation. While prior studies have focused on changes in neurons, this study is the first to systematically investigate changes in glial cells in ASD-DM.

T-3188

THE NIMH REPOSITORY AND GENOMICS RESOURCE (NRGR): A GLOBAL RESOURCE FOR THE STUDY OF THE GENETICS OF PSYCHIATRIC DISEASE

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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 a cooperative grant from NIMH to establish stem cell repositories that provide high quality patient and control iPSCs and somatic cells from a wide range of disorders. The NIMH Stem Cell Center has 376 fibroblast and 325 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 , depression, eating disorders, obsessive-compulsive 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/.

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REPROGRAMMING

T-3192

EFFICIENT DIRECT LINEAGE REPROGRAMMING OF FIBROBLASTS INTO INDUCED CARDIOMYOCYTES USING NANOTOPOGRAPHICAL CUES

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Induced cardiomyocytes (iCMs) generated via direct lineage reprogramming offer a novel therapeutic target for the study and treatment of cardiac diseases. However, the efficiency of iCM generation is significantly low for therapeutic applications. Here, we show the efficient direct conversion of somatic fibroblasts into iCMs using nanotopographic cues. Direct conversion into iCMs on nanopatterned substrates resulted in a dramatic increase in the reprogramming efficiency and maturation of iCM phenotypes compared with that on flat substrates. Additionally, the enhanced reprogramming by the substrate nanotopography was derived from changes in the activation of focal adhesion kinase and specific histone modifications. Taken together, these results suggest that nanotopographic cues can serve as an efficient stimulant for direct lineage reprogramming into iCMs.

T-3194

CRITICAL ROLE OF THE CHROMATIN REGULATOR TIP60 IN NEURONAL FATE SPECIFICATION

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Direct lineage reprogramming of a somatic cell type into another has emerged as an attractive approach for generating patient-specific cells for cell replacement and tissue repair without the complications of immune rejection and teratogenicity. The direct conversion of non-neuronal cells into functional neurons is particularly promising in the context of developing treatments and modeling of neurological disorders given the lack of access to human neurons. We have previously shown that fibroblasts can be reprogrammed directly into functional induced neuronal (iN) cells by ectopic expression of three transcription factors, Ascl1, Brn2 and Myt1l (BAM). We have also found that Ascl1 alone is sufficient to initiate the iN reprogramming process and acts as a 'pioneer transcription factor' by binding and activating inaccessible chromatin regions. However, the precise mechanism by which Ascl1 activates host chromatin and neuronal gene expression in fibroblasts remains elusive. Here we used unbiased proteomics approach to identify chromatin regulators that mediate Ascl1-driven reprogramming in MEFs and identified Tip60 acetyltransferase complex as a key player in iN cell reprogramming. We found that suppression of Tip60 via shRNA mediated knockdown prevented iN generation induced by Ascl1. This reprogramming block could be rescued by re-expression of wild type Tip60, but not catalytically deficient mutant of Tip60. We further demonstrate that the acetyltransferase activity of Tip60 is essential for proper regulation of a subset of genes during reprograming and for establishing of neuronal cell identity. By elucidating detailed mechanisms of the function of Tip60 during iN induction our study provides insights into molecular mechanisms of iN cell reprogramming and basis for future strategies of iN cell generation for biomedical and therapeutic applications.

T-3196

MITIGATING ANTAGONISM BETWEEN TRANSCRIPTION AND PROLIFERATION ALLOWS NEAR-DETERMINISTIC CELLULAR REPROGRAMMING

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Although cellular reprogramming enables the generation of new cell types for disease modeling and regenerative therapies, reprogramming remains a rare cellular event. By examining the reprogramming of fibroblasts into motor neurons and multiple other somatic lineages, we find that epigenetic barriers to conversion can be overcome by endowing cells with the ability to mitigate an inherent antagonism between transcription and DNA replication. We show that transcription factor overexpression induces unusually high rates of transcription, and that maintaining hypertranscription early in reprogramming is critical for successful lineage conversion. However, hypertranscription impedes DNA replication and restricts cell proliferation, which also promotes reprogramming. We identify a chemical and genetic cocktail that dramatically increases the number of cells capable of simultaneous hypertranscription and hyperproliferation in part through activating topoisomerases. Further, we show that hypertranscribing, hyperproliferating cells reprogram at 100-fold higher, near-deterministic rates. Therefore, relaxing biophysical constraints overcomes epigenetic barriers to cellular reprogramming.

T-3198

RAPID GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS USING A BETA-DEFENSIN3-DERIVED PEPTIDE AND INTEGRATION-FREE NUCLEOFECTION

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Somatic reprogramming from matured cells to induced pluripotent stem cells (iPSCs) is one of the best model to represent a mesenchymal-to-epithelial transition (MET). The low efficiency of iPSC generation has been improved by enhancing MET using microRNA, small molecules, and surface stiffness. However, they have some limitations like stability and toxicity. Here, we discovered a synthetic peptide from human beta-defensin-3 (hBD3) with anti-inflammatory functions and applied to iPSC generation. The generation of iPSCs was induced by nucleofection of four transcription factors (Oct4, Sox-2, KLF4 and c-Myc) and the treatment of the hBD3-derived peptide. We found that the peptide significantly increases endogenous expression of stemness genes (Oct4 and Nanog) and epitheial genes (E-cadherin and Ep-CAM) compared to non-treated group. Also the peptide reduces the time of iPSC generation compared to non-treated group. The colonies produced using nucleofection and peptide treatment has in vitro and in vivo differentiation potentials into three germ layers. Taken together, our peptide eliminates potential risks associated with the use of chemical small molecules, providing the promising technique for patient-derived iPSCs.

T-3200

CHANGES IN ENERGY METABOLISM AND SUBCELLULAR ORGANELLES IN PLURIPOTENT STEM CELLS DUE TO METABOLIC SWITCHING

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Because of their distinctive character, human pluripotent stem cells (hPSCs) undergo significant changes not only in their gene expression but also in the production and/or expenditure of energy in the form of ATP via switching of their metabolic signature from mitochondrial oxidative phosphorylation (OXPHOS) to glycolysis. This phenomenon is referred to as the Warburg effect and is a hallmark of hPSCs. The translation of functional proteins from mRNA in the cytoplasm is a major cause of energy consumption in the cell. We performed a comparative proteomics study of induced hPSCs and the fibroblasts from which they were derived, and the results suggest that mRNA translation is reduced in hPSCs. In addition, compared with somatic cells, hPSCs have large nuclei and scanty cytoplasm, which signify changes in their subcellular organelles. Our investigation of organelle function in hPSCs revealed significant changes in the mitochondrial and lysosomal functions that lead to the metabolic switch in these cells.

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T-3202

INDUCTION OF PLURIPOTENCY BY ALTERNATIVE FACTORS

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Reprogramming somatic cells to pluripotency represents a paradigm for cell fate determination. A binary logic of closing and opening chromatin provides a simple way to understand iPSC reprogramming driven by both Yamanaka factors or chemicals. Here we apply this logic to the design a seven factors combination, Jdp2, Jhdm1b, Mkk6, Glis1, Nanog, Essrb and Sall4 (7F), that reprogram MEFs to chimera competent iPSCs efficiently. RNA-and ATAC-seq reveal differences between 7F and Yamanaka Factors induced pluripotency, 7IP and YIP, in transcriptomic and chromatin accessibility dynamics(CAD). Sall4 emerges as a dominant force that can close and open chromatin with the help of Jdp2 and Glis1 in resetting somatic chromatin to a pluripotent state. These results reveal a previously unknown path between somatic and pluripotent states, open a door for cell fate control.

T-3204

VANGL2 RECEPTOR OVEREXPRESSION INCREASES APOPTOSIS AND EFFECTS INFLAMMATION AS WELL AS MIGRATION AND DIFFERENTIATIONS IN HUMAN HEK293 CELLS

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We had earlier shown that Wnt7a which is a ligand for Vangl2 receptor effects apoptosis and migration of epithelial cells. The purpose of current study is to investigate if Vangl2 is effected in wound healing and if this signaling pathway is effected when anesthetics are used. 1.Analyzing closer which molecules mediate Vangl2 signalings effect on apoptosis by investigating caspase-8 singling using live cell imaging and investigate if this signaling pathway is intervened when anesthetics are applied. 2.Analyzing closer which molecules mediate Vangl2 signalings effect on migration and see if its mediated via ezrin,paladin and FGF7 and look closer into whether this signaling pathway is intervened when anesthetics are applied. We conclude that Vangl2 overexpression/silencing effects apoptosis and hypothezise that this is done via caspase-8 signaling. We also suggest that the rearrangement of p53 and Th17 expression obtained in our preliminary experiments is a result of actin translocation from the cytoplasm into the nucleus and is probably mediated by exosomes/micro-RNAs and possibly interfered when anesthetics are applied.We further suggest that the delay in differentiation is a result of Vangl2 signalings effect on the expression of Cx43.

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T-3206

TEMPORAL RESOLUTION OF GLOBAL GENE EXPRESSION AND DNA METHYLATION CHANGES REVEALS UNIQUE FEATURES OF EPIGENETIC REMODELLING IN THE FINAL PHASES OF INDUCED PLURIPOTENCY

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Transcription factor-mediated reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) progresses via sequential events to clear epigenetic roadblocks in the path of full pluripotency acquisition. A better understanding of the global epigenome resetting involved is not only relevant to improving the quality of iPSCs, but also in understanding disease. The use of markers to characterize intermediate stages is essential to explore the underlying mechanisms, but existing markers do not provide sufficient temporal resolution of global transcriptomic and DNA methylation changes that occur during the late maturation stage of iPSC generation. The 5-methylcytosine dioxygenase Tet1 is part of the DNA demethylation machinery and is highly elevated in endogenous expression along with the master pluripotency factor Oct4 late in reprogramming. Here, we generated murine transgenic lines harboring dual fluorescent reporters reflecting cell-state specific expression of Oct4 and Tet1. By assessing reprogramming intermediates based on dual reporter patterns, we identified a sequential order of Tet1 and Oct4 gene activation at proximal and distal regulatory elements following pluripotency entry. Full induction of Tet1 marks a pivotal late intermediate stage occurring after a phase of global gene repression, and preceding full activation of Oct4 along with late naive pluripotency and germline-specific genes. Sequential activation of Tet1 further distinguishes two waves of global DNA demethylation, targeting distinct genomic features and largely uncoupled from transcriptional changes. Absence of Tet1 is compatible with reprogramming towards full Oct4 gene activation, but generates iPSCs with aberrant DNA methylation, chromosomal instability during lineage priming and defective differentiation potential. Our study captured the dynamics of global epigenome reprogramming with unprecedented precision, revealing molecular parallels and distinction from physiological reprogramming that may account for observed epigenetic differences between embryo-derived and experimentally induced pluripotent cells. Furthermore, the transcriptional logic of Tet1 expression signals a deterministic epigenetic roadmap towards generation of high-quality iPSCs.

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T-3208

PHYSIOXIC CONDITIONS WITHOUT SUBOPTIMAL TRANSIENTS DURING CELL HANDLING INCREASES HUMAN BONE MARROW MSC YIELDS

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Isolated from tissues that normally are at low oxygen levels like the bone marrow stem cell niche, Mesenchymal Stromal Cells (MSC) and the exosomes derived from them are of intense clinical interest. Many cell culturists still handle MSC in conventional room air biological safety cabinets (BSC) even though MSC are isolated from and returned to tissues with profoundly low oxygen levels. This causes stress for MSC. We have shown previously that subjecting cells to room air conditions (suboptimal transients) during cell handling negative impacts MSC yields. Here, we sought to determine what unbroken O2 level produced the best yields of human bone marrow MSC, testing 0.5%, 1%, 3%, or 18% O2, each with 5%CO2. Using the Xvivo System, all temperature and gas levels were controlled continuously, making it possible to maintain optimal low O2 conditions for the cells during all cell handling steps as well as incubation. Our null hypothesis was there would be no difference in MSC growth between the conditions. The cell handling chamber conditions were set to match incubation conditions, so each culture was in constant conditions and media were pre-equilibrated to the matching O2 levels overnight before use. Cells were counted at each passage and cells were seeded into 96-well plates and stained with crystal violet to visualize cell growth. Vessel headspace and pericellular O2 were measured before each passage. Differences were seen in cell growth with higher cumulative cell yields and faster cell growth were seen when cells were maintained continuously at 1% and 3% O2, than at 0.5% or 18%O2. Pericellular O2 levels were lower than chamber O2 levels. Cells maintained at 18% O2 senesced earlier than the other cultures. We conclude that MSC cultures can be grown to higher yields when conditions are controlled for cell handling at the appropriate tissue O2 levels.

TECHNOLOGIES FOR STEM CELL RESEARCH

T-3210

CRISPR/CAS9 GENE EDITING FOR GENERATING IPSC MODELS OF HUMAN DISEASES AND DEVELOPMENT

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Since its inception in 1998, RUCDR Infinite Biologics (RUCDR, www.rucdr.org) has provided the scientific community with the highest quality biomaterials, technical consultation, and logistical support. The stem cell lab of RUCDR provides stem cell services to researchers, the NIH and non-profit foundations. These services include the banking and distribution of source cells and induced pluripotent stem cells (iPSC) and the generation of iPSC from human somatic cells. Most recently, we have begun offering CRISPR gene editing service in human iPSCs through the use of CRISPR (clustered regularly-interspaced short palindromic repeats)/Cas9 technology. The diverse genetic background of the human subjects has hampered the usefulness of iPSCs for modeling human diseases and development. The use of the CRISPR/Cas9 system can create isogenic cell lines that will serve as better controls and help eliminate effects that are due to genetic variance rather than a biological mechanism. At RUCDR/Infinite Biologics we have developed a high throughput, cost efficient workflow for using CRISPR/Cas9 to genetically modify iPSC from affected or unaffected subjects. Using this strategy, we have generated footprint-free and precisely edited isogenic iPSC pairs harboring mutations involved in neurological disorders such as Alzheimer's Disease, Amyotrophic Lateral Sclerosis (ALS) and Parkinson's Disease. All edited iPSC lines are tested rigorously for off-target effects, homogeneity, pluripotency and genetic integrity. Data of our quality control process is included in the COA for each iPSC line we distribute.

T-3212

QUANTITATIVE LIVE-CELL ANALYSIS CHARACTERIZING MORPHOLOGY AND FUNCTION OF IPSC-DERIVED NEURONS AND SUPPORT CELLS

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Recent advances in stem cell technologies offer an exciting alternative to rodent models for investigating the human nervous system and neurological disorders. The ability to simultaneously monitor morphology and functional readouts over time is critical for thorough characterization of human induced pluripotent stem cell (hiPSC)-derived models. Typically endpoint assays of subtype-specific surface marker expression and morphological features are employed. These techniques have proven valuable, but they fail to couple insight into the ability of these cells to exhibit neuronal activity and develop mature networks. In the case of hiPSC-derived microglia, it is important to ensure that these cells are capable of critical neuroimmune functions such as phagocytosis and chemotaxis. Here we present live-cell imaging data used to quantify functional readouts of hiPSC-derived neurons and microglia and qualitatively assess morphology using the IncuCyte® for Neuroscience. Monitoring of differentiating neuro-progenitor cells (Axol Bioscience) enabled the maturation to iPSC-derived neurons to be visualized over >80 days, with marked morphological and functional development. Activity of hiPSC-derived glutamatergic and GABAergic neurons (CDI, iCell Neurons) was visualized and analyzed using the Incucyte® NeuroBurst reagent, a genetically encoded calcium indicator. While both neuronal types exhibited spontaneous activity, only glutamatergic neurons developed coordinated synaptic network activity over time in culture. To exemplify the benefit of long term monitoring of these models, the CNS.4U® (NCardia) co-culture model of hiPSC-derived neurons and astrocytes were evaluated for over 40 days. While spontaneous activity was detected after one week, correlated network bursting was only observed after 30 days in culture. Finally, hiPSC-derived microglia (Axol Bioscience) were monitored for morphological changes following differentiation and evaluated for phagocytic potential by use of a pH sensitive dye. Collectively, these data illustrate the ability to monitor and characterize hiPSC-derived neuronal cultures and support cells over time using live-cell analysis techniques.

T-3214

ENHANCING FUNCTIONAL MATURATION OF HUMAN IPSC-DERIVED NEURONS WITH NOVEL PEPTIDE AMPHIPHILE NANOSTRUCTURES

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Human induced pluripotent stem cell (iPSC)-based technologies offer a unique resource for modeling disease and regenerating complex tissues such as the central nervous system (CNS). However, iPSC models are still fraught with significant technical limitations including inefficient maturation, abnormal aggregation and reduced long-term viability of neurons. We reasoned that the lack of physiological extracellular matrix (ECM) conditions contributes to these problems and establishing a stable and bioactive ECM environment would facilitate the functional maturation of iPSC-derived neurons. To test this, we utilized peptide amphiphiles (PAs), a class of biomaterials that have the ability to assemble into supramolecular nanofibers capable of morphologically and chemically mimicking the ECM. We designed a series of PAs scaffolds containing a short bioactive peptide (IKVAV) found in Laminin-1, which plays a major role in neuronal behavior in the CNS. The newly design PAs have an identical chemical bioactivity but the supramolecular fibers that they form drastically differ in terms of mobility. Here, we have specifically explored how the molecular mobility of ECM signals impacts the maturation of different classes of iPSC-derived human neurons. We identified an IKVAV-PA that dramatically enhanced the maturation of iPSC-derived cortical and motor neurons in vitro. The beneficial activity of this IKVAV-PA strongly correlated with its intermolecular hydrogen-bonding domain, which facilitated high molecular mobility, mimicking the dynamic ECM-neuronal interactions. Neurons assembled within the high dynamic IKVAV-PA exhibited reduced aggregation, enhanced arborization, and a mature pattern of electrical activity closely resembling neurons co-cultured with primary glial cells. Using global proteomic analysis and high-resolution microscopy, we traced these effects to specific and relevant molecular pathways that were downstream of laminin-1/Integrin beta-1 signaling pathway. Our PA technology highlights the importance of the ECM in recapitulating in vivo conditions and offers a more physiological and translational platform to study the development, function and dysfunction of the CNS in disease or injury using iPSC-based approaches.

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T-3216

CORRELATION BETWEEN CELL MORPHOLOGICAL INFORMATION OBTAINED FROM DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY (D-IHM) AND FUNCTIONAL CHARACTERISTICS OF HUMAN MESENCHYMAL STEM CELLS

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In this study, we focused on the morphological information of human mesenchymal stem cells (MSCs) obtained from digital in-line holographic microscopic images (D-IHM) acquired by CultureScanner CS-1. MSCs are considered the most attractive cell source for regenerative medicine. MSCs have been highlighted because of their multi-potentialities, ease of culture and expansion in vitro. MSCs have already been utilized in clinical trials, including for GVHD, autoimmune diseases, inflammatory bowel disease and spinal cord injury, as therapeutic applications for tissue regeneration and in immune regulation. The therapeutic effect of MSCs is highly affected by the characteristic of cultured cells. Characteristics of MSCs are mainly evaluated by cell function, such as gene expression, protein expression, proliferation, differentiation and cellular energy metabolism analysis. While these have advantages in evaluating various factors, there are risks of destruction and invasion of cells during non-sterile operations. Furthermore, it is difficult to directly evaluate the cells themselves used for therapeutic administration. In contrast, the morphological evaluation of cultured cells is mainly performed by phase contrast microscopy. Morphological characteristics, such as cytoplasmic and nuclear shapes in individual cells, cell density and cell luminance are obtained through this method, but it is difficult to estimate these features objectively or further predict cell function. Therefore, we focused on the morphological analysis using D-IHM, which provide information of optical thickness and transmittance of cultured cells non-invasively and over time in a wide field of view. We have previously shown the significance of measuring the lengths of the minor axis (LMA) of cultured MSCs in phase contrast images. In smaller LMA, there is higher proliferation, migration and the secretion of regenerating factors. However, it is difficult to quantify LMA objectively in large numbers of cells. We investigated how D-IHM tended to correlate with LMA and functional characteristics of human umbilical cord-derived MSCs. We will report on the utility of morphological information obtained from D-IHM as a novel non-invasive method for predicting functional properties of MSCs.

Funding Source: Shimadzu Corporation

T-3218

LONGITUDINAL INTRAVITAL IMAGING OF TRANSPLANTED MESENCHYMAL STEM CELLS ELUCIDATES THEIR FUNCTIONAL INTEGRATION AND THERAPEUTIC POTENCY IN AN ANIMAL MODEL OF INTERSTITIAL CYSTITIS

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Mesenchymal stem cell (MSC) therapy may be a novel approach to improve interstitial cystitis/bladder pain syndrome (IC/BPS), an intractable disease characterized by severe pelvic pain and urinary frequency. Unfortunately, the properties of transplanted stem cells have not been directly analyzed in vivo, which hampers elucidation of the therapeutic mechanisms of these cells and optimization of transplantation protocols. Here, we monitored the behaviors of multipotent stem cells (M-MSCs) derived from human embryonic stem cells (hESCs) in real time using a novel combination of in vivo confocal endoscopic and microscopic imaging and demonstrated their improved therapeutic potency in a chronic IC/BPS animal model. Ten-week-old female Sprague-Dawley rats were instilled with 10 mg of protamine sulfate followed by 750 μg of lipopolysaccharide weekly for 5 weeks. The sham group was instilled with phosphate-buffered saline (PBS). Thereafter, the indicated dose (0.1, 0.25, 0.5, and 1×106 cells) of M-MSCs or PBS was injected once into the outer layer of the bladder. The distribution, perivascular integration, and therapeutic effects of M-MSCs were monitored by in vivo endoscopic and confocal microscopic imaging, awake cystometry, and histological and gene expression analyses. A novel combination of longitudinal intravital confocal fluorescence imaging and microcystoscopy in living animals, together with immunofluorescence analysis of bladder tissues, demonstrated that transplanted M-MSCs engrafted following differentiation into multiple cell types and gradually integrated into a perivascular-like structure until 30 days after transplantation. The beneficial effects of transplanted M-MSCs on bladder voiding function and the pathological characteristics of the bladder were efficient and long-lasting due to the stable engraftment of these cells. This longitudinal bioimaging study of transplanted hESC-derived M-MSCs in living animals reveals their long-term functional integration, which underlies the improved therapeutic effects of these cells on IC/BPS.

T-3220

UNIVERSAL CORRECTION OF BLOOD COAGULATION FACTOR VIII IN PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS USING CRISPR/CAS9

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Hemophilia A (HA) is caused by genetic mutations in the blood coagulation factor VIII (FVIII). Genome editing approaches can be used to target the mutated site itself in patient-derived induced pluripotent stem cells (iPSCs). However, these approaches can be hampered by difficulty preparing thousands of editing platforms for each corresponding variant found in HA patients. Here, we report a universal approach to correcting various mutations in HA patient iPSCs by the targeted insertion of the FVIII gene into the human H11 site via CRISPR/Cas9. We derived corrected clones from two types of patient iPSCs with frequencies of up to 64% and 66%, respectively, without detectable unwanted off-target mutations. Moreover, we demonstrated that endothelial cells differentiated from the corrected iPSCs successfully secreted functional protein in vitro and functionally rescued the disease phenotype in vivo. This strategy may provide a universal therapeutic method for correcting all genetic variants found in HA patients.

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T-3222

ARTIFICIAL INTELLIGENCE CAPTURES STRUCTURAL TOXICITY IN HUMAN IN VITRO CELL MODELS

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Predicting drug toxicity is an important part of the drug development process as adverse side effects are the main causes for drug attrition. In vitro human cell models, such as cells differentiated from induced pluripotent stem cells (iPSCs), can reflect human-specific physiology and pharmacology and have been increasingly investigated to evaluate toxicity early in drug development. Most cell-based imaging assays focus

on cell damage through mitochondrial deficiency or nuclear count, or functional defects such as arrhythmia or prolonged contraction time in cardiomyocytes. There is a need for new assays that can capture structural changes in cells, which may complement existing assays, improve sensitivity, and better predict clinical toxicity. We propose a novel method, PhenoTox, which uses artificial intelligence (deep neural networks) to capture subtle structural changes in cell cultures that relate to toxic drug effects. The input to PhenoTox is a collection of microscopy images captured and grouped at multiple doses and time-points for the drug of interest and a control set for each time point where no drug is applied. PhenoTox performs a series of 2-class neural network trainings comparing controls to the test conditions and generates a classification accuracy for each training. The final output is a heatmap of the z-factors across all test conditions, depicting the doses and timepoints at which structural changes have happened and how strongly they differ from controls. As part of a large ongoing study, we applied PhenoTox to characterize the effects of drugs with known toxicity profiles on both iPSC-derived hepatocytes and cardiomyocytes, including Tamoxifen, Doxorubicin, and Aspirin as a negative control. The cells were fixed and stained, and fluorescence microscopy images were collected from multiple wells with multiple locations per well. For Tamoxifen-treated hepatocytes, PhenoTox detected structural changes that correlated with loss of Cytochrome P450 3A4 activity. For Doxorubicin-treated cardiomyocytes, PhenoTox detected structural changes that correlated strongly with decreased contraction displacement. No structural change was detected for Aspirin. These results indicate that PhenoTox captures structural changes across multiple cell types that correlate with functional results.

T-3224

MURINE SCNT-DERIVED ESCS AND IPSCS WITH MISMATCHED MITOCHONDRIA TRIGGER AN IMMUNE RESPONSE IN VIVO

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The generation of human pluripotent stem cells by somatic cell nuclear transfer (SCNT) has sparked new interest in this technology since this methodical breakthrough speculated that SCNT would allow the creation of patient-matched embryonic stem cells, even in patients with hereditary mitochondrial diseases. However, herein we show that mismatched mitochondria in nuclear-transfer derived mouse embryonic stem cells (NT-ESCs) possess alloantigenicity and are subject to immune rejection. In a murine transplantation setup, we demonstrate that allogeneic mitochondria in NT-ESCs, which are nucleus-identical to the recipient, may trigger an adaptive alloimmune response that impairs the survival of NT-ESC grafts. The immune response is adaptive, directed against mitochondrial content, and amenable for tolerance induction. Mitochondrial alloantigenicity should therefore be considered when developing therapeutic SCNT-based strategies. In this context, despite their autologous nature, rejection of induced pluripotent stem cell (iPSC)-derived cells has been reported, although mechanistic details remain largely unknown. We hypothesized that new mutations in the mitochondrial DNA (mtDNA), which has far less reliable repair mechanisms than the chromosomal DNA, can give rise to neoantigens capable of eliciting an allogeneic immune response and causing rejection. Here we show that in mice, mutagenic nonsynonymous mtDNA single nucleotide polymorphisms (SNPs) generate relevant neoantigens, which initiate a highly specific immune response. This neo-antigenicity of SNPs can diminish the survival of iPSC grafts and their derivatives in autologous recipients. Thus, autologous iPSCs and their derivatives are not inherently immunologically inert for autologous transplantation and therefore techniques need to be developed to monitor mtDNA mutations and SNP enrichments during the manufacturing of autologous iPSC products.

T-3226

GENE EDITING AND MODULATION TOOLS FOR LONG NON-CODING RNA APPLICATIONS

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Approximately 98% of the human genomic sequence is characterized as non-coding RNA. A large subgroup of RNA, called long non-coding RNAs (lncRNAs), are characterized as having sequences of 200 nucleotides long or more and mediate numerous biological processes, including stem cell differentiation and maintenance. Although more and more novel lncRNAs have been identified through advancement of sequencing technologies, the biological purpose of the majority of lncRNAs remain unclear due to their low and cell specific expression

levels, varied cellular localization, shared genomic sequences with coding transcripts, and lack of effective investigating technologies. Here we evaluated several gene editing and modulation technologies that can be applied for lncRNA study: (1) RNA interference: transfection of siRNA enabled efficient repression of targeted mRNA level; (2) Cas9/RNP: Co-delivery of two synthetic gRNA with Cas9 protein into the cells resulted in gene knockouts through specific gene deletion; (3) Lentiviral based CRISPR system: we further established a dual gRNA lentiviral system that allows expression of two gRNAs for deleting user defined genomic DNA sequences. We will discuss advantages and limitations of each technology and provide useful guidance for lncRNA applications in relevant cell models, including, but not limited to, stem cells.

Funding Source: California Institute for Regenerative Medicine
Thermo Fisher Scientific

T-3228

BOVINE PLATELET LYSATE-DERIVED SERUM NEOSERA IS SAFE, LESS ETHICAL AND POWERFUL ALTERNATIVE TO FETAL BOVINE SERUM FOR THE CULTURE OF MESENCHYMAL STEM CELLS

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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 platelet lysate-derived serum “NeoSERA” .Using apheresis medical devices with closed disposable kits, sterile bovine serum NeoSERA is collected from healthy bovine receiving a regular veterinary check. After stimulation and removal of coagulated fibrin by centrifugation, NeoSERA 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§113.450), NeoSERA is finally gamma-irradiated at a dose of more than 30 kGy. NeoSERA® completely meets the standard for biological ingredients in Japan (Ministry of Health, Labour and Welfare Notification No.375 2014) and also obtained the certificate of eligibility for the raw material of regenerative medicine from Pharmaceuticals and Medical Devices Agency (PMDA, No.0417002, 2017. 4.17). Similar to blood donation, NeoSERA can repeatedly obtain from few adult bovine without sacrifice, indicating less moral problem and lot-to-lot variation. To test whether NeoSERA is useful for the expansion of mesenchymal stem cells (MSCs), a cell culture experiment was performed. 3 to 5 days after NeoSERA treatment, the proliferation of human bone marrow-, adipose tissue-, umbilical cord- and amnion-derived MSCs was significantly increased ($p < 0.05$) compared to FBS. Our results confirm that safe, less ethial and powerful adult bovine platelet-lysate-derived serum NeoSERA profoundly enhances MSC proliferation.

T-3230

UBIQUITOUS AND CELL-SPECIFIC RESPONSES TO DISSOCIATION: IN SITU FIXATION AS A TOOL TO INVESTIGATE DYNAMIC TRANSCRIPTIONAL EVENTS

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The microenvironment plays a critical role in the specification, maintenance and alteration of cell identity. It is hard to conceive a more radical niche alteration than disconnecting a cell from its tissue of origin. Nevertheless, dissociating a cell from a tissue to study its in vivo properties is a standard practice in almost every study. As most cell dissociation protocols are an order of magnitude (hours) longer than a cell's transcriptional response to stimuli (minutes) it is likely that the profiles of freshly isolated cells no longer reflect that of their in vivo counterparts. To this end, our team developed In situ Fixation, an aldehyde-based protocol that preserves a cell's transcriptome during isolation. We uncovered that standard dissociation of skeletal muscle stem cells (MuSCs) induces significant alterations on over half of the detected transcripts, shifting MuSCs from a quiescent to an activated state (Machado et al. 2017). In our most recent work, we have applied In situ Fixation to dissect the molecular events leading to MuSCs quiescence exit in a temporally resolved manner. Comprehensive time-course experiments coupled to in vivo single cell RNA-seq (scRNA-seq) on resting and injured muscle has identified early response factors involved in the transition of quiescent MuSC to activation. The work presented at this meeting, describes the first precise transcriptional cartography of MuSC quiescence and activation, featuring modules of coexpressed genes with dynamic and complex transcriptional behavior. Moreover, by scrutinizing published scRNA-seq atlases of various cell types from diverse tissues, we found signatures strikingly akin to the dissociation-induced artefact we detected in freshly isolated MuSCs. To investigate global and cell-specific responses to dissociation, methods to produce single-cell tissue atlases coupled to In situ Fixation in a high-throughput format, will be discussed. To conclude, our work aims to raise awareness about the universality and magnitude of dissociation-induced artefacts and call for a paradigm shift in how we think of freshly isolated cells. Finally, we propose In situ Fixation as an affordable, accessible and versatile method to produce artefact-free transcriptomic data for an increasingly accurate profile of cells in multicellular organisms.

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T-3232

DEVELOPING A HUMAN MESENCHYMAL STEM CELL CULTURE MEDIUM WITH HIGH GROWTH EFFICIENT, 3D CULTURE SUPPORTED, SERUM-FREE AND XENO-FREE PROPERTIES

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Human mesenchymal stem cell (MSC) therapies have been tested clinically for a variety of disorders, including Crohn's disease, multiple sclerosis, graft-versus-host disease (GVHD), bone fractures, sepsis, and cartilage defects. Despite of the remarkable clinical advancements in this field, most cell manufacturing processes still rely on the traditional culture medium containing fetal bovine serum (FBS). The highly inconsistence nature and xenogenic infection risks of the bovine serum remain a challenge for the standardization and massive production for clinics. Here, we successfully develop a medium, featured with serum-free, xeno-free components and high efficacy for MSC growth, named as AllPhase. The medium on human adipose tissue-derived mesenchymal stem cells (ADSC) and Wharton's jelly mesenchymal stem cells (WJ-MSC) enables competitive expansion performance, comparing to traditional culture medium and can maintains tri-lineage mesoderm differentiation potential after fifth passages. Furthermore, most commercial serum-free media need expansive ECM coating, like fibronectin or other commercial coating materials, to support cell adhesion. Especially, AllPhase can support ADSC expansion under 3D culture condition without microcarriers. We hope that our medium can support most bioreactors and cell industrial production for MSC clinical applications.

T-3234

ENGRAFTMENT AND PERSISTENCE OF L-MYC IMMORTALIZED NEURAL STEM CELLS IN ADULT MOUSE BRAIN

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Human neural stem cells (hNSCs) demonstrate an inherent tropism to sites of damage in the CNS that can in the context of repair can potentially be exploited for delivery of therapeutic agents promoting regeneration, and/or in situ differentiation to achieve overt cell replacement. The effectiveness of any hNSC-mediated therapy will depend on the number and viability of hNSCs reaching the target site, their appropriate engraftment, and the absence of tumorigenicity. We previously demonstrated migration of L-myc immortalized hNSCs (LM-NSC008) to sites of tumor and injury in immunodeficient (NSG) mouse brain (Gutova et al, 2016). Here we have visualized LM-NSC008 cells in optically cleared mouse brains after 2, 4, 8 and 12 months of engraftment without evidence of tumorigenicity, demonstrating their potential for long-term therapeutic deployment. Their distributions over

time was volumetrically imaged and computationally brought into registration with a Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) mouse brain atlas, and compared favorably with previously predicted migration originating in the corpus callosum. This work shows the feasibility of using 3D optical imaging on cleared tissue to evaluate engraftment, migration, distribution and fate of LM-NSC008 cells in mouse brain models for optimizing NSC-mediated cellular therapies for damaged and diseased brain. This work has also helped to validate prior computational models of NSC migration.

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T-3236

FORWARD CELLOMICS APPROACH USING MOSAIC HUMAN ORGANOID LIBRARY: FROM PHENOTYPE TO GENOTYPE

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Emerging biobanks provide a population-scale human induced pluripotent stem cells (hiPSC) library from genotyped donors, thus enabling an accessible platform to study human gene expressional variation such as gene expression quantitative trait loci (eQTL). Despite the promise of evolving organoid approach for precision analysis, conventional laboratory scale protocols are not equipped to perform large comparative analyses as culturing each organoid separately is heavily variable, costly and labor intensive. To circumvent this challenge, we approached the en masse efficacy screening strategy by developing a mosaic human liver organoid library from iPSC containing up to 20 individuals, followed by in vitro "GWAS" study to determine genotype-phenotype correlation. Specifically, we generated a mosaic human liver organoid by mixing a variety of different donor derived foregut in a single well and evaluated the Non-Alcoholic Steato Hepatitis (NASH) risk of each donor using a lipid accumulation phenotype. As a result of each organoid genotyping with a donor specific barcode, i.e. SNPs, mixed foregut gave rise to form "clonal" liver organoids from a single donor. Furthermore, diagnostic value of this approach was tested by determining sensitivity/specificity of lipid accumulation with the mosaic liver organoid library that includes lysosomal acid lipase deficiency. The area under the ROC curve was 0.888, which indicates the possibility to identify high risk donors in the mosaic organoid library. Next, we stratified them into high / low group with lipid accumulation phenotype, determined the risk SNP haplotypes for steatosis phenotype and compared odds ratio of reported NASH, which was identified through large-scale clinical cohort studies. The NASH risk SNPs PNPLA3 odd ratio was 1.81697(95% confidence interval: 1.16544 - 2.84399). In XKR 6, which should decrease serum triglyceride level, there was inverse correlation with lipid accumulation phenotype (odd rate: 0.566687, 95% confidence interval: 0.334000 - 0.951662). These results suggest the significance of population pooling approach to determine individualized basis for disease precision with relatively small sample due to minimal bias, noise and batch variation, potentially revolutionizing drug development as well as personalized therapy.

T-3238

CHARACTERISATION OF NOVEL HUMAN GENOMIC SAFE HARBOURS FOR CONTROLLED TRANSGENE EXPRESSION

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Controlled expression of transgenes in cells is essential in both therapeutic and research applications. Traditionally, such transgene expression has been accomplished via viral vector integration into the host genome, in a generally random fashion. Genome-engineering technologies, such as CRISPR/Cas9, allow for directed integration of transgenes in the cell of interest. The ideal integration sites for the foreign genetic material are 'safe harbour' sites, which allow for controlled expression of a transgene without perturbing endogenous gene expression patterns. To date, only a limited number of targeted integration sites have been reported and characterised in the human genome. These include for example, the AAVS1 site, CCR5 locus and the human homolog of ROSA26. However, none these sites meet stringent requirements for a genomic safe harbour (GHS) locus. We sought to discover putative human GSH, by first identifying loci in the human genome that are located outside DNAse clusters, gene transcription units and ultra-conserved regions, as well as >300kb away from any known oncogenes, miRNAs and lncRNAs. We further refined our list of candidate GSHs by cross referencing against a set of stable housekeeping genes (defined from the GTEx dataset) as well as against highly active chromatin compartments (inferred from published chromatin conformation data of 21 human primary tissues and cell types). Our final list has approximately 2000 putative human safe harbour loci. Using CRISPR/Cas9 targeting in H1 hES cells, we have successfully targeted two shortlisted

GSH located on chromosomes 1 and 5. Transgenes introduced to the two GSH are expressed stably and at a high level in hES cells and their integration shows no significant alteration in any of the native genes in the chromosomal neighbourhood. The two GSH are currently undergoing further in vitro characterisation via differentiation of targeted hES cells into all germ-lineages, as well as through targeting the GSH in other cell types. The uncovered GSH have the potential to enable truly targeted and controllable transgene expression without interference to the native transcriptome, a desired outcome in both research and gene therapy.

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T-3240

A STRUCTURE-BASED EXTRACELLULAR MATRIX MODIFICATION PROMOTES PARAXIAL MESODERM DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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The in vitro differentiation of human induced pluripotent stem cells (hiPSCs), which have great therapeutic potentials, was designed by recapitulating embryogenesis. However, more robust methods for lineage-specific differentiation are still under development. Considering the complexity of in vivo embryogenesis environment, extracellular matrix (ECM) should be taken into account as a key factor in concurrence with toning growth factors for lineage-specific differentiation. Here, we describe a system for differentiating hiPSCs on the new generation laminin (NGL) which is a recombinant form of a laminin-421 E8 fragment conjugated to the heparan sulfate chains (HS) attachment domain of perlecan. In this system, HS bind to fibroblast growth factor-2 (FGF2), form a stable high affinity HS-FGF2-FGFR complexes on cell surface, then strongly stimulate FGFR signaling pathway. Recapitulating embryogenesis in hiPSCs differentiation, NGL time-dependently increases the marker genes expression of primitive streak and paraxial mesoderm lineage. These effects depend on the unique structure of NGL, that could not be replaced by treating with high dose of FGF2 or coating the mixture of laminin-421 E8 and perlecan. In addition, using this xeno-free matrix, we established a highly efficient differentiation system for hiPSCs induced paraxial mesoderm lineage, subsequently, paraxial mesoderm derived myocytes and muscle stem cells, thus providing infinite source for disease modeling and regenerative medicine.

T-3242

DEVELOPMENT OF A BIOPROCESS XENO-FREE HUMAN MSCS CELL CULTURE MEDIA

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There are currently more than 1000 clinical trials involving hMSCs registered at clinicaltrials.gov, representing a steady increase of over 20% per year for the last 10 years. With a typical clinical “dose” in the range of 1E7 to 1E8 cells, this proliferation of clinical testing is driving a massive increase in demand for high quality media. In support of these high-volume clinical applications, hMSC culture media needs to be both animal origin-free and straightforward to use in downstream applications. In particular, elimination of substrate coating and media replacement steps can reduce cost and streamline operations. In response to these requirements, we focused on developing a medium formulation that allows batch culture with no media replacement, requires no substrate coating, is xeno-free and still supports high rates of hMSC proliferation. We used high-throughput formulation DOE with multiple and single factorial analysis to identify critical components of the medium, which led to 5 medium formulations. These formulations were then screened for proliferation of MSCs from 5 distinct donors to select a final formulation. Final formulation effects on MSCs were then validated on bone marrow-derived MSCs (4 donors) and umbilical cord-derived MSCs (2 donors). In addition, the final formulation was tested not only in 2D adherent culture but also in 3D suspension culture using microcarriers. We characterized the cells using a panel of assays designed to evaluate cell health and functionality. The panel included expansion kinetics (cell density), cell surface marker expression (flow cytometry analysis for CD14, CD34, CD45, CD73, CD90, CD105, and CD166), cytokine secretion (FGF, HGF, IL-8, TIMP-1, TIMP-2, and VEGF), trilineage differentiation potential (osteogenesis, adipogenesis and chondrogenesis) and immunomodulatory function (response to IFNγ stimulation). Our results demonstrate that our optimized bioprocess xeno-free cell culture medium supports high rates of proliferation across multiple hMSC culture platforms while maintaining cell robustness and functionality.

LATE-BREAKING ABSTRACTS

T-4002

IMPACT OF METFORMIN ON HUMAN PLURIPOTENT STEM CELL DERIVED NEUROECTODERM AND CEREBRAL ORGANOIDS

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Metformin is a biguanide drug and anti-hyperglycemic agent commonly used as treatment for Type 2 diabetes. It is increasingly used before and during pregnancy to treat women with pre-pregnancy diabetes, polycystic ovary syndrome (PCOS) and gestational diabetes (GDM). However, unlike insulin, metformin can easily cross the placenta. Long-term clinical studies on the impact of metformin on offspring health are still very limited. There have been extensive in vitro studies on the anti-hyperglycemic actions of metformin exposure on liver cells, skeletal muscle and adipose tissues. However, there is very little known about the extent of in utero metformin exposure and its effects on fetal development. Using human embryonic stem cells (hESCs) and human pluripotent stem cell (hPSC)-derived germ layers and cerebral organoids, we aimed to study the impact of metformin treatment on the development and function of fetal tissue. We found that metformin treatment resulted in hESCs to exhibit significant, concentration-dependent upregulation of pluripotency factors SOX2 and NANOG. Interestingly, hESC-derived neuroectoderm showed significantly reduced expression of neural markers such as SOX1, despite the upregulation of SOX2 in hESCs. Additionally, further differentiated cerebral organoids demonstrated lowered expression of the forebrain marker FOXG1 at both transcript and protein level. These findings suggest that high concentrations of metformin treatment can perturb fetal neural development. Additional experiments are currently underway to investigate the mechanisms by which metformin elicits these effects on the neural lineage.

T-4004

AGGREGATION OF HUMAN PLURIPOTENT STEM CELLS ON MICROPATTERNED SURFACE INDUCES TROPHOBLAST-LIKE INTESTINAL EPITHELIUM CELLS FOR MINI-GUT ORGANOID PRODUCTION

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There are variety of methods to obtain intestinal organoids from pluripotent stem cells. We previously reported a new method to induce highly functioned intestinal organoids (Mini-Guts) structure from human pluripotent stem cells using 1500μm diameter micropatterned surface (Uchida et al., JCI Insight 2017). However, detailed mechanism about the differentiation had remained unknown. In this study, we focused on cellular aggregation of pluripotent stem cells, more frequently observed on the micropatterned surface. Since then, we discovered the aggregation contained CDX2+/KRT7+ trophoblast-like intestinal epithelium cells, confirmed by immunostaining and human chorionic gonadotropin (hCG) secretion. This phenomenon might have been caused by decreasing OCT4 expression of the aggregated cells. To support this phenomenon, the epithelium cells of the Mini-Guts co-expressed fetus marker, such as KRT7 and AFP. Next, we drew a comparison between the pattern surface and bare-glass for the cell culture efficiency. We discovered the pattern surface led to rapid intestinal cell differentiation confirmed by villin western blotting and hCG ELISA analysis. Moreover, we also found, using transgenic iPS cells, neuroblast-marker expressing cell in the surrounded aggregated area on 2 or 3weeks from the seeding day, suggesting that the aggregation contains neuronal differentiating cells as well as intestinal differentiating cells. Based on these results, we newly invented ring-pattern shaped cell-culture surface, consisting of cell non-attachable hollow area in the center and cell attachable area. Using this surface, we induced Mini-Guts more efficiently compared to the previous circle pattern. Our results will contribute to produce intestinal organoids more efficiently and to understand the benefits of high-density culture of pluripotent stem cells for the intestinal differentiation

T-4006

THREE-DIMENSIONAL RECONSTITUTION OF MINIATURE BLADDERS WITH TISSUE STROMA THAT RECAPITULATES IN VIVO TISSUE REGENERATION AND CANCER

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Current organoid models have limitations because of the missing factors in such platforms, including mature architecture and microenvironment. Here we reconstitute the tissue stem cell-based, multi-layered miniature bladders that structurally and

functionally mimic the mammalian urinary bladders. These mini-bladders recapitulate in vivo tissue dynamics of regenerative response to bacterial infection in that, with the heightened activity of signal feedback between urothelium and the stroma and its associated increase in cell proliferation, the regenerative portions of urothelium arise from single cells through polyclonal expansion. Furthermore, using three-dimensional bioprinting technology, we develop multi-layered tumor organoids with the stroma that recapitulate the in vivo patho-physiology of patient-derived invasive urothelial carcinoma representing tumor-stromal interaction, slower drug response, immune cell infiltration, and muscle invasion. Our study thus provides a conceptual framework for the reconstitution of multi-layered, functional organoids derived from tissue stem cells or tumor cells that mimic the biology of native tissues.

T-4008

XENOBIOTIC METABOLISM IN HUMAN GUT ORGANOIDS FROM PLURIPOTENT STEM CELLS

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The absorption, distribution, metabolism, excretion and toxicity (ADMET) processes of drugs are of importance and require preclinical investigation not only in the liver but also in the intestine. Human intestinal absorption is the first gate for an orally taken therapeutic and one of important ADMET properties for drug efficacy. Various models have been developed for prediction of ADMET in the intestine. One of the most available cells is Caco-2 cells, which are colon cancer cell line. Caco-2 based monolayer culture was reported as the first barrier model and has been considered as the standard in studying intestinal disposition of drugs in vitro. However, it is different from the human intestinal cells in the expression pattern of the drug transporters. It is difficult to accurately predict absorption rate in humans, and there is a limit as a model system. In addition, the expression level of metabolizing enzymes such as CYP3A4 is very low, and so it could not be generally used for evaluation of drug metabolism. We have reported to originally generate functional human gut organoids (mini-guts) from pluripotent stem cells (PSCs) under xenogeneic-free culture conditions in vitro. The mini-guts showed the gut tube-like architecture consisted of mucosa and submucosa by histological, immunofluorescence and electron micrograph examinations. Here, we focus on intestinal metabolic activity in the mini-guts. Human drug metabolism PCR array was performed using RNA isolated single mini-gut and showed major intestinal transporters (ABCB1, ABCC2, ABCC1 ABCC3 and SLC15A1) and metabolic enzymes (CYP3A4, CYP3A5, CYP2C9 and CYP2C19) were similar expression level with normal adult intestine as control. Furthermore, induction assay of CYP3A4 and ABCB1 (P-glycoprotein) by 1, 25-dehydroxyvitamin D3 (VD3) demonstrated that markedly induced CYP3A4 and ABCB1 expression in the mini-guts. In contrast, VD3 had little effect on both expressions in Caco-2 cells. Subsequently, CYP3A4 assay with Luciferin-IPA containing a substrate for cytochrome 3A4 showed that CYP3A4 activity in mini-guts. These data suggest that mini-guts from hPSCs have intestinal absorption and metabolism ability in vitro. The mini-guts enable us to evaluate human intestinal ADMET in a dish and thus provide a novel bio-model for pharmacological testing.

T-4010

HIGH-THROUGHPUT MICROFLUIDIC PLATFORM FOR STUDYING VASCULARIZATION OF IPSC-DERIVED KIDNEY ORGANOIDS

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Kidney organoids derived from human induced pluripotent stem cells (iPSCs) represent a powerful in vitro model for studying kidney development, disease mechanisms and drug testing. Despite the great level of structural complexity reached in vitro, these kidney organoids are immature possibly due to the lack of a functional vascular system. Transplantation of kidney organoids under the kidney capsule of a mouse can significantly improve their maturation. However, alternative approaches are valuable for studying these processes in vitro. Microfluidic techniques show great potential in bridging the gap between 2D in vitro cultures and animal models. Here, we present the use of a high-throughput in vitro 'grafting' platform which allows co-culture of vessels with kidney organoids. One unit of the Mimetas Organoplate® Graft is made of two microfluidic channels in which endothelial cells can be patterned against ECM. Presence of a tissue chamber allows endothelial cell co-culture with 3D tissues. When kidney organoids are used, extensive vascular remodeling occurred with formation of a

complex 3D network of angiogenic sprouts growing towards the tissue. Moreover, vessel stabilization can be monitored overtime by real time imaging and perfusion with 150 kDa Dextran. The established kidney organoid-on-a-chip system provides a promising platform for drug testing and disease modeling.

T-4012

A TUBULAR ORGANOID-DERIVED GUT-ON-A-CHIP MODEL BY PRESERVING THE STEM CELL NICHE OF LGR5+ INTESTINAL EPITHELIA

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Microfluidic techniques are increasingly recognized as an important toolbox to add physiologically relevant cues to traditional cell culture. These cues include long term gradient stability and continuous perfusion. Microfluidic technology allows patterning of cell layers as stratified co-cultures that are devoid of artificial membranes, in order to capture complex tissue architectures found in vivo. Previously, we have introduced the OrganoPlate® platform for growing human intestinal gut tubules in a membrane-free manner. Although suitable for toxicity studies, this model uses human intestinal cell lines, such as adenocarcinoma line Caco-2, which has limited differentiation capabilities and harbors multiple gene mutations. In contrast, Lgr5+ intestinal organoids can develop crypt-villi morphology and form an epithelial barrier – features associated with gut epithelium. These organoids are usually grown as a polarized ball-like structures embedded in an ECM, with limited apical access. Here we show a human organoid gut-on-a-chip model which is composed of Lgr5+ gut epithelial cells grown inside of the microfluidic channels of the OrganoPlate®. We established a tubular shaped epithelial barrier model of the intestinal tract showing rapid cell polarization, tight junction formation and proper expression of intestinal markers. These gut tubules are suitable for high-throughput screening of compound effects through real time imaging of transport and barrier integrity. Moreover, the OrganoPlate® facilitates development of complex models of gut epithelial tubules co-cultured with endothelial vessels. These complex gut-on-a-chip models allow mimicking disease phenotypes such as inflammatory bowel diseases (IBD) and support screening for potential drug targets. Protocols have been established that allow automated readout of the barrier integrity, followed by image analysis and quantification. The combination of Lgr5+ gut organoids with the OrganoPlate® technology are a powerful combination to study physiology and disease mechanisms in patient specific gut models.

T-4014

SCALABLE MULTIWELL MICROELECTRODE ARRAY (MEA) TECHNOLOGY FOR THE EVALUATION OF CARDIAC AND NEURAL THREE-DIMENSIONAL CELL CULTURES

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Three-dimensional induced pluripotent stem cell (iPSC)-derived in vitro models, commonly referred to as spheroids or organoids, more accurately recreate the multicellular organization and structure of in vivo tissues when compared to traditional monolayer stem cell cultures. However, to effectively characterize 3D iPSC cell cultures, or to extract meaningful and predictive information from these models, new technology is required for evaluating functional cellular and network responses. For electro-active cells, like cardiomyocytes or neurons, measurements of electrophysiological activity across a networked population of cells provide a comprehensive view of function. Microelectrode array (MEA) technology offers such a platform by directly connecting key biological variables, such as gene expression or ion channel distributions, to measures of cellular and network function. Furthermore, the advent of multiwell MEA platforms has enabled scalable throughput capacity for 3D cell culture applications including toxicological and safety screening, disease modeling, and developmental biology. Neural or cardiac electrical activity can be captured simultaneously from each electrode on an MEA multiwell plate and in relation to the position of the spheroid on the array. Here, we present data supporting the use of multiwell MEA technology as an efficient non-invasive approach to capture electrophysiological activity from individual iPSC-derived spheroids. Human iPSC-derived cardiomyocytes and neural spheroids were cultured on 6-well MEA plates and monitored throughout maturation of their network connections. Cardiac electrophysiological activity, including spike amplitude and field potential duration, were recorded in response to compounds to provide information on the depolarization and repolarization of the cardiomyocyte action potential. For neural cultures, functional endpoints, such as network bursting and synchrony, were measured to define cellular activity across neural spheroids within a network. These results support the continued development and use of human

iPSC-derived cardiomyocyte and neural spheroid assays on multiwell MEA technology for high throughput drug toxicity and safety assessment, evaluation of phenotypic disease-in-a dish models, and cell development.

T-4016

REGULATION OF NEURONAL DIFFERENTIATION BY AUTS2 IN HUMAN BRAIN ORGANOIDS

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Recent genetic and genomic efforts have identified hundreds of risk genes for neurological disorders. However, the functional impact of most of these disease-associated alleles remains unclear due to the remarkable complexity of the nervous system. There is a tremendous need to define cell type-specific effects and underlying mechanisms in order to better understand disease pathogenesis, improve diagnoses, and ultimately to develop treatments for neurodevelopmental disorders. Our research seeks to address these challenges for a key risk gene, Autism Susceptibility Candidate 2 (AUTS2), and define its role in neural differentiation. AUTS2, initially reported as disrupted in a pair of twins with autism spectrum disorders (ASD), is also linked with other neurodevelopmental disorders, including intellectual disability, attention deficit hyperactivity disorder, and schizophrenia. AUTS2 maps to chromosome 7q11.2 and its expression peaks in neocortex of E13.5 mice, suggesting a role in neurogenesis. Indeed, mouse and zebrafish models of Auts2 deletion show a defect in neurodevelopment, although cellular and molecular mechanisms remain poorly understood. To understand the role of AUTS2 in neural development, we recently generated human embryonic stem cells (hESC) lacking AUTS2 that mimics the disruption in an intellectual disability patient. Using a human brain organoid in vitro culture model, our preliminary results showed that deletion of AUTS2 leads to severe defects in neuronal differentiation. In addition, our initial single-cell RNA-seq (scRNAseq) analysis identified a cell population that is lost upon AUTS2 knockout. Interestingly, these cells are enriched for FOXG1, a master regulator for cortex development through promoting neuronal progenitor cell (NPC) proliferation. Taken together, our studies have provided critical mechanistic insights toward understanding the neuronal differentiation program regulated by AUTS2, a prominent risk factor for neurodevelopmental disorders, which may lead to the development of therapeutic strategies for these diseases.

T-4018

TRANSCRIPTOMIC STATE OF HUMAN PLURIPOTENT STEM CELLS PREDICTS THE SUCCESS OF CEREBRAL ORGANOID DIFFERENTIATION

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Defects in brain development underlie many neurological diseases; yet, the critical mechanisms are not understood. Efforts to investigate these mechanisms utilize animal models. However, the human brain has distinct features from other species. Increasing evidence suggests that these human-specific features may be impacted, illustrating the need for human-specific models. One such model involves directing human pluripotent stem cells (hPSCs) to form brain-like structures termed organoids. Cerebral organoids recapitulate many aspects of human fetal brains including the formation of neural progenitor regions and cortical layering. Our lab previously developed a reproducible and efficient protocol to generate cerebral organoids. The success of cerebral organoid differentiation is influenced by the maintenance of hPSCs. Variabilities arise due to batch differences in mouse embryonic fibroblast feeders, which are required for organoid formation, and in defined media components. We seek to identify the key factors that impact neural differentiation to improve outcomes. Transcriptional analyses showed that hPSCs yielding the best organoids displayed elevated expression of TGF-beta superfamily signaling molecules and genes associated with naïve pluripotency. We are testing different growth factor combinations to enhance organoid formation under feeder-free conditions. Identifying these molecules will yield effective protocols for optimizing cerebral organoids to elucidate human neurological disease mechanisms and accelerate drug discovery.

Funding Source: UCLA-CSUN CIRM-Bridges training program (TB1-00183), UCLA Broad Stem Cell Research Center and the NICHD (K99HD096105), California Institute for Regenerative Medicine (CIRM) (DISC1-08819)

T-4020

GMP-COMPATIBLE ENGINEERED HUMAN MYOCARDIUM FOR HEART REPAIR

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Human pluripotent stem cell-derived cardiomyocytes hold great potential for bona fide remuscularization of the human heart. We have developed a process to generate large heart muscle patches for epicardial implantation. Here, we report on the transfer of our previously established research protocol to a fully GMP-compliant process for heart failure repair in patients. Methods and Results: To obtain sufficient starting material, we generated a GMP working cell bank (WCB) from human iPSC. 200 vials with 1x10E7 cells each were obtained from a GMP master cell bank. The WCB was validated to retain a high pluripotency and stable karyotype. GMP-compliant engineered human myocardium (EHM) is generated from two active substances, cardiomyocytes and stromal cells. Cardiomyocytes were obtained by directed differentiation using small molecules and growth factors. Four test runs were performed to optimize the protocol. In additional validation runs reproducible production of the clinical target dose (8x10E8 cardiomyocytes) with high purity (95±2% sarcomeric actinin-positive cells) was demonstrated per run. By immunostaining and action potential analysis we found that the majority of cardiomyocytes (26 out of 27 measured) displayed a ventricular phenotype with a prominent plateau phase (APD20/APD80>0.3). Similarly, stromal cells (Vim+, CD90+, Collagen I+) were obtained by directed differentiation of the iPSC WCB and cryo-stored until use. To generate GMP-compatible EHM patches iPSC-derived cardiomyocytes were mixed with stromal cells in a collagen hydrogel and cultured under defined, serum-free conditions yielding contracting GMP heart patches in the clinically relevant target size (3.5x3.5 cm). In conclusion,we demonstrate that engineered human myocardium can be prepared in a clinically applicable and scalable manner. The developed process is the basis for the first-in-class BioVAT-HF trial.

T-4022

ELECTROPHYSIOLOGICAL PHENOTYPE CHARACTERIZATION OF HUMAN IPSC-DERIVED NEURONAL-CELL LINES BY MEANS OF HIGH-RESOLUTION MICROELECTRODE ARRAYS

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High-resolution-microelectrode-array (HD-MEA) technology enables to study neuronal dynamics across different scales, ranging from single-axon physiology to connectivity of large networks (Müller et. al, Lab on a Chip, 2015). We have used this HD-MEA technology (26'400 electrodes at 17.5 m pitch) to compare the electrical phenotypes of 6 human neuronal cell lines (dopaminergic neurons, dopaminergic neurons α-synuclein A53T - Parkinsons disease, glutamatergic neurons, motor neurons, motor neurons SOD1-G93A -ALS, motor neurons TDP43-Q331K -ALS, all from Fujifilm Cellular Dynamics International). All neuronal lines were co-cultured with human astrocytes. Neuronal electrical activity was recorded at DIV7, DIV14 and DIV21. All neuron lines showed robust synchronized/oscillating bursting activity at DIVs 14 and 21. We used the following parameters to characterize the neuron's electrical activity: Mean Firing Rate (MFR, Hz), Mean Spike Amplitude (MSA, uV), Percentage of Active Electrodes (PAE), Burst Peak Amplitude (BP, Hz) and Interburst Interval (IBI, s). Using HD-MEA technology, we measured the following median coefficients of variation (CVs) for each parameter across developmental time points: MFR = 0.2, MSA = 0.10, PAE = 0.24, BP = 0.27 and IBI = 0.13. Furthermore, we extracted the corresponding CVs for low-resolution MEAs recordings by using the data of 16 electrodes arranged at a pitch of 300 um: MFR = 0.64, MSA = 0.28, PAE = 0.5, BP = 0.42 and IBI = 0.18. We found that the use of HD-MEA technology enables to phenotype the electrical activity of several neuronal lines with significantly lower sample-to-sample variability, as compared to state-of-the art low-resolution MEA technology. Furthermore, we isolated single-neuron electrical activity, (1) quantified network functional connectivity, and (2) characterized subcellular electrical features (e.g., axon propagation velocity along axons). We found differences in network connectivity and subcellular electrical features among the tested lines, and most importantly, between healthy lines and their diseased isogenic counterparts. We conclude that high-resolution MEA systems enable to access novel electrophysiological parameters of iPSC-derived neurons, which can be potentially used as biomarkers for phenotype screening and drug testing.

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T-4024

DRUG-INDUCED SEIZURE-LIKE ACTIVITIES IN HUMAN IPSC-DERIVED NEURONS

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Human iPSC-derived neurons are expected to be applied to toxicity evaluations in nonclinical studies. Microelectrode array (MEA), measurement system of the electrophysiological activity, are suitable to evaluate the seizure liability of drugs. We have previously reported the electrophysiological responses to several convulsive compounds using MEA in cultured hiPSC-derived neurons. However, the identification of analytical parameters to detecting seizure liability remains an important issue. In this study, we identified the parameter sets that can separate the

responses between convulsive drugs and negative control, and the responses among the several convulsants with different mechanism of action. Twelve compounds (pentylenetetrazole, picrotoxin, 4-aminopyrdine, linopyridine, amoxapine, strychnine, pilocarpine, amoxicillin, chlorpromazine, enoxacin, phenytoin and acetaminophen) selected by HESI NeuTox MEA subteam were tested at 5 concentrations for each compound. We found that the Total Firing Rate, Inter Burst Interval, Max Firing Rate (MF) and CV of MF are especially effective parameters for classification of drug responses. As a result of using the principal component analysis (PCA) method and the clustering method using four parameters, the convulsive positive compound and the negative compound were separated, and the mechanism of action of the convulsive positive compound was also classified. PCA and clustering method using the selected parameter set are useful for the prediction of seizure liability and mechanism of action of new drugs in cultured hiPSC-derived neuronal networks.

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T-4026

CELLRAFT AIR SYSTEM FOR STEM CELL ANALYSIS: DIFFERENTIATION, CRISPR AND ORGANOID WORKFLOWS

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Contemporary stem cell workflows often rely on isolating single cells for either single cell molecular analysis or clonal colony propagation. Due to stem cell-specific viability properties, isolating single cells remains a bottleneck in analytical workflows. To eliminate these cell biology limitations, Cell Microsystems has developed the CellRaft AIR System. The AIR System allows automated imaging of thousands of individual cells on a single cell culture consumable, the CytoSort Array. CytoSort Arrays come in a range of forms tailored to various laboratory workflows, each comprising thousands of microwells in which cells randomly segregate and settle. Each microwell contains a releasable plastic floor, or CellRaft, which serves as a microscale cell culture substrate. Using the AIR System software, cells on the CytoSort Array can be imaged and sorted based on fluorescence intensity in three fluorescent channels. To isolate single cells from the array, the AIR System mechanically releases the individual microscale CellRafts from each microwell and physically places them into a 96-well plate or collection tube. The CellRaft technology provides a gentle, microwell-based method for imaging, sorting and isolating stem cells for various single cell applications.

T-4028

PATTERNING HUMAN PLURIPOTENT STEM CELL FATES USING ENGINEERED MORPHOGEN GRADIENTS

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Spatiotemporal regulation of signaling pathways orchestrates diverse cellular programs, such as cell division, differentiation, and migration throughout development. Perturbing these signaling pathways by applying biomolecules of varying concentrations in cell media is a central tenant in stem cell biology. However, a method to spatially and dynamically regulate biomolecule solute patterns in vitro that is accessible to a wide range of researchers has not previously been achieved. To address this limitation, we developed a method incorporating tunable design, dynamic capabilities, and compatibility with standard 2D culture platforms. We first absorbed biomolecules into spatially-defined agarose-coated surfaces or into device-contained micromolded agarose gels. Gel surfaces loaded with biomolecules were then placed within 100um of cells in standard 2D culture. These predefined patterns of biomolecules were rapidly or sustainably transferred with high fidelity from the device to the underlying cells by diffusion. We demonstrated precise patterning of dissolved species including small molecule cell dyes, macromolecules, and viral particles. We also adapted our devices to produce gradients of morphogen signals (bone morphogenic protein 4 – BMP4, and Wnt agonist – CHIR99021) across human pluripotent stem cells. Immunostaining for lineage-specific transcription factors, Sox2, Brachyury, and CDX2, demonstrated distributions in cell fate specification that correlated with the imposed signal patterns. We further modified these distributions of cell fates by tuning the stimulus gradient geometry and amplitude. Our technology provides added control over the micron-scale spatial location of biomolecule presentation for cell fate patterning. This technology has numerous potential applications across diverse facets of stem cell biology.

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T-4030

AUTOMATED HUMAN INDUCED PLURIPOTENT STEM CELL CULTURE AND DIFFERENTIATION OF RETINAL PIGMENTED EPITHELIUM ON THE TECAN FLUENT WORKSTATION FOR PERSONALIZED DRUG SCREENING

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Cell types differentiated from human induced pluripotent stem cells (hiPSCs) provide the opportunity to generate medically important cells from individual patients and patient populations. This in turn allows drug screening and disease modelling to be carried out using more relevant and phenotypically accurate cells than traditional immortalized cell lines. However, technical complexities including lack of both scale and standardization and the lengthy time periods involved in the standard culture and differentiation of hiPSCs have limited the adoption of these technologies for personalized drug screening. The entry of reproducible end-to-end automated workflows for these processes, demonstrated on commercially available platforms, would provide enhanced accessibility of this technology to commercial pharmaceutical testing. We have utilized a TECAN Fluent automated cell culture workstation to perform hiPSC culture and differentiation in a scalable manner to generate patient-derived retinal pigmented epithelial cells for drug screening. hiPSCs derived from multiple patients with the dry form of age-related macular degeneration (AMD) were introduced into our automated workflow and the patient specific cells were cultured and differentiated into retinal pigmented epithelium (RPE). Each patient's hiPSC-derived RPE subsequently entered an automated screening workflow with a compound library targeting improved mitochondrial function. Mitochondrial respiration in the treated cells was then measured offline with the Agilent Seahorse XF system. This system performs scalable, robust, reproducible culture and differentiation of multiple individual cell lines from different patients on the TECAN Fluent platform and demonstrates the potential for end-to-end automation of hiPSC-based personalized drug testing.

T-4032

3D MIDBRAIN FLOOR PLATE MODEL FOR DIFFERENTIATION OF HUMAN PSC-DERIVED DOPAMINERGIC NEURONS

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Accurate in vitro modeling of neurological diseases requires multiple cell types of the brain to interact and develop toward mature functionality. When human pluripotent stem cells (PSC) undergo neural differentiation in 3D, self-organization of progeny cells results in organoids with brain-like structures and functions that are not observed in 2D culture. However, the increased complexity of neural organoids often comes with the costs of low throughput and poor reproducibility. Disease models for drug discovery may therefore have to temper self-organized complexity with inductive specification of desired cell types. To model Parkinsons Disease (PD), we have developed a method for differentiation of human PSC to midbrain dopaminergic (DA) neurons that combines elements of 2D dissociated culture and 3D organoid culture. Cells are efficiently specified as midbrain floor plate (FP) in 2D via an established protocol using a combination of small molecules and growth factors. FP cells are then seeded into suspension culture in defined numbers for spheroid formation and expansion, then maintained in suspension for two to five weeks of differentiation. Early differentiation of the 3D cultures is marked by morphological change and the appearance of Nurrl- and tyrosine hydroxylase (TH)-expressing DA neurons at the organoid surface. Single-cell analysis demonstrates that many neurons co-express Sox6+ TH+ and thus resemble midbrain DA neurons of the Substantia Nigra pars compacta (SNc). Replating of spheroids on extracellular matrix results in neurite outgrowth and outward migration of DA neurons. Multielectrode array (MEA) recording of replated spheroids shows spontaneous burst activity within a relatively short time, followed by gradual refinement toward coordinated rhythmic bursting. To test disease modeling in the hybrid 2D/3D system, CRISPR was used to make PD-related mutations (LRRK2 G2019S and SNCA A30P) in a Cas9-expressing iPSC line. These lines were differentiated to generate DA neurons as above, and to demonstrate sensitivity of the mutant SNc-like DA neurons to oxidative stress. In short, a hybrid 2D/3D culture system for iPSC-derived midbrain floor plate improves maturation of DA neurons and makes promising steps toward a reproducible in vitro disease model for Parkinsons.

T-4034

RSPO AND LGR-MEDIATED WNT SIGNALLING IN STEM CELLS, DEVELOPMENT AND CANCER

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Stem cells are instrumental during organogenesis, adult tissue homeostasis and promote regeneration in response to injury. Stem cell intrinsic factors as well as extrinsic cues provided by the immediate microenvironment, known as the niche, regulate organ-specific stem cell behaviors. WNT signaling mediates key developmental and homeostatic processes in mammals. During embryonic development, WNT signals induce transcriptional programs that control cell proliferation, cell survival, cell fate determination, and tissue patterning. In adults, the WNT pathway is fundamental in defining stem cell niches in several organs, which in turn maintain tissue homeostasis. Deregulation of the WNT pathway contributes to developmental defects as well as

to the initiation and progression of human diseases including several types of cancer. The R-spondin family comprises four evolutionary conserved secreted ligands. They function as key WNT enhancers by preventing the degradation of WNT receptor complexes triggered by two E3 ubiquitin ligases RNF43 and ZNRF3. Until recently, RSPOs were believed to exclusively execute this role by engaging their obligate cognate receptors LGR4, LGR5 and LGR6. Our data, however, demonstrates that the LGR4/5/6 receptors are dispensable for RSPO2 and RSPO3 functions during organogenesis. Using transgenic mouse lines, and ex vivo organoid culture techniques we aim to further identify novel WNT-related mechanisms in stem cells, development and cancer. Revealing additional layers of WNT signal modulation will undoubtedly have fundamental implications in cell biology, development and oncogenesis and may uncover novel targets for the development of more effective therapeutics against WNT-associated diseases and cancers.

T-4036

REPLACEMENT HISTONE VARIANT H3.3 MAINTAINS HSC SELF-RENEWAL AND LINEAGE DIFFERENTIATION BY SAFEGUARDING GENOME INTEGRITY

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Epigenetic signature and chromatin components serve as pivotal regulators of hematopoietic stem cell (HSC) self-renewal and lineage differentiation. Histone variant H3.3 is encoded by two genes, H3.3A and H3.3B. We report the enrichment patterns of H3.3 within adult HSCs. H3.3B is deposited at the regulatory regions and gene bodies of actively transcribed genes, promoter regions of bivalent genes, and constitutive heterochromatin regions. Deletion of H3.3A or H3.3B alone did not alter homeostatic hematopoiesis, demonstrating the compensatory effect of H3.3A and H3.3B. Inducible global deletion of H3f3a on H3.3B -/- background in adult resulted in exhaustion of HSC, imbalanced HSC differentiation towards myeloid lineage, and loss of B and T cells. Competitive transplantation assay demonstrated the cell autonomous requirement of H3.3 in maintaining HSC repopulation and lineage differentiation. Upon deletion of H3.3, HSC manifested reduced lymphoid and myeloid cell differentiation. Using in vitro endothelial-cell based coculture systems, we confirmed that H3.3 is essential for ex vivo expansion, and differentiation of adult HSPCs into B cells or CD11b+Gr1- cells. We performed Chip-seq of constitutive heterochromatin marks H3K9me3 and facultative heterochromatin marks H3K27me3 for HSCs at respective conditions. There are 2299 shared significantly reduced H3K9me3 peaks in HSCs from H3.3A-/- H3.3B-/- double knockout (DKO) mice compared with WT or BKO HSCs, among three biological replicates. Notably, there are H3K9me3 reduced mountains at the telomeric regions, suggesting the requirement of H3.3 in maintaining genome integrity. There are 174 shared significantly reduced H3K27me3 peaks in HSCs from DKO mice compared with WT or BKO HSCs. The commonly reduced H3K9me3 regions reside at LTR, LINE and intron regions, etc. Consequently, deletion of H3.3 resulted in increased death and apoptosis of HSCs, altered cell cycle progression and increased inflammatory signatures both in vitro and in vivo, in line with the premature myeloid priming of HSCs with elevated CD16/32 expression. We thus propose that H3.3 maintains the H3K9me3 levels and genome integrity at telomeric regions and repetitive regions. Loss of H3.3 induces DNA hypersensitivity, increased inflammation and premature myeloid priming of HSCs.

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T-4038

MESENCHYMAL STEM CELLS-DERIVED SECRETOME SUPPRESSES CHRONIC INFLAMMATION IN COLLAGEN-INDUCED ARTHRITIS MICE

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Secretome derived from mesenchymal stem cells (MSCs) contribute to improved recovery against tissue injury. However, the therapeutic effects of secretome are not fully understood. We investigated the potential therapeutic effects of secretome in collagen-induced arthritis (CIA) murine model representing rheumatoid arthritis (RA), and explored the mechanism and regulatory components underlying immune modulation by secretome. Secretome was produced in adipose-derived MSCs. CIA mice were injected intraperitoneally with 200 μg of secretome. Treatment-control animals were injected with 35 mg/kg methotrexate (MTX) twice weekly. Clinical activity in CIA mice, degree of inflammation, cytokine expression in the joint, serum cytokine levels, and regulatory T cells (Tregs) were evaluated.

Cytokine array performed to define immunoregulatory proteins of secetome. Mice treated with secretome showed significant improvement in clinical joint score, comparable to MTX-treated mice. Histologic examination showed greatly reduced joint inflammation and damage in secretome-treated mice compared with untreated mice. Secretome significantly decreased serum tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-12(p70) and increased IL-10 levels. Helper T cell 1 (Th1) was decreased and anti-inflammatory macrophage was increased in mice treated with secretome compared to untreated or MTX-treated mice. MSCs-derived secretome significantly suppressed joint inflammation in CIA mice and decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines. Therefore, our study suggests that the use of secretome could be an effective therapeutic approach for RA.

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T-4040

IN VIVO COMPLETE REGENERATION OF BONE DEFECTS USING PODS® CRYSTALS, A LOCALIZED AND SUSTAINED RELEASE TECHNOLOGY.

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Standard recombinant growth factors are inherently unstable, with short half-lives, which limits their utility in the lab and even more so in the clinic. In contrast, PODS® (POlyhedrin Delivery System) growth factors are a highly durable crystalline protein formulation. These protein crystals are produced in cultured insect cells by co-expressing cargo protein with polyhedrin protein, which self-assembles to encase and protect the protein of interest within each polyhedrin crystal. Thus, PODS® crystals contain intact, native, and functional protein. Resistant to many chemical and physical stresses and therefore extremely stable in storage, PODS® crystals degrade slowly over several weeks and steadily release the active cargo protein. Here, we show the complete healing of a rat calvaria bone defect with a single application of PODS® BMP-2 impregnated in absorbable collagen sponge vastly outperforms the regenerative effect of standard recombinant BMP-2. This proof of principle study demonstrates the use of PODS® crystals for therapeutic protein delivery.

T-4042

ROLE OF CD133+ PROGENITOR CELLS IN THE PATHOGENESIS OF VASCULAR REMODELING AND PULMONARY HYPERTENSION

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Pulmonary hypertension (PH) is characterized by progressive increase of pulmonary vascular resistance and obliterative pulmonary vascular remodeling that result in right heart hypertrophy, failure and premature death. Owing to the poor understanding of the underlying mechanisms of obliterative vascular remodeling, current therapies result in only modest improvement in morbidity and mortality. The underlying mechanisms of occlusive vascular lesions remain unclear. Recently, we have reported the first mouse model of PH [Tie2Cre-mediated disruption of Egln1, encoding hypoxia inducible factor (HIF) prolyl hydroxylase 2 (PHD2), designated Egln1Tie2Cre] with progressive obliterative vascular remodeling. We identified a subpopulation of lung progenitor cells expressing CD133 which was upregulated in Egln1Tie2Cre mice. Lineage tracing studies (CD133-CreERT2;Rosa26mTmG/+) demonstrated that CD133+ cell give rise to endothelial cells and smooth muscle cells in mice. Genetic depletion of CD133+ cell population in mice (CD133-CreERT2;Rosa26iDTR/+) by diphtheria toxin treatment inhibited chronic hypoxia-induced PH. RNA-seq analysis suggest that there are multiple subpopulations of CD133+ cells including CD133+/CD157+ endothelial progenitor cells, CD133+ /KLF4+ smooth muscle progenitor cells, which showed highly proliferative potential. We also demonstrated that pulmonary endothelial cells release CXCL12, and genetic deletion of CXCL12 receptor CXCR4 in CD133+ cells (CD133-CreERT2;Cxcr4f/f) protected hypoxia-induced PH in mice. Taken together, our studies demonstrated the novel role of CD133+ cells in the pathogenesis of vascular remodeling and PH.

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T-4044

REVERSIBLE SWITCHING OF LEUKEMIC CELLS TO DRUG-RESISTANT, STEM CELL LIKE SUBSETS IN CROSS-TALK WITH MESENCHYMAL STROMA

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Chemoresistance of leukemic cells has been largely attributed to the clonal evolution secondary to accumulating mutations. However, the possible impact of the bone marrow microenvironment in the development of chemoresistant subsets has not been well explored. Here, we show that subsets of leukemic blasts in contact with mesenchymal stroma undergo functional conversion into a distinct type of blasts that exhibit a stem cell-like phenotype and chemoresistance. These stroma-induced changes occurred in a reversible and stochastic manner independent of cell fusion or mitochondrial transfer. Moreover, the frequency of conversion into new phenotype was maintained at constant levels, being rapidly equilibrated among the equipotent leukemic cell population. The development of distinct leukemic subsets was driven by cross-talk whereby leukemic cells in stromal contact induces IL-4 expression in leukemic cells which in turn targets mesenchymal stroma to facilitate development of new leukemic subsets through up-regulation of VCAM-1 expression in MSCs and tight adherence to leukemic cells. The stroma-dependent generation of resistant leukemic subsets occurred both in murine and human leukemic cells including patients-derived leukemic blasts, but was abrogated on MSCs selectively depleted of self-renewing mesenchymal progenitor populations. Together, our study reveals another class of chemoresistance in leukemic blast created by stromal cross-talk and points to dynamic switching of leukemic cell functionality in concert with the microenvironment.

Funding Source: This study is supported by the NRF of Korea and funded by Ministry of Science, ICT, and Future Planning (2017M3A9B3061947) and in part by the NRF (2012R1A5A2047939)

T-4046

DEVELOPMENT OF CRANIOFACIAL SKELETAL MYOGENIC PROGENITOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Craniofacial skeletal muscle contains approximately 60 muscles, which have critical functions including eye movements, food uptake, respiration, and facial expressions. While craniofacial and limb muscles are both skeletal muscles, the embryonic origin of these two muscle types differ significantly. Skeletal muscles in the trunk and limb originated from precursor cells in segmented trunk paraxial mesoderm, referred to as somites; craniofacial muscles arise from cranial pharyngeal mesoderm during vertebrate embryogenesis. Most current skeletal muscle differentiation protocols which use induced human pluripotent stem cells (iPSCs) are based on somite-derived limb and trunk muscles developmental pathways. Since the lack of protocol for craniofacial muscles is a significant gap in the iPSC-derived muscle field, we aim to develop an optimized protocol to generate craniofacial myogenic precursor cells (cMPCs) from human induced pluripotent stem cells (iPSCs) by mimicking key signaling pathways during craniofacial embryonic myogenesis. First, we induced cranial pharyngeal mesoderm from iPSCs by modulating Wnt and bone morphogenetic protein (BMP) pathway signaling with Notch inhibition. Since second heart field also originated from cranial pharyngeal mesoderm, we suppressed cardiac lineages fates using dual inhibitions of the BMP and ROCK signaling. To isolate skeletal myogenic population, cMPCs were sorted using flow cytometry with known surface markers: HNK1-ERBB3+NGFR+. We confirmed that sorted cMPCs were expressed myogenic factor 5 (Myf5), a key marker for early skeletal myogenic precursors (99.0 ± 0.5 %). To facilitate differentiation into mature myotubes, we treated these sorted and enriched cMPCs with transforming growth factor-β (TGF- β) inhibitor and IGF. This work has established a new protocol for the generation of iPSC-derived human craniofacial muscles, which could be provide not only in vitro research tools to study muscle specificity of muscular dystrophy but also reliable cellular resources for craniofacial muscle transplantation as part of craniofacial reconstruction surgery.

Funding Source: This work was supported by a grant from the NIH grants (R01 AR071397), and the National Research Foundation (NRF) grant funded by the Korea government (NRF-2018R1A6A3A03011703).

T-4048

HUMAN IPSC-DERIVED ENDOTHELIAL CELLS EXHIBIT A MORE MATURE GENE EXPRESSION PATTERN WHEN EXPOSED TO SHEAR STRESS OR CO-CULTURE WITH IPSC-CARDIOMYOCYTES

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Endothelial cells (EC) are present in almost all tissues in the body. Besides their structural purpose, they are an integral part of the function of organs and tissues. Our aim was to explore if and how culturing human induced pluripotent stem cell (iPSC)-derived ECs in conditions better mimicking the physiology of the body changes the transcriptomic phenotype of these cells. First, ECs in the body are exposed to blood flow and shear stress. Secondly, ECs exhibit constant crosstalk with their neighboring cells, which is important for growth and homeostasis of both cell types. Due to this interaction, it is known that ECs have a different identity in different organ systems. Our hypothesis was that culturing the cells in flow and in co-culture with human iPSC-derived cardiomyocytes (iPS-CMs) would influence EC gene expression and identity to better resemble that of physiological conditions in the body. We first performed single-

cell RNA-seq (scRNA-seq) analysis on iPS-ECs in flow and iPS-ECs in static culture in order to study the effects of shear stress on the cells. We then cultured iPS-ECs together with iPS-CMs, as well as separately, and performed scRNA-seq after two days of co-culture in order to find out how the presence of iPS-CMs effects iPS-EC gene expression. Both iPS-ECs and iPS-CMs were derived from human iPS cells, which gives us access to iPS-CMs that would be difficult to obtain from human patients. Two independent iPS cell lines were used in all scRNA-seq experiments. The results reveal that ECs in flow express higher levels of notable EC markers such as CD34, ANGPT2 and ESM1 compared to ECs grown in static conditions. Endothelial cells in co-culture with CMs start expressing more cardiac endothelial markers, including CCL2. Interestingly, Notch signaling was induced in both co-culture ECs and flow ECs compared to static ECs, evidenced by increased expression of Notch target gene HES4 and Notch ligand DLL4. In addition, an extracellular matrix protein-coding gene HAPLN1 was induced in both flow and co-culture ECs. HAPLN1 has recently been indicated as a cardiac EC-specific gene. In conclusion, iPSC-derived ECs are more mature when grown under flow conditions and more cardiac endothelial cell-like in co-culture with CMs.

Funding Source: University of Helsinki, Academy of Finland, Wihuri Research Institute, Finnish Medical Foundation, Finnish Foundation for Pediatric Research

T-4050

THE ROLE OF SCIENTIFIC PAPERS CITED BY DIRECT-TO-CONSUMER STEM CELL BUSINESSES IN THE SOUTHWEST US

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Our lab has characterized 149 direct-to-consumer stem cell businesses in the Southwest United States. These clinics offer stem-cell-based interventions that have not gone through FDA regulatory approval. They mobilize a variety of forms of evidence on their websites to support their practices, including published research articles. Here we present a detailed analysis of the peer-reviewed scientific articles provided on the websites of stem cell clinics, to ascertain whether they support the treatments being offered by the clinics. Of 149 stem cell businesses operating in the Southwest US, 28 list peer-reviewed scientific papers on their websites. A total of 384 articles are cited, from 224 different scientific journals. The clinics vary widely in the number of citations they present, ranging from 1 over 85. To evaluate whether or not the research findings presented in these papers support the treatments offered by a given clinic, we read the abstract of each paper and extracted information including the condition being investigated, the source of stem cells used, whether the cells were manipulated or cultured before being administered, and details about the study itself (sample size, animal or human study, presence of a control group). The data from each paper was compared to the treatments advertised on the website of the clinic referencing the study. In total, only 2% (6/384) of papers cited were identified as directly supporting the treatments being offered by that clinic. Citing a scientific study is likely a strategy aimed at promoting credibility, but our findings suggest the need to go beyond the surface and evaluate on a case-by-case basis the connection between published findings and a given clinic's treatments. This work has implications for prospective patients as well as the Federal Trade Commission (FTC) in evaluating the scientific basis for direct-to-consumer stem cell treatments.

T-4052

HUMAN ENDOMETRIAL STROMAL CELLS EXERT IMMUNOMODULATORY RESPONSES TO INFLAMMATORY STIMULI

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Infection and inflammation can lead to Asherman's Syndrome (intra-uterine adhesions) or thin, unresponsive endometrium. Little is known about the relationship between stromal-immune interactions in the pathogenesis of infertility. The aim of this study was to determine how endometrial stromal cells (eSCs) regulate their immunomodulatory phenotype in response to inflammation. eSCs were isolated from endometrial biopsies (n=6) obtained from healthy, fertile women at cycle day 7-9. eSCs were co-cultured with interferon y (IFNy) and tumour necrosis factor alpha (TNFa) for 7 days, and cell surface expression of CD120a, CD120b, CD119, HLA I and HLA II determined with FACS. HLA II expression and the CIITA regulatory pathway were further evaluated by qPCR. Changes in secretion of interleukin (IL)-6, prostaglandin E2 (PGE2) and indoleamine-2,3 dioxygenase (IDO) activity in response to inflammation were established using ELISA and activity assays respectively. eSCs were co-cultured with allogeneic peripheral blood mononuclear cells in contact and transwell systems, with T cell differentiation and proliferation determined using FACS. eSCs constitutively expressed the IFNyR, CD119 and both TNFRI (CD120a) and II (CD120b). HLA I levels were upregulated in response to inflammation ($p=0.0038$), while no cell surface expression of HLA II was detected even with prolonged stimulation. Secreted levels of IL6, IDO and PGE2 activity were upregulated in response to inflammation ($p<0.0001$; $p=0.0022$; $p=0.0159$ respectively). eSCs suppressed the proliferation of CD4+ T cells, principally via soluble factors ($p<0.0001$). The eSC secretome modulated the CD4+ T cell phenotype, significantly suppressing numbers of central memory subsets ($p<0.0001$). In contrast direct contact cultures induced an effector memory phenotype ($p=0.0092$). No overall change in naïve or TEMRA fractions were observed. eSCs exert an immunomodulatory phenotype, suppressing the proliferation of activated CD4+ T cells and upregulating the

secretion of soluble factors including IL-6, IDO and PGE2. In contrast to different stromal cell subsets, eSCs do not induce cell surface expression of HLA II in response to inflammatory stimulation. The impact of this differential responsiveness may represent a relevant contribution to fetal-maternal tolerance.

Funding Source: This study was funded by The Swedish Research Council and Karolinska Institutet.

T-4054

BIOPROCESS DEVELOPMENT TO MANUFACTURE DOPAMINE NEURON PRODUCT BRT-DA01

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Many of the clinical symptoms of Parkinson's disease are caused by the progressive and irreversible loss of midbrain dopamine neurons. At diagnosis, patients have usually lost a majority of their midbrain dopamine neurons which causes a constellation of motor symptoms. L-dopa supplementation can transiently manage motor dysfunction by increasing dopamine levels but has long-term side-effects and does not prevent disease progression. One strategy to provide permanent symptomatic relief is to replace the degenerated cells that are lost in Parkinson's. Our approach is to manufacture authentic midbrain dopamine neurons from pluripotent stem cells for cell replacement therapy. Here, we report on our development of a closed, scaled system to manufacture billions of our dopamine neural cell product, DA01, that forms the basis of our Phase II manufacturing bioprocess. Raw material and bioprocess refinements over our Phase I bioprocess have further mitigated risk and improved process robustness. Thorough process characterization enabled an in depth understanding of DA01 through conventional quality metrics augmented with machine learning to establish the transcriptomic signature of our cell product and in-process intermediates. The combination of conventional and in silico approaches demonstrated that our scaled system makes a cell product that is nearly identical to our previous Phase I approach.

Funding Source: BlueRock Therapeutics

T-4056

ESTABLISHING HUMAN EMBRYONIC STEM CELLS MASTER CELL BANKS UNDER GMP CONDITIONS

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The increased interest in using human pluripotent stem cells for advanced therapy medicinal products (ATMP) has revealed the urgent need for safe and GMP compliant established hESC Master Cell Banks. The culture conditions for human pluripotent stem cells have evolved since 1998, leaving the use of mouse feeders and animal components behind, and instead one employ defined, feeder free culture conditions. However, the absolute majority of the derived human ES cells are made with feeders and outside the GMP environment, with animal components. Further, the starting material, the blastocysts, are not sourced according to FDA's guidelines, lacking proper donor testing, and/or sourced in non-prion free regions of the world. In order to derive human embryonic stem cells under GMP and sourcing the starting material according to also FDA's requirement we have built up the infrastructure required for a successful establishment of human ES cells for clinical applications. Initially we developed a new, xeno-free defined culture medium, that together with a defined coating substrate was successfully evaluated for derivation of hES cells. The xeno-free medium was then produced and released under GMP. We then established appropriate sourcing procedures, e.g. sourcing according to FDA's requirement and with proper donor consents that allows the future use and needed analyses as well as significant pre-screening of the donor couples before they were approved as eligible donors. In parallel we built the infra structure and processes that rendered us to become a registered tissue establishment and with a manufacturing license for deriving and banking human pluripotent stem cells from the Swedish MPA (according to the Swedish LVFS 20018:12 and LVFS 2004: 7, Eudralex Volume 4 GMP). We will present the workflow as well as data from the ongoing derivation work.

T-4058

EVOLUTIONARILY DISTINCTIVE GENE REGULATORY NETWORK OF HUMAN GERM CELL SPECIFICATION

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Germ cell specification in human occurs shortly after implantation and therefore unapproachable for experimental procedures. Our recent approach of inducing human iPS cells into primordial germ cell-like cells (PGCLCs) have established a platform to analyze the molecular mechanism behind this process. We have recently clarified the requirement of transcription factors, such as SOX17, TFAP2C and BLIMP1, expressed at proper timing is prerequisite for germ cell specification. However, what factors suffices PGCLC specification has not been elucidated. In this study, we utilized the abovementioned PGCLC differentiation protocol, and tested if an overexpression of a set of transcription factors can induce PGCLC differentiation without the addition of the key cytokine BMP4. We have observed that the overexpression of the combination of SOX17, TFAP2C and BLIMP1 is not sufficient for inducing endogenous germ cell marker genes expression, and thus have concluded that there are other factors necessary for PGCLC specification. From further screening of candidate genes, we have identified a set of transcription factors that induces the expression of endogenous transcripts of the key germ cell genes including SOX17, TFAP2C, BLIMP1 and NANOS3, which suggest the congruence to cytokine-induced PGCLCs. Our findings uncover the downstream target of BMP signaling in human germ cell specification and proved an evolutionarily distinctive molecular cascade from that of mouse.

T-4060

THE ROLE OF H3K27 DEMETHYLASES IN THE AGING 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. However, the fertile ability of SSC gradually decreases with age. The molecular mechanisms and characteristics of SSC ageing still remain unclear. We found that histone H3 lysine 27 (H3K27) demethylase, JMJD3 (KDM6B), has some roles in the regulation of SSC aging. 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. However, we also found that UTX (KDM6A), which is another H3K27 demethylase and is not detectable in wildtype undifferentiated spermatogonia, was redundantly expressed in JMJD3 null undifferentiated spermatogonia. These results suggest that not only JMJD3 but also UTX may contribute to control the spermatogonial compartment through the regulation of SSC aging. So far, expression and epigenetic profiles of JMJD3 null SSCs were analyzed to elucidate the role of H3K27 demethylases in the regulation of SSC aging. There were some genes that were downregulated in the aged SSCs but not downregulated in the JMJD3 null aged SSCs. We found a set of genes related to energy metabolism were involved in those genes. The UTX null mice and the UTX-inducible mice were generated and being analyzed. Our findings may be involved in the maintenance of diverse stem cell niches with age.

Funding Source: KAKENHI and Takeda Science foundation

T-4062

GENOMIC PROFILING WITH A NOVEL ANTIBODY REVEALS A ROLE FOR O-GLCNAC IN TRANSCRIPTIONAL REGULATION OF PLURIPOTENCY

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O-GlcNAc is a post-translational modification occurring on serine and threonine residues of intracellular proteins, and is found to participate in various biological processes. As a multi-faceted player in transcriptional regulation, O-GlcNAcylation regulates the function of many transcription and epigenetic factors. At the same time, O-GlcNAc is found to regulate pluripotency maintenance and differentiation in embryonic stem cells, and transcriptional regulation is key to pluripotency. However, the mechanisms of O-GlcNAc regulating transcription and pluripotency are not fully understood. Here, we mapped O-GlcNAc's occupancy on the mESC genome using ChIP-seq with a novel, nanomolar affinity antibody EPR19847, and identified 21099 loci bearing O-GlcNAc. We found these loci significantly enriched in active promoters marked by RNA Pol II, H3K4me3 and H3K9ac, linking O-GlcNAc to the activation of gene expression. We then profiled the expression of mESC genes under perturbations in global O-GlcNAc level and found 164 genes regulated by O-GlcNAc. These genes participate in differentiation, development, transcription and spermatogenesis,

indicating that O-GlcNAc regulates pluripotency through these pathways. These results show that O-GlcNAc participates in transcriptional regulation of mESCs, and hopefully will provide more insights into O-GlcNAc's roles in pluripotency.

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T-4064

REGULATION OF THE CHROMATIN STATE AND CORE PLURIPOTENCY FACTORS IN CANCER STEM CELLS

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Embryonic stem (ES) cells and cancer cells share many properties, including gene expression networks and the unlimited capacity for self-renewal. The naïve state of ES cells is maintained by the core pluripotency factors OCT4, NANOG, and SOX2. Previous studies have demonstrated that, like ES cells, cancer cells can harness the core pluripotency factors for their survival and self-renewal. Furthermore, a small subset of cells, also known as cancer stem cells (CSCs), are marked by these factors and pose a significant challenge to cancer treatment as they remain resistant to radiation and chemotherapeutic drugs. CSCs maintain the ability to self-renew, drive tumor heterogeneity, and contribute to an aggressive phenotype and relapse in many types of cancers. ZSCAN4 (zinc finger and SCAN domain containing 4) is an additional stem cell factor implicated in the preservation of embryonic stem cell potency and genome stability. Although it was suggested to have implications in cancer, the function of human ZSCAN4 in cancer or how it exerts its action remained unknown. In the current study, we show that ZSCAN4 marks the CSC population in head and neck squamous cell carcinoma. Our data suggest that induction of ZSCAN4 promotes the CSC phenotype by activating the core pluripotency factors and altering the CSC epigenetic profile. Consistent with this, ZSCAN4 depletion by gene knockdown leads to loss of the CSC phenotype, including decreased CSC marker expression and the impaired ability to form spheroids. Overall, our study suggests that ZSCAN4 plays a critical role in the maintenance of the undifferentiated state and survival of CSCs, indicating that ZSCAN4 is a potential therapeutic target in HNSCC.

T-4066

IDENTIFICATION IN CONDITIONED MEDIA OF STEMNESS MARKER FOR HUMAN EMBRYONIC STEM CELL DERIVED MESENCHYMAL STEM CELL USING PROTEOMICS

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Human mesenchymal stem cells (hMSCs) are multipotent adult stem cells that have ability to self-renewal and differentiate into chondrocytes, adipocytes and osteocytes as well as have immune-regulatory, have attracted much attention from researchers due to regenerative medicine implications. Especially, human pluripotent stem cell-derived mesencymal stem cells (hPSC-MSCs) is known that hPSC-MSCs have more benefits which it overcomes cellular senescence during the in vitro expansion and enhanced therapeutic effect, in comparison with hPSC-MSCs isolated from different adult sources such as bone marrow, adipose tissues, fetal liver, peripheral blood and lung and so on. To confirm the stemness in hPSC-MSCs is very important because it presents ability of hPCS-MSCs like self-renewal and multipotency. However, the stemness associated with the mechanism and biological process of hPCS-MSCs remain poorly understand as hPSC-MSCs have the heterogeneity and variation between different sources and culture condition. Therefore, we identified the stemness markers in conditioned media (CM) of hPSC-MSCs compared with CM of chondrocyte cells to improve cell quality and to detect easily than existing methods by assessing quantification of stemness markers using HPLC-MS/MS. We used SWATH-MS which is label free quantitative data-independent acquisition (DIA) to select candidate marker for stemness. In addition, multiplexed liquid chromatography-multiple reaction monitoring spectrometry was used for quantitative analysis. In total 408 proteins were identified in hPSC-MSC and 352 proteins in chondrocyte cells. The eighty-one proteins were identified with more than 1.2-fold change and p-value less than 0.05. The forty-four proteins were up-regulated and thirty-seventy were down-regulated in stroke patients comparison with healthy control. These proteins were classified into cell adhesion, extracellular matrix (ECM) and cell signaling involved in differentiation. We selected several candidate markers for stemness and quantified. These proteins were classified into cell signaling pathway (CHRD

and FST), ECM (CO1A, COL1A2 and COL1A3) and other (PAI1 and STC2). These results were regarded to be able to support whether hPSC-MSC have stemness ability for in vitro cell culture expansion.

T-4068

MODELING PICALM ALZHEIMER'S DISEASE VARIANTS IN HUMAN IPSC-DERIVED CELLS

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PICALM plays an essential role in regulating neuronal function and providing a major pathway for amyloid-beta (Aβ) clearance in vivo across the blood-brain barrier (BBB). PICALM is abundantly expressed in brain capillaries as well as in neurons in the human brain. Impaired transvascular clearance of Aβ across the BBB contributes to Aβ accumulation, accelerating neurovascular and neuronal dysfunction in the Alzheimer's disease (AD) brain. Our research has shown that PICALM is required for Aβ trafficking across the BBB through endocytotisis via LRP1, and Rab5- and Rab11-mediated vehicular transport resulting in Aβ exocytosis into circulation. Diminished PICALM levels in the brain endothelium are found in AD and are also associated with increased neuronal vulnerability to Aβ. Using CRISPR/CAS9 genome editing technology, we investigated the protective PICALM SNP rs3851179A and the risk rs3851179G variants. By successfully generating iPSC lines homologous for allelic variants of PICALM SNP rs3851179, we further differentiated these iPSCs into the cell types of the neurovascular unit: neurons, astrocytes, microglia, endothelial cells, and pericytes. We confirmed by Western blot analysis higher PICALM expression in iPSC-derived neurons and endothelial cells carrying protective rs3851179A alleles than those carrying non-protective rs3851179G alleles. In a 2D transwell model, we found that endothelial cells carrying the non-protective rs3851179G alleles cleared Aβ much less efficiently across the barrier compared to endothelial cells carrying the protective alleles. Neurons carrying protective or non-protective alleles of PICALM can also be functionally compared throughout differentiation in 3D and 2D in vitro models. By generating in vitro models with neurons, astrocytes, microglia, pericytes, and endothelial cells derived from human iPSCs with allelic variants of PICALM, we are able to explore the fundamental characteristics of AD pathology as it relates to PICALM expression.

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FRIDAY, JUNE 28, 2019

POSTER III - ODD
18:00 – 19:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

F-2001

HUMAN AMNIOTIC EPITHELIAL CELLS-DERIVED EXOSOMES RESTORE OVARIAN FUNCTION BY TRANSFERRING MICRORNAS AGAINST APOPTOSIS

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Premature ovarian insufficiency/failure (POI/POF) is one of the most common complications among female tumor patients treated with chemotherapy and requires advanced treatment strategies. Human amniotic epithelial cells (hAECs)-based therapy mediate tissue regeneration in a variety of diseases and increasing evidence suggests that the therapeutic efficacy of hAECs mainly depends on the paracrine action. This study aims to identify exosomes derived from hAECs and explore their therapeutic potential in ovaries damaged by chemotherapy and the underlying molecular mechanism. hAECs-derived exosomes exhibited a cup- or sphere-shaped morphology with a mean diameter of 100 nm and were positive for CD63 and CD9. hAECs-Exosomes transplantation increased the number of follicles and improved ovarian function in POF/POI mice. During the early stage of transplantation, hAECs-Exosomes significantly inhibited granulosa cells apoptosis and protected the ovarian vasculature from damage, and involved in maintaining the number of

primordial follicle in the injured ovaries. Enriched microRNAs (miRNAs) existed in hAECs-Exosomes and targeted genes were enriched in phosphatidylinositol signaling and apoptosis pathways. Studies in vitro demonstrated that hAECs-Exosomes inhibited chemotherapy-induced granulosa cells apoptosis via transferring functional miRNAs, such as miR-1246. Our findings demonstrate that hAECs-derived exosomes have the potential to restore ovarian function in chemotherapy-induced POF/POI mice by transferring miRNAs.

Funding Source: This study is funded by the National Key Research and Developmental Program of China (2018YFC1004800 and 2018YFC1004802), and the National Natural Science Foundation of China (No.81701397).

F-2003

DEVELOPING THE CLINICAL-GRADE PROTOCOL OF HUMAN PLACENTAL TISSUE CRYOPRESERVATION FOR HEMATOPOIETIC STEM CELLS AND MESENCHYMAL STROMAL CELLS ISOLATION

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Placenta is very attractive source of mesenchymal stromal cells (MSCs) and hematopoietic stem/progenitor cells (HSPCs). Large-scale introduction of placental-derived cells to clinical practice requires the establishing of low-temperature stock of placental tissue. Term placentas were collected after normal delivery or CS provided with women's written informed consent in the Kyiv city maternity hospital #3. One part of tissue from every specimen was processed as a native control and the other part was frozen by nine variants of cryoprotectants. The combination of different concentration of DMSO (5%, 7.5%, 10%) and sucrose (0.1 M, 0.2 M) were used for cryopreservation. Samples were frozen by special programs in the controlled rate freezer (IceCube, Austria). Colony forming units (CFU) assay was carried out on passages 0, 2, 6 for placental MSCs (pMSCs) isolated from native and thawed tissue. CFUs activity of placenta-derived HSPCs was studied using MethoCult assay (StemCell Technologies, Canada). The content of HSPCs among viable CD45+ cells from native and cryopreserved placental tissue did not significantly differ. The cryopreservation led to the alteration of CD133, CD38, and CD33 expression on HSPCs surface. HSPCs from native tissue gave rise to various types of colonies in vitro and their ratio did not significantly differ compared to ones obtained from cryopreserved samples. 5% DMSO preserved the HSPCs better in compare to 10% (without sucrose). However, HSPCs CFU recovery of samples frozen with 10% of DMSO was higher compare to ones with 5% DMSO in combination with 0.2 M sucrose. The CFU number of pMSCs for nine variants on passages 2 and 6 did not significantly differ. Results of these studies revealed the pattern of interference of two cryoprotectants influence (DMSO and sucrose) on the viability of HSPCs in placental tissue during cryopreservation. The cryoresistance of placenta-derived HSPCs and pMSCs enclosed in tissue fragments differed.

F-2005

IN VITRO, EX VIVO AND IN VIVO INFLUENCE OF 3D MICROENVIRONMENT ON ENHANCEMENT OF WJ-MSC THERAPEUTIC POTENTIAL IN CNS INJURY

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Mesenchymal stem cells (MSC) exhibit neuroprotective, angiogenic and immunomodulatory properties. Their availability, high plasticity and possibility of expansion have made MSC-based therapy one of the most commonly used in regenerative medicine. In this study we have designed 3D hydrogel scaffolds: human platelet lysate (PL) and fibrinogen (FB) and adjusted culture atmosphere to physiological oxygen concentration in order to provide the optimal microenvironment to in vitro culture and protect cells from adverse host tissue vs. transplant interaction during ex vivo and in vivo experiments. WJ-MSC in vitro cultures in different oxygen conditions (21% O2 vs. 5% O2) were tested for proliferation, viability and gene expression profile. For ex vivo studies the organotypic hippocampal slice culture (OHC) model of oxygen glucose deprivation was used to mimic an ischemic injury of neural tissue. In vivo studies were based on experimental model of brain injury induced by local ouabain injection. The effect of cell transplantation on CNS was tested in isolated animal brains at 7, 14 and 21 days after the grafting. WJ-MSC show higher survivability and increased proliferation rate in FB vs. PL scaffolds and in physiological normoxia (5% O2). 3D in vitro conditions increased expression level of

selected neurotrophins in both type of the scaffolds. Ex vivo studies indicated strong neuroprotective effect of WJ-MSC on injured hippocampal slices. WJ-MSC growing on the scaffolds and co-cultured with OHC revealed increased expression of several neurotrophins and anti-inflammatory TGF-β as well as decreased expression of pro-inflammatory IL-1β. This effect was potentiated by FB (as compared to PL) scaffolds and 5% O2 culture conditions. In vivo studies have shown increased expression of rat cytokines, e.g. NGF, VEGF-A after transplantations of WJ-MSC supported by 3D fibrin scaffolds in the injured brain region. Our results indicate significant influence of microenvironmental conditions, therefore it is necessary to optimize and standardize all aspects of preparation of therapeutically competent cell population. Moreover, the proposed models of scaffolds/cell hybrids transplantation are promising for future use in MSC-based therapy.

Funding Source: Sponsored by NCBR grant No STRATEGMED1/234261/2/NCBR/2014 and statutory funds to MMRC.

ADIPOSE AND CONNECTIVE TISSUE

F-2007

A NOVEL XENOGRAFT MODEL TO VALIDATE CAUSATIVE MUTATIONS IN PATIENTS WITH EHLERS-DANLOS SYNDROME

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The Ehlers-Danlos Syndrome (EDS) is a connective tissue disease characterized by hyperextensible skin, joint hypermobility, and cutaneous fragility. EDS is primarily caused by mutations in fibrillar collagens or genes involved in collagen protein biosynthesis. However, there are many cases in which the causative genetic defects of EDS have not been identified. To date, there is no cure for EDS with treatment limited to symptomatic care. The lack of appropriate in vitro and in vivo models that faithfully recapitulate the clinical EDS phenotype makes mechanistic studies extremely challenging, thus hindering the development of efficient therapeutic strategies. In order to develop a clinically relevant EDS model, we focused on a cohort of hypermobility type EDS patients who did not harbor known EDS-associated mutations, but exhibited classical symptoms of the disease. We isolated fibroblasts from a skin biopsy of one of these patients, and combined these cells with healthy keratinocytes. Using a silicone chamber, we grafted the cells onto an immunocompromised mouse to recapitulate the EDS skin phenotype in a xenograft model. We discovered that grafts formed with EDS fibroblasts exhibited a disorganized collagen network, a feature previously observed in the skin of EDS patients. We have compared the transcriptional profile of EDS fibroblasts with that of controls using RNA sequencing analysis, and identified a number of promising mutations and transcript variants that may be causing the phenotype in these patients. In order to validate these candidate mutations, we reprogrammed EDS fibroblasts into induced pluripotent stem cells (iPSCs). We are currently correcting these candidate mutations using CRISPR/Cas9. The corrected EDS iPSCs will be differentiated into fibroblasts, and used in our xenografting assay. If the xenografts no longer display a disorganized collagen network, this will confirm that this candidate mutation is indeed the cause of EDS in these patients; thus, confirming the usefulness of our model.

F-2009

ADIPOCYTES FROM OBESE MICE PRODUCE EGF AND PROMOTE THE SURVIVAL AND GROWTH OF PANCREATIC DUCTAL PROGENITOR CELLS IN ORGANOID CULTURE

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Obesity, a condition of excessive adipose tissue (AT), is a major health problem. AT has been recognized as an endocrine organ that secrets bioactive factors, but the repertoire remains incomplete. Obese AT can affect various differentiated cells, as well as progenitor cells in the endothelium, intestine and bone marrow. However, whether AT may also affect pancreas ductal progenitor cells is unknown. Here we examined the effects of secreted factors, collected in the conditioned medium from AT of obese or lean mice, on lean pancreatic ductal progenitor cell using an organoid culture system. We found that obese compared to lean adipocytes produced and secreted more epidermal growth factor (EGF). Recombinant EGF enhanced the survival and the self-renewal of pancreatic ductal progenitor cells in vitro. Our results uncover EGF as a previously-unrecognized secreted factor from adipocytes that may have implications for obesity-associated diseases.

MUSCULOSKELETAL TISSUE

F-2013

ROLE OF COUP-TFII AND THYROID HORMONE RECEPTOR ALPHA IN MURINE SKELETAL MUSCLE LOSS

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Myopathic changes including muscular dystrophy and weakness are commonly described in hypothyroid and hyperthyroid patients. Impaired skeletal muscle regeneration and sarcopenia with aging have been reported in a mouse model of Resistance to Thyroid Hormone (RTH) carrying a frame-shift mutation in the thyroid hormone receptors α (TRα) gene (TRα1PV). As we previously reported, TRα1PV mice present a significantly smaller pool of PAX7-positive satellite cells (SCs) in the skeletal muscle and sarcopenia with aging, that was reported to be associated with loss of SC pool. Moreover, SC function during skeletal muscle injury was impaired four days after cardiotoxin (CTX)-induced skeletal muscle injury with a decreased activation of SC and a reduced proliferation of Myf5 expressing cells. Overexpression of the nuclear orphan receptor Chicken Ovalbumin Upstream Promoter-factor II (COUP-TFII, or NR2F2) in murine satellite cells have been shown to induce a similar skeletal muscle phenotype, including skeletal muscle loss with aging, and inhibit myogenesis through modulation of Myf5 and MyoD expression. We detected a higher expression of COUP-TFII in C2C12 (a murine myoblast cell line) during proliferation, and a decline during myogenic differentiation. Moreover, we analyzed the skeletal muscle of mice at different ages and we found a higher expression of COUP-TFII in the first 2 months of life followed by a decline with age, suggesting the important role of COUP-TFII in modulating post-natal myogenesis and onset of sarcopenia with aging. In proliferating C2C12 myoblasts and SCs from wild-type and TRα1PV mice, we demonstrated via co-immunoprecipitation that COUP-TFII and TRα interact. In addition, the skeletal muscle of TRα1PV mice showed significantly higher expression of COUP-TFII compared to their WT siblings, and in TRα1PV mice COUP-TFII expression remained higher with skeletal muscle aging. These results suggest a COUP-TFII role in skeletal muscle loss with aging and impaired skeletal muscle regeneration in TRαPV mice that may be mediated by COUP-TFII-TRα interaction. These new insights can provide a therapeutic target to prevent or treat myopathies, such as sarcopenia and Duchenne-like muscular dystrophy.

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F-2015

ENHANCING MESENCHYMAL STEM CELL OSTEOGENESIS AND ADIPOGENESIS BY SMALL MOLECULES COCKTAILS

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Human mesenchymal stem cells (MSCs) hold great promises for regenerative medicine and cell therapy by their multi-potency, immune-modularity, and risk-free of tumorigenesis. Previously, we have revealed that a cocktail including six chemicals (6C, including p38 inhibitor, JNK inhibitor, PKC inhibitor, ROCK inhibitor, ErK inhibitor and GSK3βinhibitor,) are able to reprogram human somatic fibroblast to induced mesenchymal stem cells (iMSCs) within six days with a high efficiency (38%) . iMSCs fulfill all the criteria of traditional MSCs as determined by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT), including cell adhesion, marker expression, and multipotency. The iMSCs have much higher clonogenicity than fibroblasts. Additionally, iMSCs have immunomodulatory that can suppress LPS-mediated acute lung injury as effectively as bone marrow MSCs. After testing 120 different combinations, we further boosted the conversion rate from 38% to 78%. Recently, we tested these chemicals and revealed that 3 chemical- (3C), and 6 chemical-(6C) cocktail can promote cell differentiation in MSCs. Among them, in both young and aging MSCs, we found the osteogenesis markers alkaline phosphatase activity and calcium precipitation increased while we treat the MSCs with 3C. In contrast, the adipogenesis increased upon the treatment of 6C. To sum up, it suggests that the cocktails can enhance the osteogenesis or adipogenesis of MSCs, and has a great potential to treat osteoporosis or alter cell fate in the future.

F-2017

SMALL-MOLECULE RESTORATION OF DYSTROPHIN PREVENTS DYSFUNCTIONAL GLUTAMATE CLEARANCE IN IPSC MODEL OF DYSTROPHIC ASTROCYTES

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Duchenne muscular dystrophy (DMD) results, beside muscle degeneration in cognitive defects. As neuronal function is supported by astrocytes, which express dystrophin, we hypothesized that loss of dystrophin from DMD astrocytes might contribute to these cognitive defects. We generated cortical neuronal and astrocytic progeny from induced pluripotent stem cells (PSC) from DMD and several unaffected PSC lines. DMD astrocytes displayed cytoskeletal abnormalities, defects in Ca+2 homeostasis and nitric oxide signaling. In addition, defects in glutamate clearance were identified in DMD PSC-derived astrocytes; these deficits were related to a decreased neurite outgrowth and hyper-excitability of neurons derived from healthy PSC. Read-through molecule restored dystrophin expression in DMD PSC-derived astrocytes harboring a premature stop codon mutation, corrected the defective astrocyte glutamate clearance and prevented associated neurotoxicity. Moreover, we feel our results form a key component of increasingly convergent pathways that seem to be involved in multitude of neuropsychological disorders. Akin to findings in Autism iPSC models, and brain MRI studies in mdx mouse model of DMD, involvement of excitatory amino acids (Glutamate in our case) seem to be a common denominator among them all. This is, to our knowledge, the first report to demonstrate the importance of dystrophin protein in a non-myogenic setting at the cellular level in CNS. In summary, we demonstrate that: (1) Loss of dystrophin from astrocytes causes defects in astrocyte glutamate handling, (2) This results in glutamate toxicity towards normal cortical neuronal progeny, and (3) These defects could be reversed when dystrophin levels were restored, as shown here for PTC124, which allows read-through of premature termination codons and which has conditionally been approved for DMD by the EMA in 2014. We propose a role for dystrophin deficiency in defective astroglial glutamate homeostasis which initiates defects in neuronal development. Lastly, pertinent here is also the subpopulation of patients currently treated with PTC124 for myogenic defects. It will be of great interest to determine effects on cognitive function of this intervention, given that this compound can cross the blood-brain barrier.

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F-2019

MURINE SKELETAL MUSCLE STEM CELLS MAINTAIN BIOENERGETIC HOMEOSTASIS THROUGH MITOCHONDRIAL OXIDATION OF GLUCOSE DURING REGENERATIVE ACTIVATION

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Muscle stem cells (MuSCs) reside in skeletal muscle tissue and are activated by injury to initiate and drive muscle repair. In our previous work, we showed that increasing the speed of MuSC activation (exit cellular quiescence and divide) was sufficient to increase the speed of the entire process of muscle regeneration. While the biologic role of MuSC activation in muscle repair is clear, the mechanisms that regulate MuSC activation remain elusive. We found in our previous work that MuSCs upregulate genes associated with mitochondrial metabolism during injury induced activation. This suggests that MuSCs increase mitochondrial ATP production to meet the energetic demands of activation. To dissect MuSC energy metabolism, we used the Seahorse XFp extracellular flux analyzer to measure extracellular flux in oxygen and proton concentrations and determine the real-time rate of cellular ATP production. In addition, we measured changes in cellular ATP synthesis induced by 2-deoxy-D-glucose, an inhibitor of glycolysis, to determine ATP specifically generated from glucose consumption. Our data show that during the first 48 hours of activation, (1) total cellular ATP production dramatically increases by >10-fold in MuSCs, and (2) cellular consumption of glucose to generate ATP increases by >30-fold. We also found that majority of cellular ATP is produced via mitochondrial metabolism as opposed to glycolysis. Interestingly, our results show that mitochondria in freshly isolated (FI) MuSCs do not metabolize glucose to synthesize ATP, suggesting that FI MuSCs oxidize glucose exclusively through glycolysis. By 48 hours, however, we found that mitochondria carry out majority of glucose consumption (linked to ATP synthesis) in MuSCs. Collectively, these data illustrate the dynamic and dramatic metabolic transitions that occur in MuSCs as they initiate muscle repair, and advance our understanding of how metabolic cues and diet can be used to control endogenous tissue repair or augment the efficacy of regenerative medicine therapies.

F-2021

TRANSIENT ACTIVATION OF NOTCH SIGNALING ENHANCES STROMAL CELL PROLIFERATION AND SUBSEQUENT OSTEOGENESIS

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Sufficient of mesenchymal stromal cells (MSCs) is crucial for tissue growth and repair. While bone marrow-derived MSCs are an attractive cell source, their availability has been hampered by the low population of MSCs in vivo. We have shown previously that activation of Notch signaling enhances limb bud cell proliferation in vitro and in vivo. Here, we extend this knowledge to examine whether activation Notch signaling by injection of ligand jagged1 (JAG1) influences proliferation and osteogenic differentiation of bone marrow MSCs in a manner consistent with our hypothesis that transient activation of Notch signaling will promote MSC in vivo expansion leading to an enhanced osteogenic differentiation response. 10 week old WT male (n=12) and female (n=12) BL6 mice were intraperitoneal injected daily with 0.5 mg/kg of control IgG or JAG1 for 7 days followed by 6-hour in vivo BrdU labeling before sacrificed for cell and tissue collection. Immunostaining showed that expression of Notch target gene Hes1 in bone marrow cells in JAG1-injected mice was significantly increased compared to the IgG-injected control mice. Similarly, the size of BrdU positive area inside the bone marrow space was also increased in JAG1-injected mice suggesting a greater cell proliferation index than that in IgG-injected control mice. Furthermore, flow cytometry data showed a higher percentage of CD29 and CD44 positive cell population in MSCs was obtained in JAG1-treated mice when compared to IgG-treated mice. When introduced to osteogenic differentiation medium, MSCs from JAG1-treated mice exhibited a significant increased osteogenic differentiation by showing enhanced Alizarin red staining. Finally, our RT-PCR data revealed a significant increase in expression of osteogenic marker alkaline phosphatase (ALP), Runx2, osterix and osteocalicin, as well as osteogenic inducer BMP2 in MSCs from JAG-treated mice. Our data support that transient activation of Notch signaling in vivo increased bone marrow cell proliferation and MSC population. Therefore, the increased ex vivo osteogenesis of MSCs from JAG1-treated mice could be due to the increased number of true stem cells in newly isolated MSCs. Further studies to understand the effects of transient Notch activation on other cell types in multiple organs are ongoing.

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CARDIAC TISSUE AND DISEASE

F-2025

HUMAN NEONATAL C-KIT+ CARDIAC PROGENITOR CELLS IMPROVE CARDIAC FUNCTION POST-MYOCARDIAL INFARCTION BY ALTERING THE CARDIAC HEALING RESPONSE

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The c-kit+ cardiac progenitor cells (CPCs) are a promising therapy for preventing progression to heart failure after myocardial infarction (MI). In clinical trials, cell therapies based on adult CPCs (aCPCs) have demonstrated safety, but minimal efficacy. This is partly due to a heterogeneous, mostly senescent, population of cells derived from adult tissues. Recent research has shown that c-kit+ CPCs from neonatal human hearts (nCPCs) have few senescent cells, display robust proliferative capacity in vitro, and are more effective in improving cardiac function compared to c-kit+ CPCs isolated from adult human hearts in rodent MI models. We examined the ability of nCPCs to increase cardiac function and investigated the potential mechanisms for their effectiveness. nCPCs injected intramyocardially immediately post-MI in male nude rats led to significant improvements in contraction and relaxation in the left ventricle at 4 weeks after injury compared to controls. Heart failure biomarker NT-proANP and proinflammatory cytokine TNFα were significantly reduced by administration of nCPCs. Additionally, nCPC administration led to smaller scar size. As reparative effects of progenitor cells on the heart are exerted by paracrine action, we examined the effect of total conditioned medium (TCM) from several cell types in functional assays relevant to cardiac recovery post-MI. Efferocytosis, an inflammation resolution process, was significantly improved by incubation with neonatal TCM (nTCM), but not adult TCM (aTCM), mesenchymal stem cell (MSC) TCM, or fibroblast TCM. nTCM increased angiogenesis, as measured by the endothelial tube formation assay. In an anti-fibrotic assay, nTCM reduced alpha Smooth Muscle Actin protein levels, and decreased profibrotic genes COL1A1, COL1A2, COL3A1, and fibronectin. In an inflammation suppression assay, nTCM decreased secretion of proinflammatory cytokine IL-8. Overall, these data demonstrated an enhanced ability of c-kit+ nCPCs, but not aCPCs or MSCs, to improve cardiac function post-MI by promoting inflammation resolution and enhancing the cardiac healing response.

F-2027

REGENERATING THE INFARCTED PIG HEART USING HUMAN EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

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Following a myocardial infarction (MI), the damaged muscle is replaced with non-contractile scar tissue, which often leads to heart failure. In small rodent and macaque models of MI, the transplantation of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) mediates the partial remuscularization of the infarct scar leading to improvements in contractile function. However, transplantation of hESC-CMs in small non-human primates results in transient, non-lethal ventricular tachyarrhythmias (VT). We therefore tested the capacity of hESC-CMs to stably engraft in a more translationally relevant preclinical model, the infarcted pig heart. To induce infarction, we used balloon angioplasty to occlude the mid left anterior descending artery. At 3-weeks post-MI, a left lateral thoracotomy was performed and vehicle alone (n=14) or 10^9 hESC-CMs (cardiac purity of 82.3 ± 3.0%; n=13) were directly injected into the infarct zone. Animals were monitored by telemetric ECG recording and serial cardiac MRI for up to 8-weeks post-transplantation, with a small cohort of animals undergoing electroanatomical mapping (10 days post-transplantation; n=4 for vehicle and n=3 for hESC-CMs recipients). Overall, hESC-CM transplantation resulted in substantial myocardial implants within the infarct scar that matured over time, formed vascular networks with the host, and evoked minimal cellular rejection. While arrhythmias were rare in infarcted pigs receiving vehicle alone (n=14), hESC-CM recipients (n=13) experienced frequent monomorphic ventricular tachycardia before reverting back to normal sinus rhythm by approximately 4-weeks post-transplantation. Electroanatomical mapping and pacing studies implicated focal mechanisms for these graft-related tachyarrhythmias as evidenced by an abnormal centrifugal pattern with earliest electrical activation in histologically-confirmed graft tissue. These findings demonstrate the suitability of the pig model for the preclinical development of a hESC-based cardiac therapy and provide new insights into the mechanistic basis of electrical instability following hESC-CM transplantation.

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F-2029

EFFECT OF SMALL MOLECULE INDUCED BEIGE ADIPOCYTES ON CARDIOMYOCYTES AGAINST HYPOXIA/REOXYGENATION

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Beige adipocytes gained much attention as an alternative cellular target in regulating metabolic homeostasis. The development of the beige cells that has beneficial metabolic, but how thermogenic stimuli activate and control beige adipocytes is not fully understood. Here, we investigated that the effects and mechanism of isoliquiritigenin (ILG) on adipose-derived stem cells (ASCs) differentiate into functional beige/brown adipocytes. We found that ASCs derived white adipocytes are able to switch to a brown phenotype by expressing both UCP1 and CIDEA according to low dose ILG treatments. Although various pharmacological activities of ILG, the possible role of ILG in white-to beige transdifferentiation of adipose-derived stem cells have never been explored. Moreover, we demonstrated that ILG small molecule dose-response in distinct regulatory mechanisms and functions to induce the white-to-beige trans-differentiation of adipose-derived stem cells. Then, we sought to determine if small molecule induced beige adipocytes into impaired heart tissue affected surround fat in the heart. Therefore, we investigated the expression levels of proteins associated with oxidation, inflammation, and death signals in cardiomyocytes with beige cells under hypoxia/reoxygenation conditions for their roles in many physiological processes in the heart. These studies suggest that the induction of thermogenic adipocytes might offer a new approach to combating human metabolic disorders and ischemic heart diseases.

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F-2031

AUTOPHAGIC FLUX CORRECTION BY HEMATOPOIETIC STEM CELL-DERIVED MACROPHAGES IN A MOUSE MODEL OF DANON DISEASE

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Danon disease is a fatal condition without any specific therapy making heart transplantation the patient's only choice for survival. Even with heart transplantation, patients continue to suffer from skeletal myopathies. Therefore, the development of a stem cell-based systemic therapy would meet a critical unmet need. Here, we sought to assess the therapeutic potential of using Sca1+ hematopoietic stem and progenitor cell (HSPC) in the context of Danon disease which is caused by the deficiency of the lysosomal associated membrane protein type -2 (LAMP-2), a lysosomal membrane bound protein essential in autophagic flux. First, we co-cultured LAMP-2 knockout (KO) fibroblasts (FBs) with wild-type (WT) macrophages and observed the in vitro transfer of LAMP-2-positive vesicles from WT macrophages to LAMP-2 KO FBs that were near. Importantly, a decrease in the number of autophagic vacuoles (AVs) and a rescue of autophagic flux were noted in co-cultured LAMP-2 KO FBs compared to control, suggesting that fusion of autophagosomes and lysosomes was restored. Next, we transplanted LAMP-2 KO mice with WT-GFP+ mouse derived Sca1+ HSPCs. At 12-months post-transplantation, HSPC-derived GFP+/CD68+ cells were present in the hearts and skeletal muscles of HSPC-transplanted mice, demonstrating that a single infusion of HSPCs had the capacity to migrate to and integrate in the diseased hearts and skeletal muscles, and differentiate into phagocytic cells. Assessment of LAMP-2 expression in the heart and skeletal muscle confirmed that tissue engraftment of the WT-HSPC-derived macrophages resulted in the restoration of LAMP-2 expression in recipient mice. Importantly, immunofluorescence studies showed the diffuse pattern of spatial restoration of vesicular LAMP-2 expression in transplanted mice. The transfer of LAMP-2-positive vesicles from WT-donor-derived macrophages also resulted in a reduction in LC3-II levels and number of AVs in the heart and skeletal muscle tissues of recipient mice, indicating rescue of autophagic flux. Our findings may provide a new paradigm for the treatment of a wide assortment of autophagy-related disorders that collectively have a major impact on public health.

F-2033

ROLE OF TRPC7 IN REGULATING THE FUNCTIONS OF EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

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Many ion channels serve to maintain the electrophysiological function and calcium homeostasis of cardiomyocytes. Although classical sodium, potassium and calcium channels are intensively studied, many other channels may also contribute to the delicate electrophysiological regulation of cardiomyocytes. Canonical transient receptor potential (TRPC) channels are non-selective cation channel that are activated by G-protein coupled receptors. TRPC channels are widely expressed in different tissues and play important roles in the maintaining cellular homeostasis. The aim of this project is to study the function of TRPC7, the most elusive member in the TRPC family, in cardiomyocytes. Western blotting showed that TRPC7 is expressed in mouse heart. Immunocytochemistry experiments showed that the channel locates at plasma membrane at early differentiation stage of mouse embryonic stem cell-derived cardiomyocytes (mESC-CMs), but translocates from membrane to a location closer to the sarcomere during the maturation of mESC-CMs. Translocation of TRPC7 occurs during the whole process of maturation; most channels are located near the M-line in less mature mESC-CMs while they are located near the Z-line in more mature mESC-CMs. Knockdown of TRPC7 led to a decrease in calcium transients in mESC-CMs. In addition, knockdown of TRPC7 caused the disassembly of sarcomere in neonatal ventricular myocytes. Our result suggests that TRPC7 may serve multiple roles in differentiating cardiomyocytes. Further study is needed to elucidate the mechanisms of how TRPC7 regulates the functions of differentiating cardiomyocytes.

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F-2035

MONITORING STEM CELL-DERIVED CARDIOMYOCYTE MATURATION BY OPTICAL METABOLIC IMAGING

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Cardiovascular disease remains the leading cause of death in the world despite advances in treatment. Human pluripotent stem cells (hPSCs) can generate any cell lineage in vitro, including cardiomyocytes. hPSC-derived cardiomyocytes have immense potential to impact clinical care and fundamental research for cardiovascular disease. However, hPSC-derived cardiomyocytes exhibit a relatively immature phenotype. New technologies that can non-invasively quantify the maturation state in live hPSC-cardiomyocytes are needed to effectively optimize a mature phenotype. hPSC-derived cardiomyocytes undergo dramatic metabolic changes during maturation. Here, we monitor these metabolic changes that occur in hPSC-derived

cardiomyocytes during extended time in culture by multiphoton fluorescence lifetime imaging (FLIM) of the metabolic co-enzymes NAD(P)H and FAD. Changes in hPSC-cardiomyocyte metabolism during maturation were non-invasively quantified at a single-cell level with the optical metabolic imaging (OMI) parameters. Our results indicate that the lifetime of NAD(P)H increases and the redox ratio decreases with maturation over a 100-day time-course in hPSC-derived cardiomyocytes. These label-free imaging technologies could be used to test strategies to optimize the maturation of hPSC-derived cardiomyocytes in vitro.

F-2037

IN VITRO MATURED HESC-DERIVED CARDIOMYOCYTES ELECTRICALLY COUPLE AND FORM IMPROVED GRAFTS IN INJURED HEARTS

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The ability of pluripotent human embryonic stem cells (hESCs) to yield large quantities of functional cardiomyocytes (CMs) holds tremendous promise for use in cardiac regeneration. However, the immature structural, electrophysiological and contractile phenotype of available hESC-CM populations limits their translational potential. The present study was aimed at testing two hypotheses: 1) previously reported approaches to promote the maturation of hESC-CMs by culture on polydimethylsiloxane (PDMS) membranes can be upscaled for transplantation studies; and 2) PDMS-matured hESC-CMs will efficiently engraft in injured hearts and form graft myocardium with enhanced structural and functional properties. We generated transgenic hESC-CMs that stably expressed the fluorescent voltage-sensitive protein ASAP1, then comprehensively phenotyped these myocytes after 20 and 40 days of in vitro maturation on either tissue culture plastic (TCP) or PDMS. Next, we transplanted day 20 or 40 TCP vs PDMS ASAP1+ hESC-CMs into injured guinea pig hearts (n=6-9 per group). Engrafted hearts were later analyzed by ex vivo optical voltage mapping studies and histology. Relative to TCP controls, hESC-CMs on PDMS at both time-points exhibited increased cardiac gene expression as well as a more mature structural and electrophysiological phenotype in vitro. Although CMs from both substrates showed similar capacity for engraftment (graft area by histomorphometry), graft formed using PDMS-matured myocytes had more mature histological properties, with better-aligned cardiomyocytes and increased sarcomere lengths and gap junction expression. Most importantly, graft formed with PDMS-matured myocytes showed enhanced electrophysiological properties by optical mapping based on ASAP1 fluorescence, including better host-graft electromechanical integration and more rapid and uniform conduction velocity. We demonstrate that hESC-CMs matured on PDMS can be produced in large quantities (scale of 10e8 to 10e9 CMs). Moreover, PDMS-matured myocytes form large intramyocardial grafts with enhanced cardiac structure and greatly improved electrical function, thereby establishing that CM maturation prior to transplantation meaningfully improves outcomes in vivo.

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F-2039

GENOME-EDITED IPSC AND PIG CARDIOMYOPATHY MODELS REVEAL MUTANT RBM20 FORMS MISLOCALIZED GRANULES TO DOMINANTLY DISRUPT GLOBAL SPLICING

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Dilated cardiomyopathy (DCM) is the most common indication for heart transplantation. Among over 50 DCM causal genes identified so far, the gene RBM20 has emerged as a key regulator of cardiac splicing in both early cardiogenesis and inherited dilated cardiomyopathy. The pathogenic RBM20 mutations display a striking recurring pattern of eight tightly clustered single amino acid DCM-associated substitutions in the arginine-serine (RS) domain of RBM20, but the pathogenic mechanism of these clustered mutations is unknown. To understand the role of specific RBM20 alleles, we generated genome-edited human induced pluripotent stem cell (iPSC)-derived cardiomyocytes with heterozygous or homozygous R636S mutation or a functional knockout. In addition to both mutant and loss-of-function alleles mimicking the contractile phenotypes of dilated cardiomyopathy patients, R636S mutation resulted in RBM20 mislocalization from the cardiomyocyte nucleus to the cytoplasm as prominent granules. Global transcriptome analyses revealed distinct subclasses of RBM20 target splicing defects, including pathogenic mutation-specific, dosage dependent and heterozygous mutation-dominant splicing. Providing in vivo context, we independently confirmed global and splice-event specific observations in the hearts of genome- edited pigs with the same R636S mutation. Our results highlight novel pathogenic mechanisms in splicing factor-mediated cardiomyopathy, through both splicing-dependent and independent pathways, that will rationally guide future therapeutic development.

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F-2041

SINGLE-CELL TRANSCRIPTOME ANALYSIS DURING CARDIOGENESIS REVEALS BASIS FOR ORGAN LEVEL DEVELOPMENTAL ANOMALIES

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Organogenesis involves integration of myriad cell types with reciprocal interactions, each progressing through successive stages of lineage specification and differentiation. Establishment of unique gene networks within each cell dictates fate determination, and mutations of transcription factors that drive such networks can result in birth defects. Congenital heart defects are the most common malformations and are caused by disruption of discrete subsets of progenitors, however, determining the transcriptional changes in individual cells that lead to organ-level defects in the heart, or other organs, has not been tractable. In this work, we employed single-cell RNA sequencing to interrogate early cardiac progenitor cells as they become specified during normal and abnormal cardiogenesis, revealing how dysregulation of specific cellular sub-populations can have catastrophic consequences. A network-based computational method for single-cell RNA-sequencing that predicts lineage specifying transcription factors identified Hand2 as a specifier of outflow tract cells but not right ventricular cells, despite failure of right ventricular formation in Hand2-null mice. Temporal single-cell transcriptome analysis of Hand2-null embryos revealed failure of outflow tract myocardium specification, whereas right ventricular myocardium differentiated but failed to migrate into the anterior pole of the developing heart. We found dysregulation of retinoic acid signaling, responsible for anterior-posterior cardiac patterning, that was associated with posteriorization of anterior cardiac progenitors in Hand2-null mutant hearts and ectopic atrial gene expression in outflow tract and right ventricle precursors. This work reveals transcriptional determinants in individual cells that specify cardiac progenitor cell fate and differentiation and exposes mechanisms of disrupted cardiac development at single-cell resolution, providing a framework to investigate congenital heart defects.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

F-2043

EFFECTIVE GENERATION OF ENDOTHELIAL CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Cardiovascular disease affects millions of people. Induced pluripotent stem cells (iPSCs) derived functional endothelial cells (ECs) are emerging as unlimited resource for cell and gene therapy for cardiovascular diseases. Therefore, there is an urgent need for developing robust and efficient approaches to differentiate iPSCs into mature ECs. Here we reported an effective protocol for generating mature and functional human endothelial cells from iPSCs for mechanistic studies, drug screening and vascular therapy. Integration-free iPSCs were generated from human peripheral blood mononuclear cells (PBMC). The stemness of the iPSCs were confirmed by staining of the stem cells markers using specific antibodies as well as embryoid body formation. iPSCs were differentiated into endothelial cells using a diversity of combinations of growth factors and small molecules targeting the key signaling pathways of the endothelial lineage development. The expression of the endothelial lineage markers, such as CD31 (PECAM1) ,CD144 (VE-cadherin) and vWF (Von Willebrand factor) was examined by immunofluorescence staining and flow cytometry. In vitro tube formation and Dil fluorescent dye-labeled acetylated low density lipoprotein(Dil-ac-LDL) uptake assay were performed to demonstrate the function of the differentiated cells.iPSCs 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 cells from iPSCs displayed the phenotypes and function of the primary endothelial cells. CD144 and vWF were positively detected by immunofluorescence microscopy and 98% of the cells expressed the endothelial lineage markers CD31 and CD144 determined by flow cytometry. The differentiated cells also assembled into well-defined vessel-like structures in tube formation assay in vitro. Dil-ac-LDL uptake assay showed that the hiPSCs-derived ECs were able to take Dil-ac-LDL.We established an effective and robust strategy for endothelial cells differentiation from iPS cells. The iPSC-derived endothelial cells demonstrate the phenotype and function of primary human endothelial cells.

F-2045

ANGIOTENSIN 2 ATTENUATES THE BIOACTIVITIES OF HUMAN ENDOTHELIAL PROGENITOR CELLS VIA DOWNREGULATION OF BETA2-ADRENERGIC RECEPTOR

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Cross talks between the renin-angiotensin system (RAS), sympathetic nervous system, and vascular homeostasis are tightly coordinated in hypertension. Angiotensin II (Ang II), a key factor in RAS, when abnormally activated, affects the number and bioactivity of circulating human endothelial progenitor cells (hEPCs) in hypertensive patients. In this study, we investigated how the augmentation of Ang II regulates adrenergic receptor-mediated signaling and angiogenic bioactivities of hEPCs. Interestingly, the short-term treatment of hEPCs with Ang II drastically attenuated the expression of beta-2 adrenergic receptor (ADRB2), but did not alter the expression of beta-1 adrenergic receptor (ADRB1) and Ang II type 1 receptor (AT1R). EPC functional assay clearly demonstrated that the treatment with ADRB2 agonists significantly increased EPC bioactivities including cell proliferation, migration, and tube formation abilities. However, EPC bioactivities were decreased dramatically

when treated with Ang II. Importantly, the attenuation of EPC bioactivities by Ang II was restored by treatment with an AT1R antagonist (telmisartan; TERT). We found that AT1R binds to ADRB2 in physiological conditions, but this binding is significantly decreased in the presence of Ang II. Furthermore, TERT, an Ang II-AT1R interaction blocker, restored the interaction between AT1R and ADRB2, suggesting that Ang II might induce the dysfunction of EPCs via downregulation of ADRB2, and an AT1R blocker could prevent Ang II-mediated ADRB2 depletion in EPCs. Taken together, our report provides novel insights into potential therapeutic approaches for hypertension-related cardiovascular diseases.

F-2047

TRANSDIFFERENTIATION OF HUMAN ADULT FIBROBLASTS INTO AUTHENTIC ENDOTHELIAL CELLS

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Previously, we reported direct conversion of adult fibroblasts (FBs) into endothelial cells (ECs) using defined factors in mice. Here, we assessed whether this approach can be applied for direct conversion of human adult ECs to authentic ECs. We tested whether 5 defined factors (Foxo1, Er71, Klf2, Tal1, Lmo2) for mouse induced ECs (iECs) could convert human dermal FBs (HDFs) to ECs. 28 days after infection of lentiviruses expressing each gene to HDFs, ECs defined by VE-cadherin expression on FACS were detected (32.1±5.1%). Interestingly, 2 factors were dispensable, and only 3 factors (factor X, Y, Z) were necessary and sufficient to make human iECs (49.4±3.5%). To enhance the efficiency, a lentivirus expressing 3 factors plus GFP altogether were made using 2A system. Unexpectedly, VE-cadherin+/GFP+ cells induced by this virus was integrated with VE-cadherin-/GFP+ and HDF control together in hierarchical analysis in whole transcriptome sequencing, which meant VE-cadherin+ cells were not completely converted yet. The proportion of cells expressing another endothelial specific marker, CD31, together (VE-cadherin/CD31 double positive (DP) cells) was only 5.2±0.6%. To get complete conversion, several means were tried. Among them, rosiglitazone (MET inducer) treatment, prolonged incubation after VE-cadherin sorting, and suppression of some FBs specific transcription factors (TFs) using siRNA worked. However, 2nd stage infection of other endothelial specific TFs, shear stress, treatment of VEGF, SB431542 or Wnt modulators, time dependent expression using Tet-on system did not. Final protocol could convert 19.6±3.0% of HDFs into DP cells 6 weeks after infection. DP cells showed characteristics of authentic human ECs (Matrigel tube formation, Ac-LDL uptake, lectin binding, NO production, IF staining for EC markers, characteristic EC morphology on optical and electron microscope, and whole transcriptome sequencing). Our iEC protocol showed the most efficient EC conversion rate, compared with the protocols suggested by other groups. Furthermore, unlike other studies in which EC-like cells were defined as only one single marker, our study suggests a new perspective that the use of double markers can purify mature iECs which exclude immature iECs that arise during the direct conversion process.

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HEMATOPOIESIS/IMMUNOLOGY

F-2049

EX VIVO ACTIVATION OF HEAT SHOCK FACTOR 1 (HSF1) PROMOTES SUSTAINED HEMATOPOIETIC STEM CELL SELF-RENEWAL

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The inability to maintain and expand somatic stem cells in culture represents a major barrier to their use in cell-based therapies. As such, there is significant need to uncover why somatic stem cell self-renewal is diminished ex vivo, and to discover conditions that support stem cell growth in vitro. We recently discovered that hematopoietic stem cells (HSCs) exhibit low protein synthesis in vivo, regardless of whether they are quiescent or undergoing self-renewing divisions. Low protein synthesis is necessary for HSCs, as modest (~30%) increases in protein synthesis impair HSC self-renewal. In the present study, we determined that cultured HSCs rapidly

upregulated genes that promote translation and exhibited a ~2000% increase in protein synthesis. Increased protein synthesis overwhelmed protein quality control systems within HSCs, caused an imbalance in protein homeostasis, and was associated with nuclear translocation of Hsf1. Hsf1 is the master regulator of the heat shock pathway, and induces transcription of heat shock proteins that coordinate protein folding, trafficking and degradation to sustain protein homeostasis in response to proteotoxic stress. Inactive Hsf1 is typically sequestered in the cytoplasm through binding to Hsp90 and TRiC, and was rarely seen in the nucleus within HSCs in vivo. Genetic deletion of Hsf1 exacerbated HSC depletion in vitro, but had little effect on HSC function in vivo. These data indicated that Hsf1 promotes ex vivo HSC maintenance, and raised the possibility that increasing Hsf1 activation could enhance HSC self-renewal. To test this, we cultured purified HSCs in the presence of a Hsp90 or TRiC inhibitor that each promoted Hsf1 nuclear translocation, and assessed HSC function in serial transplantation assays. Over the 10-day culture period, HSCs proliferated extensively and fully retained serial long-term multilineage reconstituting activity. The positive effect of both Hsp90 and TRiC inhibitors on HSC growth was completely ablated in the absence of Hsf1. Furthermore, Hsf1 activation reduced the unfolded protein load and partially rebalanced protein homeostasis. These findings indicate that maintaining protein homeostasis is a key factor in promoting ex vivo stem cell self-renewal, and reveal new strategies that enable sustained HSC growth in culture.

F-2051

FORGOTTEN GEMS: HUMAN CD34- HEMATOPOIETIC STEM CELLS

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Human HSC research effort has been largely restricted to CD34+ cells both in prenatal and adult life. However, recent evidence has highlighted that both adult bone marrow and umbilical cord blood contain CD34- SCID-repopulating cells (SRCs). CD34- SRCs exhibit distinct in vivo repopulation kinetics and capacity to produce functional CD34+ HSCs, and accordingly have been speculated to reside at the apex of the human haematopoietic hierarchy. However, only one out of 5000 cells within the CD34-/CD38-/CD93+ population is estimated to have in vivo repopulation capacity, which has significantly hindered efforts to elucidate the function and molecular mechanisms regulating this HSC compartment in comparison to CD34+ HSCs. We thus aim to enhance our ability to purify bona-fide CD34- HSCs and further expand the knowledge of this immature stem pool. We have successfully identified an additional positive selection marker, enriching for CD34- SRCs by 20-fold, thus facilitating our efforts to elucidate the functionality and molecular pathways maintaining this population. Through serial transplantations of enriched CD34- SRCs in NSG and enhanced NSG mouse models (NSG-S, NSBGW, and NSG-hmSCF), we observed more potent and efficient repopulation and lineage commitment kinetics. Resulting data and single cell-transcriptomic profile of this population represent a further step toward a better understanding of its role in human hematopoiesis. With these new data we have asserted the importance of human CD34- HSCs. In view of their highly quiescent nature, yet robust population capacity, it is easy to speculate that the CD34- HSCs hold enormous therapeutic potential that cannot be ignored.

F-2053

IDENTIFICATION OF A MACROCYCLIC DITERPENE COMPOUND WITH COMBINATORIAL DIFFERENTIATING EFFECT VIA PKC ACTIVATION AND BET INHIBITION IN PRIMARY HUMAN MONOCYTIC LEUKEMIA

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Safer and more efficient therapy for patients with acute myeloid leukemia (AML) is needed and differentiation therapy is an appealing option to eradicate leukemic stem cells blocked in differentiation. With the aim of identifying new AML differentiating molecules, we screened 528 natural compounds on primary AML samples in our co-culture system mimicking the niche. Compounds that upregulated the expression of the myeloid differentiation markers CD11b/CD15 were considered as positive hits. Among those, a macrocyclic diterpene called H4 upregulated expression of CD11b (DMSO 22±5%, H4 66±8% SEM), which was accompanied with a clear decrease in nuclear-cytoplasmic ratio, on patient cells with AML subtype M5. No toxicity was observed to healthy control cells. In order to unravel the signaling pathways contributing to the differentiation response, we performed a combinatorial treatment with H4 and 176 molecules with defined drug targets. Inhibition of PKC, RTKs,

JNK and Raf/MEK/ERK impeded the differentiation-promoting effect of H4 with complete block upon combined treatment with the PKC inhibitor GFX109203X. Furthermore, PKC-GFP-isoforms (α, β ,ε, δ) were translocated to the plasma membrane within 60-120 seconds upon H4 treatment. Demonstrating that H4 induced myeloid differentiation is mediated by PKC activation. Gene expression profiling on primary M5 AML cells treated for 16h with H4 revealed a significant enrichment for MYC target genes. The increase in MYC transcription was confirmed by qPCR and was accompanied by an increased protein translation rate (OP-Puro incorporation assay). To evaluate the role of MYC activation upon H4 treatment, MYC expression was inhibited using the BET inhibitor CPI-203 in combination with H4. The combination increased the H4 induced differentiation capacity synergistically from a 3-fold (H4 or CPI alone) to 11-fold increase in CD11b MFI in comparison to DMSO. A similar trend was seen in two additional patient samples of M5 AML. In summary, the compound H4 promotes differentiation by activation of PKC. Combinatorial treatment with BET inhibition results in stronger promotion of differentiation with reduced expression of MYC. Thus, the combination of PKC agonists and BET inhibitors has therapeutic potential that warrants further studies as a differentiation option for AML.

Funding Source: This work was supported by the Swedish Cancer Foundation, the Swedish Research Council, the Swedish Society for Medical Research, and Kamprad foundation.

F-2055

MOLECULAR CHARACTERIZATION OF T CELL RECEPTOR AND HLA IN T-CELL DERIVED IPSCS

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As the immunotherapy market continues to grow, significant progress has been made in treating diseases and cancer by leveraging the potential of immune cells, particularly T cells. Autologous CAR T cell therapy has shown high levels of complete remission in treatment of blood-based cancers, but the personalized medicine is hard to scale. Off-the-self, allogenic CAR T treatments derived from donor T cells are being explored as a potential to address issues with the autologous treatments. CAR T cell therapies could be advanced through the use of induced pluripotent stem cells (iPSC). iPSCs derived from T cells offer advantages over traditional somatic cell iPSCs by conservation of T cell receptor (TCR) through reprogramming. Additionally, the ability to engineer chimeric antigen receptors (CAR) into iPSCs, which can then be expanded and differentiated back to T cells, provides a cost-effective method to generate large number of allogeneic CAR-T cells. The therapeutic success of such allogeneic T cell therapies can be further increased with high-resolution HLA typing to reduce the risk of any post-transplant complications. In this study, we utilize qPCR and next generation sequencing tools for the comparison and thorough characterization of TCR and HLA in iPSCs derived from Fibroblast, PBMC and T cells. Since reprogramming is carried out from a heterogeneous population of T cells, detailed phenotyping of the starting donor cells was performed. The parent and resulting iPSC clones that were subjected to comprehensive characterization to confirm pluripotency were used for downstream molecular characterization. To determine the T cell sub types that were reprogrammed, a qPCR assay was developed to assess the germline configuration of iPSCs. Next Generation Sequencing-based Immune Repertoire was used to generate high-throughput sequencing data of TCR populations. These assays revealed the diversity, or the number of unique TCR sequences, present in the starting T cells and the resulting iPSC clones. Further, HLA typing was carried out on the parental cells and iPSC clones to qualify the cells for use in immune therapy applications. The development a robust workflow to characterize the starting immune cell population and the iPSC lines will benefit the development of translational therapies.

F-2057

GENERATION OF HYPOIMMUNOGENIC HUMAN PLURIPOTENT STEM CELLS

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Polymorphic human leukocyte antigens (HLA) form the primary immune barrier to cell therapy. In addition, innate immune surveillance impacts cell engraftment, yet a strategy to control both, adaptive and innate immunity, is lacking. Here we employed multiplex genome editing to specifically ablate the expression of the highly polymorphic HLA class Ia and class II in human pluripotent stem cells (hPSCs). Furthermore, to prevent

innate immune rejection and further suppress adaptive immune responses, we expressed the immunomodulatory factors PD-L1, HLA-G, and the macrophage 'don't-eat me' signal CD47, from the AAVS1 safe harbor locus. Utilizing in vitro and in vivo immunoassays, we found that T cell responses were blunted. Moreover, NK cell killing and macrophage engulfment of our engineered cells was minimal. Our results describe an approach that effectively targets adaptive as well as innate immune responses and may therefore enable cell therapy on a broader scale.

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F-2059

EPIDERMAL GROWTH FACTOR REJUVENATES AGING MOUSE HEMATOPOIETIC STEM CELLS

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Aging hematopoietic stem cells (HSCs) display distinct abnormalities such as myeloid skewing, decreased repopulating capacity, and leukemia predisposition. Radiation exposure potentiates aging of the hematopoietic system. Our lab demonstrated that EGF promotes HSC regeneration after radiation. Therefore, we hypothesized that EGFR signaling may rejuvenate aging HSCs. We discovered that aged >18-24 month old C57BL/6 mice have decreased levels of EGF in blood compared to young 2-4 month old mice and decreased expression of EGFR on bone marrow (BM) ckit+sca-1+lin- (KSL) stem/progenitor cells. Aged BM KSL displayed increased DNA damage in culture with thrombopoietin, SCF and Flt-3 ligand (TSF) compared to young KSL while EGF treatment decreased DNA damage in aged BM KSL compared to TSF. Treatment with EGF also increased colony forming capacity of aged BM KSL and increased primary donor cell engraftment compared to TSF. We next tested whether systemic administration of EGF for 4 weeks could alter the hematopoietic characteristics of aged mice. EGF treatment decreased BM myeloid skewing and increased CD3 T cell content in aged mice compared to saline-injected controls. Furthermore, EGF treatment of aged mice increased functional HSCs capable of competitive multilineage engraftment of recipient congenic mice in primary and secondary transplants. We next sought to determine if deficiency in EGFR signaling could accelerate hematopoietic aging in mice by using a doxycycline-inducible, hematopoietic cell specific EGFR dominant negative mutant model (SCL-tTA;EGFR-DN mice). Aged mutant mice displayed increased myeloid skewing and generated significantly decreased colony forming cells, compared to age-matched, EGFR-expressing mice. Mechanistically, BM KSL cells from aged mutant mice demonstrated increased senescence and increased expression of p16 compared to young mice. Additionally, in vivo treatment of aged C57BL/6 with EGF decreased reactive oxygen species level compared to saline, and EGF stimulation of aged BM KSL cells in vitro resulted in decreased phosphorylation of p38. These studies suggest that EGF/EGFR signaling declines with age and that reactivation of EGFR signaling via EGF treatment can ameliorate clinically relevant features of hematopoietic aging, including HSC self-renewal capacity.

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F-2061

THE ROLE OF BASIC HELIX-LOOP-HELIX TRANSCRIPTION FACTOR SWITCHING IN DETERMINING CHROMATIN LANDSCAPE AND T CELL FATE COMMITMENT IN MOUSE EARLY T CELL DEVELOPMENT

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For hematopoietic precursors establishing their T cell identity in mouse thymus, a key question is how the precise coordination of key transcription factors activates the T lineage program and shuts off the precursor program. To identify the key regulatory changes in a non-biased way, we profiled the global chromatin accessibility changes during the T lineage commitment process using ATAC-seq. Many regulatory elements that became accessible during commitment were highly enriched for E protein heterodimer E2A-HEB binding motifs. Using E protein

inhibitor ID2 to block the E2A-HEB activity in a committed pro-T cell line, we observed that E2A-HEB is indispensable to maintain the chromatin accessibility of T cell specific elements and to activate their associated T cell genes. Paradoxically, E2A-HEB expression levels are nearly constant throughout the developmental process, and ID2 level is nearly absent. We therefore have tried to identify the mechanism that must be counteracting E2A-HEB activity before commitment, specifically testing the precursor-specific form of E2A heterodimer, E2A-Lyl1, and its cofactor Lmo2. Forced expression of Lmo2 and Lyl1 in primary early committed T cells induces closing of commitment specific sites and loss of T lineage commitment, evident by the downregulation of T cell gene expression, including Bcl11b, Rag1 and Cd3e. More strikingly, it shows dramatic backward chromatin and transcriptome reprogramming to a precursor-like state, with the reopening of precursor-specific sites and the reactivation of many precursor genes including PU. 1, Bcl11a, c-Kit. These results indicate that in precursors, E2A-Lyl1-Lmo2 can not only establish the precursor specific chromatin landscape and activate precursor genes, but can also strongly compete against existing HEB proteins and suppress E2A-HEB activity on initiating T lineage program. Our work suggests that the E2A heterodimer partner switching during early T cell development is crucial in determining T lineage progression through regulating the chromatin landscape both before and after commitment. Our work also suggests new strategies of hematopoietic reprogramming.

F-2063

INHIBITION OF PROTEIN TYROSINE PHOSPHATASE-SIGMA PROMOTES HEMATOPOIETIC REGENERATION VIA ACTIVATION OF RAC1 SIGNALING PATHWAY

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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 regarding the function of protein tyrosine phosphatases (PTPs) in regulating HSC fate. We previously discovered that receptor protein tyrosine phosphatase-sigma (PTPσ) was highly expressed by murine and human HSCs and constitutive deletion of PTPσ caused a marked increase in HSC repopulating capacity in vivo (Quarmyne et al. J Clin Invest 2015). We hypothesized that pharmacologic inhibition could increase HSC repopulating capacity or regenerative capacity following injury. Utilizing a small molecule screen, we identified small molecule, 5483071 (Chembridge), with predicted PTPσ inhibitory activity. We synthesized a chemical homologue, DJ001, and demonstrated that DJ001 had strong PTPσ inhibitory activity in vitro (IC50 = 1.54μM). Systemic administration of the PTPσ inhibitor, DJ001, to irradiated mice promoted HSC regeneration, accelerated hematologic recovery and improved survival compared to control, irradiated mice. Similarly, following chemotherapy, DJ001 administration accelerated hematologic recovery in mice. DJ001 displayed high specificity for PTPσ and antagonized PTPσ via unique non-competitive, allosteric binding. Mechanistically, DJ001 suppressed radiation – induced HSC apoptosis via activation of the RhoGTPase, RAC1, and induction of BCL-XL. DJ001 concordantly induced HSC proliferation after irradiation via RAC1 – dependent induction of CDK2. Treatment of irradiated human HSCs with DJ001 also promoted the regeneration of human HSCs capable of multilineage in vivo repopulation in NOD/SCID IL2 receptor-gamma null (NSG) mice. These studies demonstrate the therapeutic potential of a selective, small molecule PTPσ inhibitor for human hematopoietic regeneration.

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PANCREAS, LIVER, KIDNEY

F-2065

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 signaling logics underlying renal epithelial cell differentiation. 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 a complex combination of signaling agonists and/or antagonists that 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. NPHS1 or 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. The cells will not only provide a valuable tool to understand the signaling inputs required for podocyte development/homeostasis but will also provide a critic tool towards developing treatments for human kidney diseases characterized by podocyte dysfunction.

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F-2067

DEFINING PROGENITOR TYPES AND PROGENITOR PROGRAMS IN STRATIFICATION OF THE MOUSE AND HUMAN RENAL COLLECTING SYSTEM

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In the adult kidney, the collecting duct is responsible for maintenance of water and pH homeostasis. These two important functions are carried out by distinct cell populations, the principal cells and the intercalated cells. Recently generated single RNA-seq (scRNA-seq) data in the laboratory illuminates diversity in these cell types in relation to their position along the cortical-medullary axis of the kidney. During development, a progenitor pool within each branch tip of the arborizing ureteric epithelium lays down the cellular template for the epithelial network of the collecting system. The mechanisms responsible for the tight temporal and spatial regulation of mature cell fates originating from this progenitor pool are not well understood. We are combining lineage tracing with scRNA-seq and in vivo expression studies to better understand how molecular and cellular changes within the progenitor pool relate to diverse developmental outcomes in assembly of the mature collecting system. Further, we are exploring signaling networks and transcriptional mediators to identify relevant regulatory mechanisms. We expect these approaches will give new insights into basic mechanisms of progenitor programming with potential relevance beyond the kidney model. An understanding of these programs is essential for improving current kidney organoid models which lack a functional collecting duct epithelium.

F-2069

FOXA TRANSCRIPTION FACTORS SPECIFY HUMAN ENDODERM-DERIVED ORGAN LINEAGES BY PRIMING SIGNAL-RESPONSIVE ENHANCERS

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During development, cell fate decisions are driven by transcription factors (TFs) that initiate cell type-specific gene expression programs. Earlier studies of differentiation of human embryonic stem cells (hESCs) into pancreatic cells have suggested epigenetic priming of pancreatic enhancers as a mechanism by which pancreatic genes are primed in early endodermal intermediates prior to pancreas induction.

However, the mechanism that underlies priming of lineage-specific enhancers remains unclear. We tested the hypothesis that enhancer priming is initiated by FOXA family TFs which have the ability to engage with condensed chromatin. We show that pancreatic enhancers are broadly occupied by FOXA TFs and that association of FOXA TFs with pancreatic enhancers correlates tightly with a gain of the H3K4me1 histone modification characteristic of primed enhancers, as well as a gain in ATAC-seq signal, indicative of accessible chromatin. Supporting a role of FOXA TFs in pancreatic lineage specification, FOXA1-/-/FOXA2-/- lines fail to initiate expression of pancreatic master regulator genes. Analysis of pancreatic enhancers in FOXA1-/-/FOXA2-/- hESC-pancreatic progenitors further revealed a lack of H3K27ac and H3K4me1 deposition and ATAC-seq signal. To explore the mechanism by which FOXA TFs activate pancreatic enhancers, we examined FOXA occupancy throughout the differentiation time course. This analysis revealed FOXA recruitment to a subset of pancreatic enhancers prior to enhancer activation and pancreatic gene expression. Compared to the remainder of pancreatic enhancers, this enhancer subset was enriched for retinoic acid receptor (RAR) motifs and binding of the RAR subunit RXR. These findings suggest that priming by FOXA TFs is limited to a subset of pancreatic enhancers that are signal responsive. Using a hESC-based model of liver development, we determined that the association of early FOXA recruitment and signal-dependency also exists at hepatic enhancers. Consistent with liver induction being BMP-dependent, “FOXA-primed” liver enhancers were enriched for motifs of BMP-dependent SMAD TFs. Combined, our analysis suggests a role for pioneer TFs in selectively priming signal-dependent enhancers with broader activation of the entire enhancer repertoire occurring through other mechanisms.

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F-2071

STEM CELL DERIVED PANCREATIC ISLETS FOR TRANSPLANTATION INTO ANTERIOR CHAMBER OF RABBIT EYES

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Type 1 Diabetes is a rapidly expanding endocrine disease affecting millions of people worldwide. Patient with type 1 Diabetes can be successfully treated with transplantation of cadaveric pancreatic islets, but this approach is limited by shortage of donor pancreatic tissue and low viability of islets post transplantation due to inflammation after blood contact. Transplantation of human pluripotent stem cell (hPSC) derived islets into the anterior chamber of eye (ACE) can potentially solve these problems by utilizing a renewable cell source and the possibility of non-invasive longitudinal evaluation post transplantation. In this study, we differentiated hPSCs into pancreatic islet-like cell aggregates. The islet-like aggregates were then incorporated into 3D porous scaffolds made of recombinant spider silk protein. The cell aggregate containing silk matrices were injected into ACE of a large-eyed pre-clinical animal model. Engraftment and maturation into functional islets in vivo were examined one month after transplantation. Our results show that the injected aggregates were integrated onto the iris tissue and became vascularized. The aggregates closely resemble human islets in both size and morphology, and contain mainly mono-hormonal endocrine cells expressing either insulin, glucagon or somatostatin. No major adverse effects on eye morphology and function were found during the transplantation period. Glucose stimulated insulin secretion (GSIS), transplant rejection and tolerance, and biosafety are currently under investigation. Together these results indicate that hPSC-derived islets could be utilized instead of primary human pancreatic islets for transplantation therapy for diabetes in the future.

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F-2073

GENERATION OF HUMAN IPSC-DERIVED PANCREATIC DUCTAL CELLS FOR IN VITRO DISEASE MODELLING OF PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic Ductal Adenocarcinoma (PDAC), which makes up the majority of pancreatic cancer cases, begins as a tumor in the pancreatic duct. Each year, more than 350, 000 people are diagnosed with PDAC and about 96% of them die. The 5-year survival rate of PDAC is very low, and even with surgical removal of the tumor, the 5-year survival rate only increases to about 15%. Even so, due to rapid metastasis and resistance, surgery and standard treatments like chemotherapy are not effective. While there has been advancement in discerning the molecular background of the disease, there is limited understanding of early drivers – genetic and environmental – PDAC transformation, mainly due to limited models of human PDAC. Here, we aim to develop an in vitro model of PDAC by first establishing a robust protocol for generating human induced pluripotent stem cell (hiPSC)-derived pancreatic ductal cells. Our lab has previously developed a consistent and effective protocol to generate pancreatic progenitors across multiple iPSC cell lines. Using this protocol, we have collected cells from pluripotency stage throughout the sequential induction of definitive endoderm, posterior foregut, and pancreatic progenitor, to find a pivotal time-point to direct cells toward ductal lineage. Our results indicate a temporal window with the highest gene expression of ductal progenitor markers, such as SOX9, KRT19, HNF1B, and CA2, which is the best period to drive the PDX1+NKX6.1+PTF1A- pancreatic bipotent trunk progenitors toward the pancreatic ductal lineage. Notch, BMP, EGF, FGF, Shh signaling pathway modulation was tested to determine the optimal generation of pancreatic bipotent trunk progenitors, in favor of generating ductal epithelium from iPSC-derived pancreatic progenitors, and differentiated ductal cells were functionally assayed to assess enzymatic activity by measuring carbonic anhydrase (CA) levels. Our next goal will be to derive PDAC patient iPSCs from a panel of familial PDAC patients, differentiate to ductal cells and expose them to different microenvironments, such as hypoxia, and/or assess their interaction with the immune system, which are all known to be implicated in PDAC, to develop a more comprehensive and reliable disease model.

F-2075

SOX9+/PTF1A+ CELLS DEFINE THE TIP PROGENITOR DOMAIN WITHIN THE HUMAN DEVELOPING PANCREAS

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The developing mammalian pancreas is a highly organized structure, in which subpopulations of progenitor and committed cells can be identified based on their molecular profile and location, after branching morphogenesis has initiated. The pancreatic multipotent progenitor cells (MPCs) in early mouse embryos are known to express several key transcription factors, including Pdx1, Sox9, and Ptf1a, which are indispensable for their establishment, maintenance and proliferation. Recent advances have been made in understanding human pancreatic development, however, little is known regarding the presence of pancreatic progenitors, particularly in the second trimester, and their characteristics. We have identified pancreatic human multipotent progenitor cells (hMPC) between 13.5 and 17.5 weeks of gestation, which are characterized by the expression of SOX9 and PTF1A. These cells reside within the tips of the branching epithelium and were isolated by combining an RNA-based probe technology with fluorescence activated cell sorting. These cells are in a proliferative state, in the process of branching morphogenesis, and their pool decreases over time. RNA-sequencing profiling revealed that SOX9+/PTF1A+ cells are enriched for genes characteristic of MPCs rather than committed cells and show activation of NOTCH and WTN/β-catenin signaling pathways, known regulators of the MPC niche and pancreatic specification. The SOX9+/PTF1A+ cells thus display the full spectrum of characteristics that define the MPC niche, including maintenance of key transcription factors expression, proliferative and branching capabilities. Based on our results, we propose that SOX9+/PTF1A+ cells in the second trimester human pancreas are uncommitted MPC-like cells that reside at the tips of the expanding pancreatic epithelium, directing self-renewal and inducing pancreatic differentiation. This novel insight into the genetic signature of human fetal MPCs will allow for further understanding of the molecular programs and interactions that sustain the human pancreatic MPC niche. It will also help improve pluripotent stem cell differentiation protocols and allow for better manipulation of the stem cell-derived ß-like cell for regenerative medicine and cell replacement therapy.

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EPITHELIAL TISSUES

F-2079

DORSOVENTRAL DIFFERENCE IN TRACHEAL BASAL STEM CELLS

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Airway epithelium is composed of mucociliary epithelium and basal stem cells underneath. Mucociliary epithelium secretes mucous to capture viruses, noxious substances, and allergens, and ciliated cells move them out from airway to protect lung from infection or inflammation. When mucociliary epithelium is damaged, basal stem cells proliferate and give rise to both secretory cells and ciliated cells to repair mucociliary epithelium. In the previous study, we showed that proliferation and differentiation of basal stem cells are regulated by mesenchymal cells during repair of airway epithelium. Airway is surrounded by cartilages and smooth muscles, which are mainly located at ventral side and dorsal side of trachea, respectively. Thus, we hypothesized that behavior of basal stem cells could be different in ventral side and dorsal side of trachea. To observe the difference in proliferation of basal stem cells in ventral and dorsal trachea, we utilized the 3D clonal organoid culture system. This assay revealed that basal stem cells from dorsal trachea showed higher colony forming efficacy superior to those from ventral trachea. In vivo clonal analysis using lineage tracing model for basal stem cells (Keratin5-CreER; Rosa-Confetti) showed that there are more clones and clones contain more cells in dorsal trachea compared to ventral trachea. Since the distribution of blood vessels is not different between ventral and dorsal trachea, characteristics of basal stem cells itself is thought to be cause the difference in proliferation. Gene ontology analysis from RNA-seq data revealed that genes expressed in basal stem cells of dorsal trachea are enriched in wound healing and response to wounding while those in ventral trachea are enriched in response to external stimulus and immune response. Interestingly, basal stem cells in ventral trachea expressed Myostatin, which inhibits the growth of smooth muscle cells, and HGF, which facilitates cartilage repair. This is consistent with a model in which basal stem cells in dorsal trachea are a main source for repair of airway epithelium and basal stem cells in ventral trachea protect airway from infection and also maintain cartilages. Taken together, these results revealed different roles in basal stem cells for the maintenance of airway and airway epithelium.

F-2081

BMAL1-DRIVEN PERIPHERAL CLOCKS RESPOND INDEPENDENTLY TO LIGHT

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Organisms adjust their daily physiology to anticipate the environmental changes resulting from the Earth rotating around its own axis. At the cellular level, circadian regulation is established by a Bmal1-dependent molecular network that oscillates in a self-sustained manner and imposes daily oscillations on the expression of thousands of genes. In turn, circadian synchronization of cells within tissue depends on environmental entrainment cues, such as changes in light levels. However, it is still unclear whether the circadian clocks of different tissues respond independently to environmental signals, or rather require interactions with each other to achieve a synchronized rhythmicity. This is a relevant issue, since clock alterations can have drastic physiological consequences, such as shortened mammalian lifespan and changes in the predisposition to tumorigenesis. Here, we show that, unexpectedly, light can synchronize the Bmal1-dependent core circadian machinery in the epidermis, in the absence of Bmal1-driven clocks in other tissues. Strikingly, this circadian rhythmicity is lost in constant darkness, indicating that Bmal1 provides a “memory” of time to synchronize peripheral clocks when external entraining signals are absent. Importantly, we find that tissue autonomous Bmal1 suffices for sustaining tissue homeostasis in otherwise arrhythmic and prematurely-aging animals. Our results, therefore, support a two-branched model for the daily synchronization of peripheral tissues: an immediate response branch, whereby light can entrain circadian clocks without any commitment of other Bmal1-dependent clocks; and a memory branch that uses other Bmal1-dependent clocks to “remember” circadian rhythmicity in the absence of external cues.

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F-2083

OPTIMIZING CULTURE CONDITIONS FOR EXPANSION OF HUMAN EPIDERMAL STEM CELLS FOR SKIN REGENERATION

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The human epidermal stem cells (EpiSCs) are particularly suited for cellular therapy due to their accessibility and low immunogenicity. The conventional means of expanding EpiSCs uses the support of feeder layer cells. There are still superior techniques needed to culture these cells, ideally without the feeder layer. Therefore, the present study attempted to optimize in vitro culture methods for establishing a pure population of EpiSCs. Human skin samples were obtained with informed written consent taken priorly. The isolation and expansion of EpiSCs was performed by suspension and explant methods using different culture media (alpha-MEM, DMEM/F12 and EpiLife) with supplementation of fetal bovine serum (FBS at 0, 10 and 20%) and growth factors [2 ng/ml keratinocyte growth factor, (KGF) and 2 ng/ml epidermal growth factor (EGF), 1% human keratinocyte growth supplement (HKGS)]. Among the culture methods, explant culture showed the attachment of cells in alpha-MEM at 20% FBS supplementation by exhibiting a typical “cobblestone” epithelial pattern of growth at passage 0, but did not retain the similar morphology at passage 1. In contrast, no cells were firmly attached in suspension culture either in alpha-MEM or DMEM/F12 in all concentrations of FBS. Interestingly, a higher number of proper EpiSCs from suspension culture was observed when alpha-MEM was supplemented with FBS and growth factors, such as KGF, EGF and HKGS. In EpiLife media with HKGS, primary culture did not support the attachment of cells. Whereas, the EpiLife media supplemented with KGF, EGF and HKGS resulted in proper attachment of EpiSCs with typical morphological features. Following this, EpiSCs established from alpha-MEM and EpiLife supplemented with various growth factors were analysed for the proliferation rate and the expression of stem/progenitor and keratinocyte specific markers, such as p63, K5, K14, K15, integrin β1, K1, K10 and filaggrin. The analyses showed that all EpiSCs had high clonogenic potential with varied levels of expression of selected markers. In summary, our findings illustrated that established EpiSCs in alpha-MEM and EpiLife media with growth factors possess plasticity with high proliferation ability. Thus EpiSCs may represent an ideal source for skin regenerative medicine applications.

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F-2085

CELL EXTRACT FROM HUMAN LABIAL GLAND STEM CELLS RESCUES THE HYPOFUNCTION OF IRRADIATED SALIVARY GLANDS IN A MOUSE MODEL.

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Nowadays, adult stem cells play an important role in tissue repair and regeneration. However, cell-based therapies still possess potential risks, such as tumorigenesis and immunogenicity. The main objective in this study was to test the therapeutic effect of a cell-free/ cell extract therapy to restore the hypofunction of irradiation-induced salivary glands (SGs). Human labial gland derived-stromal (mesenchymal) stem cells (LSC) were harvested by explant culture method. Stem cell properties of LSC were characterized and confirmed by flow cytometry. Then LSCs were lysed into labial glands stem cell extract (LSCE) by three freeze-thaw cycles. Either LSCE or normal saline (as a vehicle control) was injected intravenously into mice post-local irradiation to SGs (13Gy). Using explant culture method of small pieces of human labial glands, we successfully harvested a large number of LSCs with self-renewal and multipotent differentiation capacities. Our results demonstrated that several angiogenesis-related factors, such as FGF-1/ -2, KGF and VEGF, were detected in LSCE. For the in vivo study, LSCE restored 50%-60% of saliva secretion, protected acinar cells, blood vessels and parasympathetic nerves, promoted the cell proliferation and up-regulated the expression of tissue repair/ regeneration proteins and genes. These findings improved our understanding of human LSCs and indicated the potential therapeutic application of labial gland stem cell extract (LSCE) to treat irradiation-induced salivary hypofunction for head and neck cancer patients.

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F-2087

TRANSCRIPTOME PROFILING IN AMELOBLAST-LIKE CELLS DERIVED FROM ADULT GINGIVAL EPITHELIAL CELLS : IDENTIFICATION OF AMELOBLAST-SPECIFIC CELL SURFACE MARKERS

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Dental enamel is the highly mineralized tissue covering the tooth surface and is formed by ameloblasts. Ameloblasts have been known to be impossible to detect in adult tooth because they are shed by apoptosis during enamel maturation and tooth eruption. Owing to these, little was known about appropriate cell surface markers to isolate ameloblast-like cells in tissues. To overcome these problems, epithelial cells were selectively cultivated from the gingival tissues and used as a stem cell source for ameloblastic differentiation. When gingival epithelial cells were treated with a specified concentration of BMP2, BMP4, and TGFb-1, the expression of ameloblast-specific markers was increased, and both the MAPK and Smad signaling pathways were activated. Gingival epithelial cells differentiated into ameloblast-like cells through epithelial-mesenchymal transition. By RNA-Seq analysis, we reported 20 ameloblast-specific genes associated with cell surface, cell adhesion, and extracellular matrix function. These cell surface markers might be useful for the detection and isolation of ameloblast-like cells from dental tissues.

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F-2089

A MOUSE MODEL OF PRECISION RADIATION INDUCED CHRONIC HYPOSALIVATION FOR HUMAN SALIVARY STEM CELL TRANSPLANTS

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Radiation leads to salivary dysfunction and xerostomia (also known as hyposalivation or dry mouth), a major unmet medical need among head and neck cancer survivors. A cure for this chronic debilitating condition is “non-existent”. Since this condition is ‘irreversible’, treatment methods for regeneration of salivary glands and physiological secretion of saliva from reconstituted glands are urgently needed. Stem cells have been identified in salivary gland, which are capable of differentiating into functionally competent salivary units. A preclinical model to test such cells is currently lacking. We have developed the first precision-X-radi ation induced mouse model of radiation induced chronic hyposalivation. We have used highly immunodeficient transgenic NSG-3GSM mice expressing three human cytokines including human stem cell factor. NSG-3GSM mice owing to Prkdcscid mutation are radiosensitive, thus we exploited the stereotactic targeting and dose delivery capabilities of X-Rad SmART system to radio-ablate salivary glands and induce xerostomia. Further, we have established an orthotropic survival surgery procedure to enable transplant of salivary stem cells into radio ablated sub-mandibular glands of these mice to study regeneration and restoration of salivary functions. This protocol includes neck incision and injection of cells in buffered-trypan blue dye into right and left sub-mandibular glands under anesthesia. Mice recovered from surgery remained healthy. Moreover, we have established a robust method for reflex saliva flow measurement using Schirmer strips in irradiated and sham mice. Our data from the se measurements, demonstrates significant X-ray dose-dependent reduction in saliva production, where 10 Gy irradiated mice showed ~50% reduction in saliva production and this remained consistent for over a period of six months. In summary, we have developed the first humanized mouse model that mimics a challenging, chronic lifestyle problem in cancer survivors and plan to use this robust platform to measure regenerative outcome following patient-derived salivary cell transplants.

EYE AND RETINA

F-2091

IPSC BASED CELLULAR MODEL USED TO CHARACTERIZE THE MOLECULAR PATHOLOGY OF CORNEAL ENDOTHELIAL DYSTROPHY

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Congenital hereditary endothelial dystrophy (CHED) is a rare disease caused by biallelic mutations in the SLC4A11 gene. The disease is clinically characterized by cloudy cornea that may be present from birth or may be infantile in onset. Because of the endothelial dystrophy the patient's vision degrade in time. We have used direct and genome sequencing to screen six probands with CHED, collectively we identified four novel and seven previously reported disease-associated SLC4A11 variants. To analyze the effect of c.2240+5G>A on pre-mRNA splicing, and thus to prove pathogenicity of the variant, we have developed a corneal endothelial-like (CE-like) cell model differentiated from autologous induced pluripotent stem cells (iPSCs) via neural crest cells exposed to B27, PDGF-BB and DKK-2. CE-like cells were demonstrated to express several endothelial cell-specific markers including SLC4A11. Total RNA was extracted, and reverse transcriptase PCR was performed. The c.2240+5G>A variant was demonstrated to introduce a cryptic splice donor site leading to an insertion of six bp and the subsequent introduction of a premature stop codon (p.Thr747*). This proof-of-concept study highlights the potential of using iPSC derived CE-like cells to investigate the pathogenic consequences of SLC4A11 disease-associated variants.

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F-2093

GENERATION OF RETINAL PIGMENT EPITHELIAL CELLS FROM MOUSE CHEMICAL INDUCED PLURIPOTENT STEM CELLS BY SMALL MOLECULES

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Somatic cells can be reprogrammed into pluripotent stem cells by pure chemicals, without genetic manipulation. Therefore, chemical induced pluripotent stem cells (CiPSCs) provide a promising approach for generating clinically desirable cell types for regenerative medicine. Retinal pigment epithelium (RPE) cells derived from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been clinically translated. However, ESC-derived RPE cells face ethical and possible immune rejection problems. The safety of exogenous gene also limits the clinical application of iPSC-derived RPE. In this study, we report highly efficient generation of CiPSCs from mouse fibroblasts and differentiation into RPE cells using chemically defined conditions. We utilize a cocktail of small molecules to generate pluripotent stem cells can be generated from mouse somatic cells without genetic manipulation. The CiPSCs resemble ESCs in terms of their morphology, gene expression and differentiation potential. Then, the CiPSCs were differentiated into functional RPE cells by a cocktail of small molecules and a three-dimensional (3D) culture system. . The results showed that differentiation toward RPE fate within 4 weeks. The RPE cells displayed characteristics of apical basal polarity, cellular structure and gene expression tight junctions, phagocytose photoreceptor outer segments similar with native RPE. We report here development of a high-efficient, small molecule-based methodology for generation of RPE from mouse fibroblast, which we suppose more suitable for clinical application. Alternatively, CiPSCs may be generated from patient-derived somatic cells and provide a readily accessible source of autologous RPE cell grafts.

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F-2095

MODULATION OF OSMOTIC STRESS-INDUCED TRPV1 EXPRESSION RESCUES HUMAN IPS-DERIVED RETINAL GANGLION CELLS THROUGH PKA

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Transient receptor potential vanilloid 1 (TRPV1), recognized as a hyperosmolarity sensor, is a crucial ion channel involved in the pathogenesis of neural and glial signaling. Recently, TRPV1 was determined to play a role in retinal physiology and visual transmission. In this study, we sought to clarify the role of TRPV1 and the downstream pathway in the osmotic stress-related retina ganglion cell (RGC) damage. First, we modified the RGC differentiation protocol to obtain a homogeneous RGC population from human induced pluripotent stem cells

(hiPSCs). Subsequently, we induced high osmotic pressure in the hiPSC-derived RGCs by administering NaCl solution and observed the behavior of the TRPV1 channel and its downstream cascade. We obtained a purified RGC population from the heterogeneous retina cell population using our modified method. Our findings revealed that TRPV1 was activated after 24 hours of NaCl treatment. Upregulation of TRPV1 was noted with autophagy and apoptosis induction. Downstream protein expression analysis indicated increased phosphorylation of CREB and downregulated brain-derived neurotropic factor (BDNF). However, hyperosmolarity-mediated defective electrophysiological activity, morphological change and apoptosis of RGCs, CREB phosphorylation, and BDNF downregulation were abrogated after concomitant treatment with the PKA inhibitor H89. Collectively, our study results indicated that the TRPV1–PKA pathway contributed to cellular response under high levels of osmolarity stress; furthermore, the PKA inhibitor had a protective effect on RGCs exposed to this stress. Therefore, our findings may assist in the treatment of eye diseases involving RGC damage.

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F-2097

PURIFICATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORNEAL EPITHELIAL CELLS USING CELL TYPE-SPECIFIC ADHESIVENESS ON LAMININ ISOFORMS

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The products differentiated from human induced pluripotent stem cells (hiPSCs) which replace damaged tissues, are expected as a novel treatment for intractable diseases. Previously, we established a multilayered structure that reproduces the whole eye development, generated from hiPSCs on laminin-511 fragment (LN511E8). Our recent work showed that LN isoforms largely affects ocular cell differentiation from hiPSCs. For realization of hiPSC-based therapy, purification of the target cells is an important step. In this study, we examined use of LN isoforms for purification of hiPSC-derived corneal epithelial cells (iCECs) and manufacturing iCEC sheets. We found that differentiated ocular lineage cells, derived from hiPSCs have unique adhesion specificities and growth characteristics on distinct LN isoforms. iCECs and the other type cells showed specific cell adhesion to LN332, 411, 511 and LN211E8, respectively. Furthermore, LN332E8 promoted proliferation for epithelial cells including iCECs, but not for non-epithelial cells. By using the combination of specific adhesion of LN isoforms and magnetic separation, we successfully fabricated iCEC sheet with high purity without using fluorescence activated cell sorting (FACS). Thus, use of specific LN isoforms contributes to establishment of a simple and efficient method for manufacturing of iCEC sheets.

F-2099

TRANSPLANTATION OF HUMAN RETINAL PIGMENT EPITHELIAL SHEETS IN THE MACAQUE MONKEY EYE

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Age-related macular degeneration (AMD) the most common cause of vision loss in the industrialized country and the third cause of blindness globally. Retinal pigment epithelial (RPE) cell death occurs before the majority of vision loss occurs implying that if these lost RPE are replaced, AMD progression can be mitigated. Adult human donor derived RPE (ahRPE) as a source for cell therapy for AMD is promising as this would allow HLA matching and has already been shown rescue vision in a rat model RPE dysfunction. Here we explored a surgical approach

for transplanting a sheet of ahRPE on a porous polyester scaffold under the macula of the macaque monkey. We followed the transplant for three months and evaluated retinal physiology and function. We found transplantation of ahRPE underneath the macula is safe and preserves vision and normal retina physiology.

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F-2101

ISOLATION, CHARACTERIZATION AND COMPARISON OF HUMAN EXTRAOCULAR MUSCLE-DERIVED STROMAL CELLS IN NORMAL INDIVIDUAL AND PATIENTS WITH THYROID ASSOCIATED OPHTHALMOPATHY

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Mesenchymal stem cells (MSCs) are promising cell for various purposes like tissue engineering, regeneration and gene therapy. MSCs isolated from human extraocular muscles can be easily expanded in vitro, and can undergo multilineage differentiations, such as adipogenesis, chondrogenesis, osteogenesis, and even neuronal and myogenic differentiation. This study aimed to isolate, characterize and compare the extraocular muscle derived stromal cells (MDSCs) from normal individual and patients with thyroid-associated ophthalmopathy (TAO) patients. Extraocular muscles were obtained during strabismus surgery. Flow cytometric analysis was done to determine CD surface antigens, such as CD31, CD34 CD45, CD73, CD90, CD44 and CD59. We quantified various cytokines secreted from MDSCs such as, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL17A, TNF-α, IFN-γ and GM-CSF by using multi-analyze ELISA array kit. We performed Oil Red O staining for adipogenesis, Alzarin Red for osteogenesis and Alcian blue for chondrogenesis and real time PCR to measure the mRNA levels for myogenesis. Our results showed MDSCs from normal and TAO patient have similar potential tendency of characterization in terms of surface antigens and secretions of cytokines. Also, there was no significant difference between the tendency of MDSCs from normal and TAO patients in terms of multilineage differentiation, such as adipocytes, osteocytes and chondrocytes. However, myogenic differentiation of MDSCs from normal human showed higher than TAO patients. In conclusion, MDSCs from human extraocular muscles in normal individuals showed similar properties with TAO patients, and are good candidate for stem cell based therapies for treating various disorders

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F-2103

CHARACTERIZATION OF RETINAL ORGANOIDS BY 2-PHOTON MICROSCOPY PRIOR TO TRANSPLANTATION INTO RETINAL DEGENERATE RAT MODELS

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Stem cell-derived retinal organoids develop similar cell types and structures as in vivo tissue. Previous studies have shown that retinal organoid sheet transplantation improves visual function in retinal degenerate rats. Evaluating organoid quality and characteristics prior to transplantation is of substantial importance as it may directly determine the quality of its differentiation, integration in the host, and restoration of function. Traditional methods to monitor organoid development includes empirical observation through brightfield microscopy and histological immunostaining. Brightfield microscopy is highly user-dependent and histology requires organoid destruction. Therefore, a quantitative, non-invasive real-time imaging technique is needed for discerning tissue objectively. In this study, we applied 2-photon microscopy approaches to evaluate organoids: fluorescence lifetime imaging microscopy (FLIM) and hyperspectral imaging (HSpec), both using 740nm pulsed excitation. Organoids were produced and provided by AIVITA Biomedical Inc. FLIM phasor analysis of endogenous NADH provided indicated free and bound NADH distribution, which identifies the cells as glycolytic or oxidative and reflects the metabolism of the tissue. HSpec fluorescence emission light in the range of 420 nm to 690 nm analyzed via the phasor approach highlighted the distribution of retinol. We found that distinguishing between high- and low-quality organoids were very subjective by brightfield microscopy alone. However, FLIM and HSpec showed different structural and developmental stages of organoids while highlighting metabolic features and retinol distribution. Retinal photoreceptors are more glycolytic with higher retinol concentration, thus, the increased distribution of free NADH and retinol is a marker for photoreceptor cells. FLIM and HSpec are two powerful label-free imaging techniques to monitor retinal organoid development. Our future goal is to develop a standard criterion to evaluate organoid quality comprehensively and ultimately enhance the survival rate and efficacy of retinal sheet transplantation.

Funding Source: California Institute for Regenerative Medicine: TRAN-1 Grant 10995

STEM CELL NICHES

F-2105

FROM SKIN TO NERVOUS SYSTEM: KERATINOCYTE DERIVED NEURAL CREST STEM CELLS, AN AUTOLOGOUS MULTIPOTENT CELL SOURCE FOR NEURODEGENERATIVE DISEASE TREATMENT

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Neural crest (NC) cells play a central role in development of the peripheral nervous system, craniofacial skeleton, and skin pigmentation due to their broad multilineage differentiation potential into neurons (Neu), Schwann cells (SC), melanocytes (MC), and smooth muscle cells (SMC). Recently, we identified an easily accessible source of multipotent NC stem cells from human inter-follicular keratinocyte (KC) cultures (termed KC-NC) isolated from glabrous neonatal foreskin. Using small molecules FGF2 and IGF1 as well as inhibition of TGF-β1 we are able to maintain SOX10+/FOXD3+ KC-NC in vitro. Genome wide transcriptomic analysis, as well as single cell RNA-seq showed upregulation of NC-specific genes and cell differentiation trajectories indicative of the epidermal origin of these cells. Clonal analysis verified the clonal multipotency of KC-NC towards all NC specific lineages (Neu, SC, MC, SMC) with varying efficiencies. Each NC derivative was also examined using functional assays, including electrophysiological tests for neurons, L-Dopa assays for MC and contractile force generation for SMC. Most notably, upon transplantation into chick embryos, KC-NC migrated along stereotypical pathways contributing to all NC-derivatives including Neu, SC, SMC and putative MC. Surprisingly, KC-NC could also be derived from aged adult donors (67-93 years old), maintained their multipotency in vitro and contributed to all NC derivatives in vitro and in ovo. In addition, CpG methylation analysis showed that the epigenetic age of adult KC-NC was significantly lower than that of KC and the chronological age of the donors. We are examining the regenerative potential of KC-NC by transplantation into the corpus callosum of the shiverer mouse model (shi/shi), an established model of demyelinating disease. Our data so far show that KC-NC migrated extensively and continued to proliferate a few weeks post-transplantation. Current efforts focus on long-term transplantation experiments to determine the potential of KC-NC for axonal myelination. Given the accessibility of human skin and the high proliferation capacity of KC and KC-NC, these cells represent a potentially useful source of autologous stem cells for studying or treatment of neurodegenerative diseases.

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F-2107

FEEDER-FREE CULTURE OF NAIVE HUMAN PLURIPOTENT STEM CELLS IN NORMOXIC CONDITIONS

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The ‘naïve’ or ground state in pluripotent stem cells (PSCs) is distinctively different from traditional human PSC culture, relating to an earlier embryonic stage. The key characteristics of naïve pluripotency are: hypomethylation, double X chromosome activation, cloning capability, contribution to chimeras, and LIF-dependency. Human PSCs have shown resistance towards reversion to the ground state, with the different protocols described requiring feeder cells and hypoxic conditions, and reportedly showing DNA instability over long-term culture. The complex culture conditions also complicate their handling, with a selection step typically required before any analysis technique. We have previously shown that a human serum-derived protein, Inter-alpha inhibitor (Ial), supports human PSCs in coating-free conditions for long-term culture without loss of pluripotency or DNA instability. Here we present successful generation and culture of human and mouse naïve PSCs in coating-free conditions using the addition of Ial to the medium. In mouse PSCs, the cells show increased genomic stability, naïve marker expression and differentiation potential. In human PSCs, reversion to the naïve state is achieved without mouse feeder cells, and the method is compatible with normoxic conditions. Two different strategies are used: RSeT Medium (StemCell Technologies) and 2iGöY. For the adequate validation of this human naïve PSC culture method, Super-Resolution confocal microscopy has been used to show overall decreased DNA

methylation and distinct naïve marker expression in human PSCs reverted using lal. Integrin engagement and expression is also assessed, showing lower focal adhesion formation and subsequent signalling in lal culturing conditions, with naïve cells presenting overall decreased integrin expression. Phospho-proteome studies are also performed to investigate early signalling events linked to lal-mediated attachment of both traditional and naïve hPSCs. After thorough characterisation, this new culture method has the potential to stream-line naïve human PSC culture for high-throughput and pharmaceutical applications, as lal greatly simplifies the culturing method and is readily available at clinical grade.

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F-2109

CHRONIC SENESCENCE AFTER SULFUR MUSTARD EXPOSURE RESULTED IN A SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE

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Chronic wound healing disorder after sulfur mustard (SM) exposure is still not completely understood. As mesenchymal stem cells (MSC) are essential for wound healing, it is our hypothesis that SM induces chronic senescence in MSC. Such cells would be able to persist over long time periods and secrete proinflammatory cytokines known as the senescence-associated secretory phenotype (SASP). Therefore, in this initial study it was assessed whether SM is able to induce chronic senescence in MSC and whether changes in their secretome correspond to SASP which may be an underlying cause of the wound healing disorder. Human MSC were obtained from the bone marrow of donor femoral heads. Their quality was determined using differentiation techniques and specific cell surface markers by flow cytometry (CD14-/CD34-/CD45-/CD105+/CD106+). The cells were exposed to single doses of SM (1 - 40 µM) or H2O2 (200 µM) and the senescence-associated β-galactosidase (SA-β-gal) was stained with X-gal substrate up to 31 days afterwards. Cell culture supernatants were collected at different time points and analyzed for over 70 chemokines, cytokines and growth factors using Bioplex assay. Senescence induction was increasing with time and concentration. Single dose exposure resulted in a stable senescence about 21 days later verified by SA-β-gal staining. Secreted factors of senescent cells were observed to be early (i.e. MCP-1), only intermediate (i.e. IL-12 (p70)), late (i.e. CXCL9) or constantly elevated (i.e. IL-6), compared to correspondent controls. Moreover, the two analytes sTNF-R1 and CXCL16 showed differences between the SM- and the H2O2-induced senescent cells. In conclusion, SM single dose exposure is sufficient to induce chronic senescence in human MSC. These SM-induced senescent MSC may be unable to fulfil their regenerative role and contribute to the wound healing disorder. This is in line with our results, that these cells secreted a variety of proinflammatory cytokines. The time-dependence as well as the SM-induced specific upregulation of some analytes provide deeper insight into SM-induced senescence. Further research is necessary to prevent the secretion of these cytokines or selectively remove senescent cells which would be an innovative treatment strategy after SM exposure.

F-2111

HUMAN PERINATAL STEM CELL-DERIVED MATRIX SUPPORTS EXPANSION AND MAINTENANCE OF PLURIPOTENCY IN HUMAN STEM CELLS

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Human induced pluripotent stem cells (iPSCs) hold promise for the study, diagnosis, and treatment of a variety of diseases based on their theoretical ability to proliferate indefinitely and differentiate into any cell type found in the body. However, in practice it is difficult to achieve mature phenotypes in differentiated cells, and iPSC cultures are notoriously inconsistent and difficult to maintain. Recently, a variety of substrates have been proposed to improve the consistency, ease-of-use, and cost of culturing pluripotent stem cells. It is now widely accepted that the microenvironment is critical for determining cell fate. Previously, our group has reported the development of scalable methods for production of bone marrow mesenchymal stem cell-derived matrices (BM-ECM) for isolation and expansion of mesenchymal stem cells without loss of stemness. Recently, we have reported tissue-specific differences in matrices that are critical for determining cell fate and function in vitro. We hypothesized that highly potent perinatal stem cells may create a matrix that better supports the maintenance of pluripotent stem cells. To test our hypothesis, we produced a cell-derived matrix from human amniotic fluid stem cells (AF-ECM). While possessing many of the same major components, the AF-ECM is distinct in structure and composition from BM-ECM. Relative to BM-ECM, the AF-ECM is an order of magnitude less stiff ($p<.00001$), twice as adhesive ($p<.001$), and much less rough ($p<.0001$). Importantly, these differences result in distinct function. While the BM-ECM does not support iPSC culture,

AF-ECM supports proliferation of iPSCs comparable to that of Matrigel. Furthermore, AF-ECM also supports expansion and/or maintenance of other more mature cell types, including mesenchymal stem cells, chondrocytes, cardiomyocytes, and endothelial progenitor cells. Together, these data suggest that AF-ECM may possess extracellular cues that are ideal for the maintenance of iPSCs, as well as their differentiated progeny.

F-2113

DECODING THE MOUSE TOOTH DEVELOPMENT IN VIVO BY LARGE-SCALE SINGLE CELL RNA-SEQ

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Tooth develops from a serial of epithelial and neural crest-derived mesenchymal interactions throughout the initiation, bud, cap and bell stages with several signaling pathway families. The odontogenic potential is believed to shift from the dental epithelium to the dental mesenchyme at bud stage. However, the key signals for pulse-on the odontogenic potential in the regenerative organ germs are still unclear. Here we performed single-cell RNA-seq on the mouse dental development in vivo. We analysed the transcriptome profiles of ~96,000 cells of the dental regions in mouse embryo from day 10 to day 16. Cell developmental trajectories were clearly divided into the dental epithelial and mesenchymal paths. Besides, we revealed the key dental signaling pathways between the dental epithelium and mesenchyme, which activate the odontogenic potentials. Our study demonstrated the detailed molecular mechanisms in mouse tooth development. In addition, we discovered a small population of “seed” cells for dental development by the large-scale single-cell RNA-seq technique. Collectively, our study suggests the possible signaling pathway candidates for the tooth regeneration.

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F-2115

REDUCING EDGE EFFECT IN 96-WELL PLATE MSC SCREENING ASSAYS BY CONTROLLING THERMAL CONDITIONS DURING CELL PLATING

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The standard 96-well plate is often used for stem cell screening or tox assays. Researchers avoid using edge wells for cells, eliminating over one third of assay space, because of high variability. Others have reported that increased humidity or extended periods of time at room temp during cell settling can reduce edge effect. We previously reported that plating cells at constant 37°C, which is less stressful for cells, also reduces edge effect. Here we extend those studies to test the ability of thermal control of plating conditions to reduce variability in human bone marrow Mesenchymal Stromal/Stem Cells (MSC) toxicity assays. Our hypothesis was that when chamber temperatures were controlled to a constant 37°C during cell plating and settling we would see no difference between the variances in cell density in groups of wells including and excluding edge wells. We used the Xvivo System to provide fully controllable gas and temperature levels for both cell incubation and handling. We performed 24-hour MSC toxicity assays with low concentrations of disinfectants including isopropyl alcohol and Spor-Klenz (peracetic acid, acetic acid, and hydrogen peroxide). Using the HoloMonitor M4 microscope, we showed that cell settling in edge wells was random when MSC were plated at constant 37°C, unlike the directional rolling seen in edge wells when cells were plated at room temp and transferred to the incubator. Using standard MTT-based metabolic and crystal violet cell density assays, we showed that MSC cells were affected in a dose-dependent manner by exposure to commonly used disinfectants. Using a one-tailed F-test for this analysis, we saw no significant difference in variance when edge wells were used along with non-edge wells. We concluded that constant 37°C for cell plating and settling may improve reproducibility of 96-well based cell assays and save money, materials and time.

CANCERS

F-2117

THE WNT CANONICAL PATHWAY CONTROLS CELL NUTRITION BY ACTIVATING MACROPINOCYTOSIS AND LYSOSOMAL DEGRADATION OF EXTRACELLULAR PROTEINS

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Activation of the Wnt pathway is at the core of many human cancers. During canonical Wnt signaling, the Lrp6 and Frizzled bind Wnt and the complex is endocytosed. GSK3, Dishevelled and Axin are sequestered inside the intraluminal vesicles of late endosomes, also known as multivesicular bodies (MVBs). Formation of MVBs is required for Wnt signaling, by reducing levels of GSK3 in the cytosol. Recently, we have found that Wnt causes a great increase in levels of non-receptor mediated endocytosis of BSA-De-Quenched (BSA-DQ), a marker that fluoresces upon degradation in lysosome and that the activity of protein arginine methyltransferase 1 (PRMT1) is required for Wnt activation. From these and other experiments involving siRNAs Wnt emerges as a global regulator of endocytosis. We now report our discovery that the increased protein uptake was caused by macropinocytosis, in which membrane ruffles accompanied by F-actin reorganization folded over large amounts of extracellular fluid within minutes of Wnt treatment. PRMT1 activity and the ESCRT machinery were necessary for Wnt signaling. The endocytic uptake of high molecular weight tetramethylrhodamine dextran (TMR dextran 70 kDa, which has a hydrated diameter of 200 μM) was used as a macropinocytosis marker and was inhibited by ethyl-isopropyl amiloride (EIPA) and amiloride (a diuretic commonly used in the clinic that inhibits of plasma membrane Na+/H+ exchanger). The emerging connection between Wnt signaling, arginine methylation, lysosomal trafficking and endocytosis will lead to novel therapies for human cancers and stem cells in which Wnt signaling is activated.

F-2119

AUTOLOGOUS IMMUNOTHERAPY USING CANCER STEM CELLS AS ANTIGENIC SOURCE

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In certain conditions, populations of progenitor cells can restart the growth-proliferation cycle and produce cells with regenerative scope. This stem cell niche is dormant until proper signals trigger re-entry into the proliferation cycle. The re-entry signals can originate from events such as trauma, cell damage, or microorganism aggression. Genetic variants or acquired mutations can perturb the cell cycle, resulting occasionally in survival and proliferative advantages. Cells in the tumor stem cell niche, or tumor initiating cells (TIC) are in low frequency, usually representing < 0.5% of the cells in the tumor mass and may only temporarily express neoantigens, thus eluding an immune response or becoming subject to immune-editing. In previous trials in patients with metastatic melanoma, vaccines designed to express antigens from TICs were associated with minimal toxicity, and encouraging survival. The vaccine consisted of autologous dendritic cells loaded with antigens from autologous TICs (DC-TC). In a single-arm trial 54 patients had a projected 5-year survival rate of 54%,. In a randomized phase II clinical trial patients treated with DC-TC had superior survival compared to those treated with a tumor cell vaccine (median 43 vs 20 months, 3-year OS 65% vs 25%, and a 70%reduction in the risk of death. In contrast, similar therapies using whole tumor preparations or single peptide antigens failed to show significant survival benefit in other trials. We concluded that for the success of the immune therapy using ex-vivo loaded antigen-presenting cells, the isolation and purification of the autologous TIC was a critical step in manufacturing. Our manufacturing methods to isolate and amplify TICs consist of dissociating the tumor, exposing the cells to a serum-free media, and promoting TIC spheroids formation. The growth media lacks differentiation signals usually found in serum and is enriched with mTOR pathway stimulating factors. In addition, spheroid formation encourages paracrine and juxtacrine signaling that is essential for stem cell niche maintenance. Here we present the characterization of the generated TIC population by commonly recognized phenotype markers for stem cells, by DNA exome and methylation analysis, as well by a tumorigenesis assay in NOD/SCID mice.

F-2121

CALCIUM CHANNELS AS NOVEL THERAPEUTIC TARGET OF OVARIAN CANCER STEM CELLS

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Ovarian cancer has been known as the most lethal gynecologic malignancy and the overall 5-year survival rate for epithelial ovarian cancer is only almost 30%. Epithelial ovarian cancer shows drug resistance, frequent recurrence and poor prognosis. Although chemotherapy removes most cancer cells, a few cancer stem cells (CSCs) still remain, and could be a major contributor of drug resistance. To isolate compounds with an therapeutic effect on cancer stem cells in ovarian tumor, we used ovarian cancer stem-like cells (CSLCs) via sphere culture of A2780 epithelial ovarian cancer cells for screening FDA-approved library based on a high-throughput screening (HTS) system. Four compounds (known as calcium channel blockers, CCB) which block voltage-gated calcium channels were selected to inhibit the proliferation of ovarian CSLCs. Interestingly, voltage-gated calcium channels are overexpressed in ovarian CSLCs, whereas down-regulation of calcium channel genes reduced the properties of ovarian CSLCs. The treatment of CCBs decreased sphere formation and viability of ovarian CSLCs as well as induced apoptosis. In addition, CCBs destroyed stemness and inhibited AKT and ERK signaling pathway in ovarian CSLCs. Furthermore, their effects on combination therapy with cisplatin showed synergistic effect

on the inhibition of ovarian CSLCs viability and proliferation. Taken together, the four calcium channel blockers can be potential therapeutic drugs for prevention of ovarian cancer recurrence.

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F-2123

MOLECULAR NETWORK PATHWAY MECHANISM IN DRUG RESISTANCE, CANCER AND STEM CELLS

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Molecular network pathways interact with each other and regulate cellular phenotypes. Epithelial-mesenchymal transition (EMT) plays an important role in the acquisition of cancer stem cell (CSC) feature and drug resistance. To reveal the molecular signaling network pathway mechanism, gene expression in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC) have been analyzed and compared. Microarray analysis was performed using total RNA purified from MSCs and diffuse-type GC in GeneChip U133Plus 2.0 platform. The gene expression profiling demonstrated that gene expression of cadherin 1 (CDH1), erb-b2 receptor tyrosine kinases (ERBBs), and patched 1 (PTCH1) were up-regulated in diffuse-type GC compared to MSCs, whereas CDH2 and fibronectin 1 (FN1) were down-regulated. Wnt/beta-catenin signaling, as well as ERBB and PTCH1 signaling networks, involved in EMT, CSCs and drug resistance, have been investigated and profiled in bioinformatics. In conclusion, the EMT-related molecular network pathways have been revealed in MSCs and diffuse-type GC, which may contribute into the elucidation of mechanism in the drug resistance of CSC population.

F-2125

THE bHLH FACTOR E47 DOWNREGULATES ONCOGENIC C-MYC IN PANCREATIC CANCER THROUGH EPIGENETIC MODIFICATIONS

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Dysregulation of c-MYC is a key factor in the malignant transformation of pancreatic ductal adenocarcinoma (PDA). Recent studies have shown: high levels of MYC drive down expression of bHLH transcription factor, E47 in the pancreas. Moreover, the most common mutations in noncoding regions are binding sites of E47 and its homologs suggesting this protein may serve a tumor-suppressor role in pancreatic cancer. Consistent with this, our data indicate that restoring the transcriptional activity of E47 can induce growth arrest in PDA cells by downregulating the number of MYC RNA transcripts. To better understand the molecular mechanism of this reciprocal relationship between MYC and E47, we performed ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing). One finding is that the chromatin accessibility around E47 enhancer regions is diminished during pancreas pathogenesis. In contrast, chromatin accessibility of a discrete regulatory element near the 3' end of MYC locus is elevated in cancerous cells compared to pre-cancerous cells, and this corresponds with the increased amount of MYC transcripts in cancer. Remarkably restoration of E47 activity in pancreatic cancer reduced the chromatin accessibility of the regulatory element in the MYC locus. Thus, we hypothesize that E47 is acting as a tumor suppressor by downregulating MYC transcription through epigenetic modifications. Further efforts we want to further investigate the functional interplay between E47 and MYC during the progression of pancreatic cancer.

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F-2127

ANALYSIS OF CHEMOTHERAPY SENSITIVITY IN HUMAN PLURIPOTENT STEM CELLS

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The study of cancer has benefited dramatically from the availability of tumor tissues and the development of cancer cell lines. Nevertheless, the cancer genome complexity of and differences between species frequently limit clinical translation. In contrast, human pluripotent stem cells (hPSCs) offer a reliable and efficient platform with the advantage of a normal and uniform genetic background. Resistance to anti-cancer drugs represents a major barrier for an efficient treatment and subjects a significant number of patients to ineffective treatment and to excessive chemotherapy side-effects. To assess the potential of hPSCs as a tool to predict anti-cancer drugs sensitivity, we exposed the cells to 107 different chemotherapies from the Approved Oncology Drugs Set, obtained from the NCI. These drugs target over 20 different pathways and mechanisms of actions affecting malignant processes. As expected, hPSC sensitivity (monitored by cell viability assays) varied between the different groups ranging from low to extreme sensitivity. Significant sensitivity has been observed to the Topoisomerase-inhibitors group and the epigenetic inhibitors (DNA methyltransferase and histone deacetylase inhibitors) group. We then evaluated the predictive power of hPSCs compared to human cancer cell lines (hCCls), utilizing Sanger's Genomics of Drug Sensitivity in Cancer and the NCI's Growth Inhibition Data. This comparison consisted of 88 shared drugs among the independent studies. This analysis produced a significant positive correlation between hPSCs and hCCls (averaged between all cancer cell lines). This high correlation highlights the usefulness of hPSCs in drug response prediction. In contrast, differences have been identified between hPSCs and hCCls in response to epigenetic and topoisomerase inhibitors, highlighting the unique sensitivity of undifferentiated cells to these substances. Together, our results demonstrate the potential of hPSCs in anti-cancer drug research as well as offer new avenues to the study of hPSC and hCCLs cellular differences.

F-2129

TRIB2 IS AN IMPORTANT REGULATOR OF CANCER STEM CELL-PROPERTIES IN OVARIAN CANCER CELLS

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It is important to find the target gene of recurring cancer cells to reduce ovarian cancer. The tribbles homolog 2 (TRIB2) interacts and modulates signaling pathway in significant cellular processes. The relevance of TIRB2 in relation to the tumorigenic potential of ovarian cancer is currently unknown. In the current study, we find that TRIB2 promote cancer stem cell property in ovarian cancer cells through WNT signaling. TRIB2 knockdown in A2780 ovarian cancer cells decreased sphere forming ability, expression of stemness and EMT related genes, cell migration, drug resistance, whereas TIRB2 overexpression of stemness and EMT related genes, cell migration, drug resistance.TRIB2 knockdown considerably decreased tumor size. Furthermore, TRIB2 overexpression increase promoter activity of TOP-Flash, Beta-catenin stability and nuclear beta-catenin accumulation. These results suggest that TRIB2 function as a transcriptional factor for beta-catenin and as and oncogene by enhancing cancer stem cell property in ovarian cancer cells. Together, these data identify that TRIB2 can be important target for developing a therapeutic agent for ovarian cancer patients.

NEURAL DEVELOPMENT AND REGENERATION

F-3001

THE KYNURENINE PATHWAY OF TRYPTOPHAN METABOLISM MODULATES MOUSE NEURAL STEM CELL PROLIFERATION

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The search for molecules which critically regulate neural stem cell (NSC) proliferation is ongoing, underpinning future production of cell lineages for therapy, while helping understand why innate repair in neurodegenerative and neuroinflammatory diseases fails. Our ongoing research has investigated a role of the kynurenine pathway (KP) in healthy metabolism and neurological diseases. The KP critically regulates bioavailability of the essential amino acid tryptophan, and is induced by interferon treatment. In MS the KP is dysregulated, producing high levels of metabolites including the potent neurotoxin Quinolinic acid. We investigated the hypothesis that modulating the KP by interferon treatment drove changes in NSC proliferation. E14 mouse neurospheres were generated and cultured. Agonists, antagonists or siRNAs to KP enzymes were used to dissect the pathways. Interferon-gamma (IFN-g) significantly upregulated (4.1 fold) indoleamine-2,3-dioxygenase (IDO-1; the initial rate-limiting enzyme that metabolises Tryptophan) mRNA in NSCs cultured in proliferative conditions, and to a lesser extent with differentiation. NSCs express all KP enzymes and notably, IFN-g upregulates other KP enzymes and has the most prominent effect on mRNA of Kynureninase (KYNU), then partial IDO-1, full IDO-2 and Kynurenine Monooxygenase (KMO). 10 IU/mL IFN-g leads to impaired NSC proliferation (*p=0.0323), and alteration of the metabolic state of NSCs including their NAD+/NADH ratio (representing cellular energy levels) via Trp depletion (needed for protein biosynthesis). KP metabolites themselves can modulate NSC proliferation, as co-treatment with the neuroprotective Kynurenic Acid partially reversed the reduced proliferation in IFN-g-treated NSCs. IFN-beta negligibly affected IDO-1 levels, but induced IDO-2, and significantly decreased proliferation and downstream enzyme KMO. This study is the first characterization of KP enzyme expression in NSCs. We show that KP enzymes play a specific role in the biology of NSCs and tryptophan metabolism, including the dominant regulation of the KP by interferons. Selective KP inhibition could minimize cell death and impaired regeneration during inflammatory episodes and optimize NSC proliferation and differentiation with direct therapeutic applications.

Funding Source: This study was supported by the National and Health Medical Research Council (NHMRC), St. Vincent's Clinic Foundation Sydney, and the University of New South Wales (Sydney, Australia).

F-3003

RAT HIPPOCAMPAL NEURAL STEM CELL MODULATION USING PDGF, VEGF, PDGF/VEGF AND BDNF

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Neural stem cells have become the focus of many studies as they have the potential to differentiate into all three neuronal lineages. This may be utilised to develop new and novel ways to treat neurological conditions such as spinal cord and brain injury, especially if the stem cells can be modulated in vivo without additional invasive surgical procedures. This research aimed to investigate the effects of the growth factors Vascular Endothelial Growth Factor, Platelet Derived Growth Factor, Brain Derived Neurotrophic Factor and Vascular Endothelial Growth Factor/Platelet Derived Growth Factor on hippocampal derived neural stem cells. Cell growth and differentiation were assessed using immunohistochemistry and glutaminase enzyme assay. Cells were cultured for 14 days and treated with different growth factors at two different concentrations 20ng/mL and 100ng/mL. At 2 weeks, cells were fixed, and immunohistochemistry was conducted to determine cellular differentiation using antibodies against GFAP, Nestin, OSP and NF200. Cell media supernatant was also collected during treatment to determine glutaminase levels secreted by the cells as an indicator of neural differentiation. VEGF/PDGF at 100ng/mL had the greatest influence on cellular proliferation of HNSC, which also stained positively for Nestin, OSP and NF200. In comparison, HNSCs in other treatments had poorer cell health and adhesion. HNSC in all treatment groups displayed some differentiation markers and morphology but this is most significant in 100ng/ml VEGF/PDGF treatment. VEGF/PDGF growth factor combination produced the optimal effect on the HNSCs inducing the differentiation pathway exhibiting oligodendrocytic and neuronal markers. This is a promising finding that should be further investigated in brain and spinal cord injury

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F-3005

CENPJ REGULATES CILIA DISASSEMBLY AND NEUROGENESIS IN THE DEVELOPING MOUSE CORTEX

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Primary cilia are microtubule-based protuberances that project from the eukaryotic cell body to sense the extracellular environment. Ciliogenesis is closely correlated to the cell cycle and defects of cilia are related to human systemic diseases such as primary ciliary dyskinesia. However, the role of ciliogenesis in cortical development remains unclear. Here, we demonstrate that Cenpj, a protein that is required for centriole biogenesis, plays a role in regulating cilium disassembly in vivo. Depletion of Cenpj in neural progenitor cells results in long cilia and abnormal cilia disassembly. Radial glial cell (RG cells) with Cenpj depletion exhibit uncompleted cell division, reduced cell proliferation, and increased cell apoptosis in the developing mouse cerebrum cortex, leading to microcephaly. In addition, Cenpj depletion causes long and thin primary cilia and motile cilia in adult neural stem cells and reduced cell proliferation

in the subventricular zone. Furthermore, we show that Cenpj regulates cilia disassembly and neurogenesis through Kif2a, a plus-end-directed motor protein. These data collected from mice of both sexes provide insights into how ciliogenesis plays roles in cortical development and primary microcephaly induced by Cenpj mutations in humans.

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F-3007

LINEAGE ANALYSIS OF EMBRYONIC NEURAL PRECURSORS FROM THE SUBPALLIAL GERMINAL ZONE AT SINGLE CELL RESOLUTION

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We define two distinct types of neural stem cells (NSCs) and their downstream neural progenitor cells (NPCs) from the embryonic ventral germinal zone (GZ) using clonal lineage tracing and single cell transcriptomics. Primitive (p)NSCs express Oct4 but do not express GFAP, and mouse embryo-derived pNSCs form clonogenic neurospheres proliferate rapidly when grown in LIF. pNSCs arise earlier in development than GFAP-expressing definitive (d)NSCs that form clonogenic neurospheres in FGF2/EGF. To assess the differences in functional outputs of both NSC types, we performed clonal lineage tracing within clonal neurospheres grown in either LIF or EGF/FGF2, to enrich for neural progenitor cells (NPCs) directly downstream pNSCs or dNSCs, respectively. PNSCs from the E17.5 ventral forebrain GZ gave rise to more unipotent neuronal progenitor cells than dNSCs, and dNSCs gave rise to more unipotent astrocyte progenitor cells. Both NSCs gave rise to bipotent NPCs that produce neurons and astrocytes, which consistently proliferated more than unipotent NPCs. Surprisingly, pNSCs give rise to these more unipotent neuronal progenitor cells that expressed GFAP+ before they became post-mitotic neurons. These clonal progenitor lineage tracing data allowed us to construct a hierarchy of progenitor subtypes downstream of pNSCs and dNSCs. To validate this hierarchy, identify markers for distinctly specified NPCs, and assess single cell RNA expression differences between these NPCs, we performed Drop-seq on E17.5 neural stem and progenitors cells from clonal neurospheres grown in either LIF or FGF2/EGF. UMAP-based Monocle 3alpha and p-Creode served as additional methods to map out the transition states of NPCs. These bioinformatic lineage analyses revealed a unique pathway for making OB interneurons downstream of pNSCs compared to other neuronal cell types. Combined, these data provide single cell resolution of NPCs present in the pre-natal brain, including NPCs downstream of rare pNSCs that would likely be missed from population level analyses in vivo.

F-3009

DIRECT CONVERSION OF HUMAN EMBRYONIC STEM CELL-DERIVED GLIAL PROGENITORS INTO MIDBRAIN DOPAMINERGIC NEURONS

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Direct in vivo conversion is emerging as a novel route for generating new neurons for brain repair. Oligodendrocyte progenitor cells (OPCs) present an interesting target for direct neuronal conversion since these cells are replenishable and since they are found in relative abundance throughout the adult brain. To date, all in vivo conversion studies have been conducted using resident rodent glia and the outstanding question whether human glia can be converted into neurons within the adult brain remains to be resolved. To address this question we have differentiated human embryonic stem cells (hESCs) into glial progenitor cells (GPCs), to obtain a human glial cell source that is highly expandable and possible to cryopreserve. Using these hESC-derived GPCs we have identified a conversion factor combination that efficiently convert human GPCs into midbrain dopaminergic (mDA) neurons, the cell type that selectively degenerates in Parkinson's disease (PD). The resulting neurons express markers characteristic of DA neurons and are functionally mature. Building on our in vitro data, we are now transplanting our hESC-derived GPCs into a rat model of PD to convert them into mDA neurons in vivo using a doxycycline inducible system. This study will provide us with novel insight of the possibility to directly convert human glial cells into subtype specific neurons in vivo, and to assess the therapeutic potentials of this approach.

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F-3011

SPINAL CORD INJURY: EFFECT OF BIOMATERIAL AND IMMUNE RESPONSE ON HUMAN NEURAL STEM CELLS FATE

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Spinal Cord Injury (SCI) is a devastating condition, which results in loss of sensory, motor, and reflex function below the level of injury. Following the initial injury, disruption of the blood spinal cord barrier allows infiltration of peripheral immune cells from the blood into the spinal cord tissue. We have recently shown that immune cells and their components can influence the outcome of reparative strategies, including human neural stem cell (hNSC) transplantation, resulting in changes in fate, migration and efficacy. Additionally, we have demonstrated that implantation of a poly (lactide-co-glycolide) (PLG) biomaterial bridge can lead to regeneration of the sensory and motor axons including the corticospinal tract (CST) in association with recovery of function. My overall goal is to test the combinatorial effect of two regenerative approaches, implantation of a poly (lactide-co-glycolide) (PLG) biomaterial bridge and transplantation of hNSC. However, PLG bridges become extensively cellularized after implantation, including recruitment of macrophages and other innate immune cells, suggesting the potential for the innate immune response to modulate hNSC within the PLG-bridge. Additionally, macrophages are the dominant cell population within the implanted PLG-bridge during axonal regeneration. These macrophages contribute to the biodegradation of PLG-bridge and remain in proximity to the regenerating fibers, suggesting that PLG-bridges may support regeneration by modulating the immune environment. Accordingly, we have investigated the combined effect of PLG and immune cues on hNSC fate in-vitro to understand the synergetic outcome of this combinatorial approach. Thus, as a secondary objective we studied the immunomodulatory effect of the PLG scaffold on innate immune cell intrinsic properties that can alter stem cell fate. These studies provide insight into the interaction between PLG, hNSC, and innate inflammatory cells in vitro in order to enhance in vivo testing of combinatorial administration of PLG bridges to promote axonal regeneration and hNSC to support myelination of new axons after SCI.

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F-3013

SOXC TRANSCRIPTION FACTORS ARE CRUCIAL REGULATORS OF SENSORY PROGENITOR DIFFERENTIATION IN THE MOUSE ORGAN OF CORTI

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All vertebrates use sensory organs containing hair cells to perceive sound and motion. These sensory receptors are rare in number and after damage do not regenerate, leading to permanent hearing loss and balance disorders. The cells that give rise to the organ of Corti in the mammalian inner ear first exit the cell cycle within a well-defined prosensory domain and then initiate a process of differentiation that gives rise to a stereotyped mosaic of hair cells and supporting cells. The mechanisms governing prosensory fate commitment are not known, but are important for a full understanding of the failure of regeneration in this system. Sox genes are well-established regulators of cell fate determination. We show that during sensory epithelia development in the organ of Corti, two members in the SoxC family, Sox4 and Sox11, are highly expressed in the sensory progenitors at the onset of hair cell differentiation. Heterozygous knockout of SoxC genes causes partial hearing loss and vestibular dysfunction, while homozygous loss blocks progenitor cell differentiation towards the hair cell fate. Using transcriptomic and epigenomic analysis, we characterized the

transition from proliferating progenitors to differentiated hair cells, and assayed the effects of the loss on SoxC genes on this process. By assessing chromatin accessibility (ATAC-seq), we observe that the potential regulatory elements associated with hair cell fate-commitment emerge in the postmitotic progenitor cells, and are enriched for Sox transcription factor binding motifs. Using conditional inactivation of Sox4 and Sox11 genes in the inner ear, combined with Chip-seq analysis, we demonstrate that accessibility of these newly emerged regulatory elements is directly dependent on SoxC gene expression. Single cell sequencing confirms that conditional loss of SoxC genes leads to downregulation of the predicted prosensory gene targets in the organ of Corti progenitor cells. Consistent with this observation, overexpression of SoxC prior to cell fate determination enhances sensory differentiation. Our results reveal that through chromatin remodeling, and consequent hair cell-specific gene expression, SoxC transcription factors are crucial for sensory cell fate determination in the progenitor population of the organ of Corti.

F-3015

ANATOMICAL AND SINGLE CELL TRANSCRIPTOMIC PROFILING OF THE DEVELOPING ENTORHINAL CORTEX NEURONAL CIRCUIT IN THE PIG

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The entorhinal cortex (EC) acts as a gateway for information traveling in and out of the hippocampal formation and is important for spatial memory. The stellate cell (SC) resides in layer II (LII) of the medial EC, projects to the dentate gyrus and contributes to both the grid and border cell phenotypes. However, very little is known about the molecular identity of SCs. SCs express reelin (RELN+) and are negative for calbindin (CALB1-) and this differentiates them from the other main principle neurons. We, therefore, decided to probe the developing EC to identify key growth factors and cytokines that are critical for the formation of SCs in the pig. We selected the pig as a model since its advantageous compared to the rodent in reflecting human EC development due to the pig brains larger size, gyrencephalic anatomy and longer gestation period (gestation length 114 days). Nissl staining and postmortem structural MRI indicated that entorhinal-like cells present at the superficial border of LII at E50 and a more developed EC architecture from E60 onwards. We characterized cortical lamination by performing immunohistochemistry using antibodies against SATB2, CTIP2, GFAP, BLBP, SOX2, PAX6, TBR1 and TBR2 during EC development. We identified the formation of the EC between E26 and E33 and a distinct LII forming from E33 to E39. Interestingly, LV/LVI formed after LII, and prior to LIII/IV at E39 to E50. LIII/IV was first observed at E60 indicating the cortex forms disparately to normal cortical lamination in a sandwich-like manner. Furthermore, we identified a population of RELN+/CALB1-/MAP2+/CTIP2+ neurons at the superficial border of LII at E60, which we presume to be the SCs. The percentage of these cells in LII increased from 1.87% of the population in the cortical plate at E60 to 51.77% within LII by E100. Single-cell RNA sequencing data was performed on EC, which has led to the identification of unique gene signatures for 4 progenitors, 10 excitatory neuron and 7 inhibitory neuron populations. This study has led to the characterization of the developing EC in a new species and documents when SCs arise in the EC. We are now using this data to develop in-vitro protocols to produce SCs from iPSCs.

NEURAL DISEASE AND DEGENERATION

F-3017

NEURAL STEM CELLS FOR DISEASE MODELING AND EVALUATION OF THERAPEUTICS FOR TAY-SACHS DISEASE

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Tay-Sachs disease (TSD) is a rare neurodegenerative disorder caused by autosomal recessive mutations in the HEXA gene that encodes β-hexosaminidase. Deficiency of β-hexosaminidase results in accumulation of GM2 ganglioside, a glycosphingolipid, in lysosomes. Currently, there is no effective treatment for TSD. In this study, we generated induced pluripotent stem cells (iPSCs) from two TSD patient dermal fibroblast lines and further differentiated them into neural stem cells (NSCs), which exhibited the disease phenotype of accumulations of GM2 ganglioside and other lipids in lysosomes. Treatment with recombinant human Hex A enzyme abolished the lipid accumulation in these cells. We also found that hydroxypropyl-β cyclodextrin (HPβCD) and δ-tocopherol significantly ameliorated the lipid accumulations in patient cells. A combination of HPβCD and δ-tocopherol significantly decreased the lipid accumulation to levels comparable to wild-type cells. Furthermore, a drug combination screening of a small compound library was performed to identify compounds that enhance the effect of HPβCD in the patient cells. These results provide insights to better understand disease pathophysiology and suggest a potential drug development path for treatment of Tay-Sachs diseases.

F-3019

SYNERGISTIC EFFECTS OF EXERCISE AND CELL THERAPY FOR PARKINSON'S DISEASE MODEL RATS

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Cell transplantation is expected to be a promising treatment for Parkinson's disease (PD), in which re-innervation of the host striatum by the grafted dopamine (DA) neurons is essential. Especially, the dorsolateral part of the striatum is important because it is the target of midbrain A9 DA neurons, which are degenerated in PD pathology. The effect of exercise on survival and maturation of the grafted neurons has been reported in several neurological diseases models, but never in PD models. In this study, we transplanted the ventral mesencephalic neurons of embryonic (E12.5) rats into the striatum of adult chronic PD model rats, and combined treadmill training as exercise after transplantation. Then the survival and neurite extension of the grafted DA neurons were evaluated. Exercise significantly increased the number of survived DA neurons. Moreover, it promoted the neurite extension from the graft toward the dorsolateral part of the striatum. These effects are supposed to be mediated through the modulation of neurotrophic factors and inflammatory cytokines in the host brain environment. This study indicates a beneficial effect of exercise after cell transplantation in PD.

F-3021

BREAST CARCINOMA AMPLIFIED SEQUENCE 2 (BCAS2) REGULATES ADULT NEUROGENESIS THROUGH B-CATENIN

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Breast carcinoma amplified sequence 2 (BCAS2) regulates β-catenin gene splicing; the loss of BCAS2 expression in forebrain (BCAS2 cKO) show the impaired learning and memory with β-catenin declination. Due to β-catenin regulates adult neurogenesis, here we extensively found that BCAS2 cKO mice showed low Sox2+-neuron stem cells (NSC) proliferation and self-renew. Similarly, the stereotaxic intracranial injection of lentivirus-shBCAS2 to knockdown BCAS2 in hippocampus; and confirmed BCAS2-regulating adult neurogenesis via β-catenin. Moreover, AAV-BCAS2 gene therapy to cKO could rescue the proliferation of Sox2+-NSCs with the increasing β-catenin expression. Collectively, BCAS2 regulating adult neurogenesis through a β-catenin are autocrine and paracrine effects. Furthermore, the lithium treatment to BCAS2 cKO mice reveals the improvement the spatial learning and memory capabilities coupled with increasing β-catenin expression and restoring the declined number of NSC number and proliferation, thereof, BCAS2 cKO mice can act as a neuron degeneration model for future drug screening.

F-3023

EFFECTS OF TDP-43 LOCALIZATION ON STRESS RECOVERY IN STEM CELL-DERIVED IN VITRO HUMAN MOTOR NEURONS

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Tar DNA-Binding Protein 43 (TDP-43) cytoplasmic aggregates are known to be associated with several neurodegenerative conditions, including Amyotrophic Lateral Sclerosis (ALS). Although TDP-43 mutations are found in roughly 4% of familial ALS patients, TDP-43 positive inclusions are found in 97% of sporadic and familial ALS cases, suggesting that TDP-43 mutation is not the only contributor to the proteinopathy. Previous work has additionally shown that cytoplasmic TDP-43 levels will transiently increase in response to physical, proteotoxic, and oxidative stress. However, the effect of TDP-43 subcellular localization on human motor neurons' ability to adapt to these stressors has yet to be tested. We hypothesize that TDP-43 cytoplasmic-nuclear shuttling is required to mount the appropriate compensatory response that ensures neuronal survival and proper recovery in the face of stress. In this study, we examine the capacity for axonal outgrowth after axotomization in the context of expressing different structural variants of TDP-43 from the AAVS1 safe harbor locus in an in vitro system of human motor neurons derived from HUES3 human embryonic stem cells. We believe that these findings represent basic characteristics of TDP-43 regulation that will be informative to multiple neurodegenerative contexts.

F-3025

INVESTIGATING THE POTENTIAL OF HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS TO TARGET NEUROVASCULAR DYSFUNCTION AFTER TRAUMATIC BRAIN INJURY

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Traumatic Brain Injury (TBI) is the leading cause of morbidity and life-years lost in North America thus advancement of clinical therapeutics is an urgent issue. Primary injury occurs when the brain is impacted with sufficient force to cause trauma; a secondary injury phase that is composed of cellular and molecular mechanisms follows and contributes to neurovascular dysfunction and axonal breakdown among other events. Human umbilical cord perivascular cells (HUCPVCs) are a source of mesenchymal stromal cells (MSCs); they express the pericyte markers NG2 and CD146 and PDGFR-b. The use of HUCPVCs in the treatment of TBI has the potential to contribute to through paracrine signaling, and through the structural stabilization of damaged vasculature. Collectively, the pleiotropic properties of HUCPVCs make them a potential cell-based therapeutic approach to target multiple pathophysiological pathways associated with secondary injury after TBI. Rats subjected to a fluid percussion injury (FPI) and systemically infused with 1.5 x 106 cells at 1.5h post-injury were sacrificed at acute time points. Vascular leakage was assessed using an Evan's blue assay and expressed in microgram of dye per gram of tissue. At 24h and 48h vascular leakage was 6.4 microgram and 15.5 microgram vs. 1.7 microgram in sham rats. HUCPVC treated rats had 5.5 microgram and 3.3 microgram at 24 and 48 hours, respectively. Vascular density assessed by RECA-1 immunohistochemistry at 24h and 48h demonstrated a reduction by 40% in injured animals relative to sham and cell-treated animals. Cortical tissue at the injury site was extracted at 24h and 48h for western blot analysis to examine the expression of tight junction complexes as an indicator of vascular dysfunction. Expression of occludin was increased 400% and 200% in FPI and cell treated animals respectively at 24h and was comparable to sham by 48 hours. However, evaluation of the Occludin-ZO1 complex formation by immunoprecipitation and western blot analysis indicated only a 100% increase in complex formation at 24h in FPI animals but a 650% increase in cell treated animals. The infusion of HUCPVCs following modelled TBI injury was associated with reduced vascular leakage suggesting a potential therapeutic strategy to address vascular disruption after TBI.

Funding Source: PSI Foundation

F-3027

THE STRENGTH OF CLINICAL DATA IN INFORMING IPSC-DERIVED MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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In amyotrophic lateral sclerosis (ALS), disease heterogeneity is often described as one of the largest problems impacting the effective study and treatment of disease. A deeper understanding of the source of disease heterogeneity may aid in elucidating underlying disease mechanisms. By matching clinical data to patient-specific pathophysiological outcomes, we can begin to understand the drivers of disease processes. With this in mind, fibroblasts from ALS patients with familial mutations in SOD1 and C9orf72 genes, as well as a patient with no known mutation (termed a sporadic ALS patient), were reprogrammed into iPSCs. Patients met the revised El-Escorial criteria for ALS, and clinical demographics were collected at the time of enrolment. ALS patient iPSCs, along with iPSCs generated from age- and sex-matched controls, were differentiated into

lower motor neurons. Neurons displayed all characteristic markers of this cell type, and morphologically resembled lower motor neurons. Evaluation of cellular electrophysiological (3Brain - Biocam X), as well as metabolic (Agilent - Seahorse) phenotypes was performed to align with disease-associated pathology. Fibroblasts were also incorporated into the analysis pipeline to determine if disease-specific phenotypes were uniform between fibroblasts and neurons. In conclusion, we have established a valuable resource of iPSC lines that display hallmark pathophysiological features of ALS. The strength of this resource is exemplified through the matching of in vitro cellular phenotypes with extensive clinical data. These models are now being used to investigate the role of neurometabolic defects in ALS, with specific interest in biochemical pathways that drive pathogenic change. Such pathways are also being specifically targeted with lead compounds in an attempt to ameliorate disease-associated phenotypes.

F-3029

POOLED KINOME WIDE CRISPR/CAS9 SCREEN IN HUMAN STEM CELL DERIVED NEURONS TO IDENTIFY PHARMACOLOGICAL TARGETS FOR ALS/FTD

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative diseases that overlap in their clinical presentation, pathology and genetics. So far, no effective treatment is available. Hexanucleotide-repeat expansions in the C9ORF72 gene are the most common cause of ALS/FTD. The nucleotide-repeat expansions are translated into dipeptide-repeat (DPR) proteins, which contribute to neurodegeneration. In addition, neuroinflammation is widely regarded as chronic cell stress which promote the pathogenesis of ALS/FTD. Cell reprogramming technology enabled the generation of human induced pluripotent stem cells (hiPSCs) to model disease in a dish. The recent developed CRISPR/Cas9 genome editing has expanded the scope and reliability of genetic deletion screens of the human genome. As kinase activation is crucial for neuronal survival and are good targets for novel drug development, we here perform a kinome-wide CRSIPR/Cas screen to identify novel candidate drug targets for ALS/FTD. We created iPSC lines of different genetic backgrounds with a doxycycline inducible CRISPR/Cas9 expressing cassette in the AAVS1 locus. We have transduced the sgRNA kinome wide library in the cells. Following activation of Cas9 with doxycycline and following incubation in the presence and absence of neuroinflamatory cytokines and DPR. To identify the kinases, that when eliminated, enable neurons to survive, we have collected surviving cells and currently performing next generation sequencing (NGS) to identify the sgRNAs that have been depleted or are enriched. This screen combined with ALS/FTD related validation will enable us to discover kinases involved in neuron degeneration and lay the foundation for discovering novel pharmacological targets for ALS/FTD.

F-3031

A GERMLINE HOMOZYGOUS MUTATION IN HUMAN OXIDATION RESISTANCE 1 GENE CAUSE DEVELOPMENTAL DELAY, EPILEPSY AND CEREBELLAR ATROPHY

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Oxidation Resistance 1 (OXR1) is a highly conserved gene that plays an essential role in antioxidation in eukaryotes, from yeast to human. Here using whole exome sequencing, we identified three patients with a homozygous mutation in OXR1 that leads to clinical developmental delay, epilepsy and marked

cerebellar atrophy with onset in early childhood. Patient derived lymphoblasts showed impaired cell proliferation, increased apoptosis, and abnormally high sensitivity to oxidative stress with elevated oxidative DNA damage. The patient iPSC-derived neuroepithelial cells and brain organoids display defects in neural aggregate formation, neurite outgrowth and neuronal differentiation, as well as increased apoptosis. RNA sequencing analysis reveals that OXR1 regulates the transcriptional networks important for ROS protection, regulation of apoptosis and neuronal development. The core pathways involved in neuronal differentiation, proliferation and axonal targeting as well as axon guidance are dys-regulated in OXR1 deficient neurons. The landscape of histone modification has been altered in OXR1 deficient neurons. These findings provide the first description of a human disease associated with OXR1 deficiency, indicating an essential role for OXR1 in neuronal protection and brain development.

F-3033

GENETICALLY CORRECTED IPSC-DERIVED NEURAL STEM CELL GRAFTS DELIVER NAGLU-IGFII FUSION PROTEIN TO AFFECT CNS DISEASE IN SANFILIPPO B MICE

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Sanfilippo syndrome type B (mucopolysaccharidosis type IIIB [MPS IIIB]) is a recessive genetic disorder that severely affects the brain and is caused by a deficiency in the enzyme α-N-acetylglucosaminidase (NAGLU), leading to intralysosomal accumulation of heparan sulfate. There are currently no treatments for this disorder. We carried out ex vivo, lentiviral correction of Naglu–/– neural stem cells derived from Naglu–/– mice (iNSCs) using a modified NAGLU, consisting of this enzyme fused to a receptor binding peptide of IGFII. This enables receptor-mediated uptake and delivery to lysosomes, a process that is very inefficient for natve NAGLU. Corrected iNSCs secreted a functional NAGLU-IGFII enzyme that could be taken up by deficient cells. Following long-term transplantation into Naglu–/– mice, we detected NAGLU-IGFII activity in all engrafted animals. Transplanted Naglu–/– mice showed a significant restoration of Naglu activity, and a decrease in storage material, astrocytosis, and microglial activation, with beneficial effects extending along the rostrocaudal axis of the brain. Our results demonstrate long-term engraftment of iNSCs in the brain that are capable of cross-correcting pathology in Naglu–/– mice. Our findings suggest that genetically engineered iNSCs could potentially be used to deliver modified enzymes and treat MPS IIIB.

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F-3035

DISEASE MODELLING OF ZIKA WITH HUMAN NEURAL STEM CELLS

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Zika virus caused worldwide concern by its association with severe fetal brain injury including, but not limited to, microcephaly in the fetus of infected pregnant females, with an epidemic in Brazil in 2015. Using human fetal neural stem cells (hfNSC) from several anatomical locations of a developing central nervous system in the second trimester, we explored their role in disease modelling by investigating their infection with Zika. Regionally derived hfNSC (15-21 wks) were derived as neurospheres and cultured on laminin coated plates for 7-14 days before infection with the virus at MOI of 10 for 2 hours. Fresh media is added after removal of virus and collected after 96 hours and analysed, alongside with the infected cells. Cells were also replated on coated dishes for 7 days before harvested for analysis. Viral infectivity was assessed by detection of virus antigen with FACS and effects of infectivity ascertained by qPCR and immunocytochemistry. An average of 10.2±6.7, 7.9±3.5 and 4.6±1.9% of the forebrain derived hfNSC were found infected with the French Polynesia (FP), Brazilian (PE) and Singapore strains of Zika virus respectively. An average of 15.9±4.5, 7.6±2.2 and 10.4±3.8% of the midbrain derived hfNSC were found infected with the same Zika strains respectively. This illustrates that hfNSC were infected at varying degrees with the different strains of ZIKV (Range: 0.63-29.8%) with the higher infection rates observed from infection with the French Polynesia strains. In the forebrain derived hfNSC, Zika infection resulted in a significantly lowered neuronal differentiation by means of neurofilament-M expression (34.4±4.6 (mock-infected) vs 6.0±3.5 (PF, p-value=0.004) vs 0.8±0.8% (PE, p-value=0.002)). In the midbrain derived hfNSC, an increased

differentiation into oligodendrocytes by means of PDGFRα expression was observed (42.9±2.0 (mock-infected) vs 97.6±1.0 (PF, p-value<0.001) vs 78.8±3.3% (PE, p-value<0.001)). These studies demonstrated the use of hfNSC in parsing out the effects of congenital ZIKA infection on human neural cells at a clinically relevant gestation and could be useful for in vitro studies on related work, such as vaccine testing or determining the effects of viruses before in vivo work.

F-3037

ESTABLISHMENT OF A PARKINSON'S DISEASE NOG MOUSE MODEL FOR EVALUATING IN VIVO FUNCTIONS OF HUMAN IPS CELL-DERIVED DOPAMINERGIC NEURONS

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Parkinson's disease (PD) is a neurodegenerative condition characterized by symptoms, such as shaking at rest, muscular rigidity, akinesia, and postural instability. As PD is caused by degeneration and loss of dopaminergic neurons in the substantia nigra, human ES/iPS-derived dopaminergic neurons are a potential source for cell transplantation therapy. Many research groups have reported successful differentiation of functional dopaminergic neurons from human ES/iPS cells and, in 2018, the first clinical trial of human iPS-derived dopaminergic neurons for PD patients was announced in Japan. In the present study, we aimed to establish a novel model for evaluating in vivo functions of human iPS-derived dopaminergic neurons using an immunodeficient mouse. The NOG mouse, which is severely immunodeficient, was established by introducing the IL2rγnull gene of IL2rγ knockout mice into immunodeficient NOD/ShiJic-scid mice and backcrossing 10 times. As various human cells can be engrafted in NOG mice, a PD model NOG mouse could be used to evaluate in vivo functions of human iPS-derived dopaminergic neurons as well as tumorigenicity. Moreover, the NOG mouse makes it possible to evaluate in vivo functions using a small number of cells. Based on previous work, we injected 6-hydroxydomaine (6-OHDA) into the left striatum of NOG mice. Four weeks later, we administrated apomorphine and scored the number of contralateral rotations. As a result, 6-OHDA-treated NOG mouse demonstrated clockwise rotations (>7 rpm/min). Immunohistochemical analysis revealed clear degeneration of tyrosine hydroxylase-expressing dopaminergic neurons in the striatum of 6-OHDA-treated NOG mice. We are currently examining the transplantation of human iPS-derived dopaminergic neurons into the striatum of 6-OHDA-treated NOG mice. We expect that PD model NOG mouse will be a simple, low-cost model for evaluating in vivo functions of human iPS-derived dopaminergic neurons.

ORGANOIDS

F-3041

DIFFERENTIATION AND CULTURE OF EXOCRINE HUMAN PANCREATIC ORGANOIDS FROM STEM CELL-DERIVED PANCREATIC PROGENITORS

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The exocrine compartment makes up approximately 98% of the human pancreas and is composed of enzyme producing acinar cells connected by a network of bicarbonate-secreting ductal cells. Pancreatic cancer, chronic pancreatitis and cystic fibrosis are severe pathologic conditions which arise in the exocrine tissue but in vitro models for their study and the development of efficient treatments are lacking. Recent publications describe the in vitro differentiation of human pluripotent stem cells into pancreatic exocrine organoids and demonstrate their value as a physiological 3D model for the investigation of pancreatic pathologies and development. To standardize the generation of these organoids across multiple labs using different hPSC lines, we are developing the STEMdiff™ Pancreatic Exocrine Organoid Kit. Embryonic (H9, H1) and induced pluripotent (M001) stem cell lines previously maintained in mTeSR™1 were seeded as monolayers at a density of 2.6–3.7x105 / cm3 and directed through 12–14 days of differentiation to sequentially generate definitive endoderm, primitive gut tube, foregut endoderm and PDX1+/NKX6.1+ pancreatic progenitor cells. Pancreatic progenitors were harvested and seeded into 50 µL Corning® Matrigel® domes and subjected to a 10–14 day differentiation protocol to promote exocrine pancreatic cell

differentiation. The cells formed E-cadherin+/EpCAM+/ZO-1+ polarized organoids with a central lumen, basolateral cell-contacts, and a collagen IV+/laminin+ basement membrane. The organoids also express progenitor (PDX1, NKX6.1), ductal (CFTR, CA2, SOX9, KRT19), and acinar (amylase) cell markers and contain aldehyde dehydrogenase-expressing cells, as detected by the ALDEFLUOR™ Kit. Organoid cultures can also passage every 8–14 days for a minimum of 3 passages. Our results demonstrate that the STEMdiff™ Pancreatic Exocrine Organoid Kit is an efficient tool to generate exocrine pancreatic tissue.

F-3043

EFFICIENT, REPRODUCIBLE AND HIGH-THROUGHPUT-COMPATIBLE PROTOCOLS FOR DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL LINES INTO KIDNEY ORGANOIDS

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The ability to differentiate human pluripotent stem cells (hPSC) into kidney organoids provides a next generation cell-based assay and platforms for basic research studies, patient-specific disease modeling and nephrotoxic drug screening. However, current protocols for the derivation of kidney organoids from hPSCs are highly variable and are not standardized. We developed the STEMdiff™ Kidney Organoid Kit, containing specialized serum-free media to enable efficient and reproducible differentiation across multiple human embryonic and induced pluripotent stem cell lines (H1, H9, WLS-1C and STiPS-M001), previously maintained in mTeSR™1. Cells were seeded into Corning® Matrigel® coated 96-well plates. After 24 hours, adherent cells were overlaid with an additional layer of Corning® Matrigel®, which resulted in the formation of cavitated hPSC spheroids within the next 48 hours. On the following day, differentiation of cavitated hPSC spheroids was initiated by switching media from mTeSR™1 to the STEMdiff™ Kidney Organoid Kit. During the next 18 days of differentiation, cells were directed through stages of late primitive streak, posterior intermediate mesoderm, and metanephric mesoderm to give rise to kidney organoids that are composed of podocytes, proximal and distal tubules. Samples were collected prior to and post differentiation and analyzed by immunocytochemistry, RT-qPCR and/or flow cytometry to verify lineage-specific marker expression. Cavitated hPSC spheroids display a uniform expression of pluripotency markers OCT4 and SOX2 prior to the start of differentiation. All tested hPSC lines are highly efficient in generating self-organizing kidney organoids that form convoluted tubular structures with typical nephron-like segmentation. Kidney organoids reliably establish with efficiencies of 98.7 ± 4.1 (mean ± SD; n = 1 - 3 per cell line) organoids per cm2. Organoids, analyzed on day 18, express markers of podocytes (PODXL, NPHS1), proximal (LTL, CUBN) and distal tubules (CDH1, GATA3) (n = 8). In summary, STEMdiff™ Kidney Organoid Kit supports highly efficient and robust differentiation of hPSCs into kidney organoids in high-throughput-compatible microwell plates, which can be used for nephrotoxic compound screening.

F-3045

UTILIZING PATIENT-DERIVED BRAIN ORGANOIDS TO MODEL NEURAL NETWORK PATHOLOGY IN EPILEPSY

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Human pluripotent stem cell-derived brain organoids recapitulate many aspects of human brain development and cytoarchitecture, and could provide invaluable insights into neurological diseases such as epilepsy. To realize this potential, we generated interneuron-rich ganglionic eminence (G) and excitatory neuron predominant cortical (C) organoids and fused these structures to create complex brain organoids with excitatory-inhibitory neuron interconnections. We then utilized live two-photon calcium imaging methodologies and extracellular electrode recording of local field potentials to determine the electrophysiological signatures of fusion organoids. We showed that human embryonic stem cell derived C+G fusion organoids display unique inhibitory interneuron-modulated spontaneous neural network activities including complex rhythmic oscillations. Moreover, human induced pluripotent stem cell-derived fusion organoids from a patient with Rett syndrome, a genetic disorder highly associated with epilepsy, had epileptiform-like activity

and altered network oscillations compared to isogenic control organoids derived from the same patient. We next leveraged the fusion technique to mix isogenic control (C) with Rett (G) and vice versa, and subsequently showed a predominant role of interneurons in the Rett epileptiform electrophysiologic signatures. Finally, treatment of Rett fusions with the anti-seizure medication sodium valproate reduced spike frequency without rescuing oscillatory activity, while treatment with the p53 inhibitor pifithrin-α reduced spike frequency and rescued some oscillatory activities. Together, these findings show that we have developed methodologies for reproducibly measuring complex neurophysiologic activities in human brain organoids. Moreover, we generated data that suggest the presence of ictogenic networks in Rett organoids that recapitulate electrographic features of epilepsy. This opens the door to deeper exploration of both Rett-associated and other seizure disorders and for novel therapeutic interventions.

F-3047

EFFICIENT ESTABLISHMENT AND GROWTH OF HUMAN INTESTINAL ORGANOID-DERIVED MONOLAYERS

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Organoids are a cutting-edge tool for regenerative medicine, disease modelling and drug screening. The STEMdiff™ Intestinal Organoid Kit and IntestiCult™ Organoid Growth Medium (OGM; Human) are used for the derivation and expansion of human intestinal organoids from human pluripotent stem cells (hPSC) and primary tissues, respectively. The focus of the current study is to develop protocols for growing and differentiating organoid-derived intestinal cells in a monolayer culture, and to compare the electrophysiological properties of these cells to the commonly used Caco-2 cancer cell line. To do this, primary human intestinal organoids were seeded in IntestiCult™ OGM onto Corning® Matrigel® -coated tissue culture and transwell plates. After 7 days, the cultures were analyzed by immunocytochemistry, transepithelial electrical resistance (TEER) measurements to measure barrier integrity, and by Ussing Chamber analysis to measure ion transport. Organoid-derived monolayers are composed of VIL1-, EpCAM-, E-cadherin- and claudin-expressing enterocytes that have a polarized morphology complete with tight junctions and a brush border, as well as MUC2 expressing goblet cells. Intestinal organoid-derived monolayers have measured TEER values averaging 358.5 ± 22.6 Ω*cm2 (SEM; n=12), compared to an average of 301.1 ± 22.3 Ω*cm2 (SEM; n=10) for comparable Caco-2 monolayers, demonstrating that these cells have equivalent or greater barrier function. Treatment with the cAMP elevating agents IBMX and forskolin increase the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) by a change in short-circuit current (ΔISC) of 40.3 ± 7.62 µA/cm2 (SEM; n=6) in human colonic monolayers, significantly more than 6.7 ± 0.61 µA/cm2 (SEM; n=4; p=0.04) for Caco-2 monolayers. Additionally, treatment with the CFTR inhibitor-172 reduced CFTR activity by a ΔISC of 80.6 ± 5.96 µA/cm2 (SEM; n=6) compared to 15.5 ± 1.98 µA/cm2 (SEM; n=4; p<0.0001) in Caco-2 cells, demonstrating increased sensitivity in organoid-monolayer compared to Caco-2 cultures. The new IntestiCult™ OGM monolayer protocol provides a novel experimental platform that better mimics the in vivo intestinal epithelium, demonstrated by performance of functional assays that matches or exceeds those of the cell lines currently in widespread use.

F-3049

THE EFFECTS OF MICROGRAVITY ON MICROGLIA 3-DIMENSIONAL MODELS OF PARKINSON'S DISEASE AND MULTIPLE SCLEROSIS

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Exposure to microgravity and radiation, as occurs in the International Space Station (ISS), causes significant mechanical unloading of mammalian tissues, resulting in rapid physiological alterations. While many studies have focused on the impact of microgravity on the cardiac and musculoskeletal systems,

microgravity is also known to have a significant impact on the central nervous system. Research using a variety of tissue types has demonstrated that microgravity increases proliferation and delays differentiation of stem cells. Cell-cell interactions are critical for neuronal communication, but to date no studies have yet evaluated these effects in human cells in microgravity. Taking advantage of the potential of the induced pluripotent stem cells (iPSC) to differentiate into any cell type of the human body, we propose to study 3D neuroglial cell cultures derived from iPSCs of patients with Parkinson's disease and multiple sclerosis, to analyze the migratory capability of iPSC-derived microglial cells in microgravity. Based on this, we will evaluate retrospectively cell–cell interactions and migratory capabilities of iPSCs-derive microglial cells from patients with Parkinson's disease (dopaminergic neurons) and multiple sclerosis (cortical neurons), versus co-cultures of the same cell types from healthy control subjects. The cells will be maintained on the International Space Station (ISS) for one month. This groundbreaking study will be the first long-term cell culture experiment conducted in microgravity using human patient-derived iPSCs to study Parkinson's disease and multiple sclerosis. This research will provide valuable insights into the mechanisms by which neuronal cells mature in 3D cultures, factors influencing migration of glial cells in organoids, and changes in gene expression that may play a role in these disease processes, and may well have an impact on discovery and development of biomarkers and therapeutics.

F-3051

TRANSPLANTATION OF HUMAN EMBRYONIC STEM CELL-DERIVED CEREBRAL ORGANOID AFTER LONG-TERM CULTURE INTO MOUSE MOTOR CORTEX

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Cell replacement therapy is an expected future treatment for reconstructing the neural circuits of cerebral cortices injured by stroke or trauma. The transplantation of cerebral organoids derived from pluripotent stem cells is attracting attention because cerebral organoids can generate three-dimensional structures of the cerebral cortex. Recent studies reported that the transplantation of human embryonic stem cell (hESC)-derived cerebral organoids into mouse cerebral cortex promoted graft survival with vascularization and axonal growth to the host brain. However, it remains unclear which developmental stage of the organoid is optimal for the transplantation. Additionally, it is unclear whether cerebral organoids can reconstruct motor pathways of a damaged motor cortex following transplantation. To clarify these problems, we induced cerebral organoids from hESCs using SFEBq (Serum-free Floating culture of Embryoid Body-like aggregates with quick reaggregation) method. We transplanted the cerebral organoids at early (day 42–43) and late (day 71–84) developmental-stage into cerebral cortices of early postnatal mice. Cerebral organoids at both developmental stages survived and promoted axonal growth to the host brain. Late-stage cerebral organoids did not cause tumor-like graft growth and were considered more suitable for the transplantation. Finally, we transplanted late-stage cerebral organoids into the motor cortex of adult mice. The engrafted cerebral organoids provided fiber extensions along the corticospinal tract when the graft was transplanted one week after lesioning the motor cortex. We confirmed that hESC-derived cerebral organoids at the late developmental stage promote axonal growth along the corticospinal tract without tumor-like graft growth after transplantation into the lesioned motor cortex of adult mice. Our approach is an important step for the reconstruction of motor pathways after cerebral cortex injury.

F-3053

DECIPHERING THE ROLE OF TSC1 IN A CORTICAL ORGANOID MODEL OF TUBEROUS SCLEROSIS

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Tuberous sclerosis complex (TSC) is a developmental disorder caused by a mutation of one of the two tumor suppressor genes, TSC1 or TSC2. Protein products of those genes form a TSC1/TSC2 complex, which plays a pivotal role as a negative regulator of the mTOR pathway. One of the hallmarks of TSC pathology is the formation of cortical tubers (i.e., benign lesions with abnormal glial and neuronal cells) that disrupt the classical six-layered cytoarchitecture of the cortex and can cause epileptic seizures. First signs of the disease can be seen already during the early stage of human fetal development. So far, experimental studies on TSC were largely conducted in mice. However, human and mouse cortical development differs significantly, creating a need for appropriate human model systems. Here we address this need by generating TSC1-deficient human iPSC lines using CRISPR/Cas9 technology and subjecting these lines to cortical

differentiation in 2D and 3D organoid cultures. In agreement with the role of TSC1 in the regulation of mTOR signaling, TSC1-deficient cortical cultures show evidence of increased mTOR signaling, including elevated phospho-S6 levels and an increase in cell size. When compared to isogenic controls, TSC-1-deficient cortical organoids display unaltered overall growth rates as well as stratification into a ventricular zone-like area and neuronal cell layers. However, a more in-depth analysis of division patterns revealed a significant increase in the number of cells with vertical cleavage planes at the expense of horizontal cleavage planes in TSC1-deficient organoids. These observations may serve as an entry point for dissecting potential alterations in the dynamics of neural stem cell proliferation and differentiation associated with TSC1-deficiency and impaired mTOR signaling. Our data underline the notion that cerebral organoids provide a valuable tool to decipher pathomechanisms underlying the emergence of human cortical malformations.

F-3055

HUMAN SLICED NEOCORTICAL ORGANOIDS ESTABLISH SPECIFIED UPPER AND DEEP CORTICAL LAYERS

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Brain organoids, human pluripotent stem cell(hPSC)-derived self-organizing three-dimensional (3D) tissues with cell types and cytoarchitecture resembling the embryonic human brain, have emerged as valuable model systems to investigate human brain development and disorders. Previously, we have developed methodologies to generate forebrain organoids that resemble human cerebral cortex development around mid-gestation period with remarkable fidelity. Engineering mature cortical organoids representative of late gestation stages could fill the current gaps in our knowledge of human corticogenesis due to the inaccessibility of fetal tissues in the third trimester. However, due to a lack of functional vascular circulation system, growth and maturation of cortical organoids are limited by an insufficient supply of oxygen and nutrients via diffusion. Here we report a slicing method to bypass the diffusion limit by exposing the organoid interior while maintaining its structural integrity, which leads to the generation of organoids representative of human late-stage neocortical development. Substantially reduced cell death and sustained cell proliferation allow the progenitor and neuronal layers to expand larger beyond previous size limits and produce well-separated upper and deep cortical neuronal layers. Within the cortical layers, spontaneously active neuronal networks fire coordinated bursts across long distance. We further identify the critical role of WNT/β-Catenin signaling in regulating cortical neuron fate specification and layer separation, which is disrupted by a mutated risk gene for psychiatric disorder in patient iPSC-derived organoids. Overall, our sliced neocortical organoids introduce a new approach to overcome diffusion limit in 3D cultures, and offer an instrumental platform for investigating previously-intangible human-specific features of late-stage brain development.

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F-3057

SCALABLE AND HIGH-FIDELITY DRUG INDUCED LIVER INJURY SCREEN USING HUMAN IPSC-LIVER ORGANOIDS

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Billions of dollars are lost annually from drug development in the pharmaceutical industry due to the failures of drug candidates in initial screens, and nearly a third of drugs are later withdrawn from the market. Preclinical prediction of which compounds have drug induced liver injury (DILI) risk in humans is still a significant challenge in drug development. Here, we developed an method to generate unique human pluripotent stem cell derived liver organoids (HLOs), that contain cells from multi-lineages, secrete albumin, and have CYP enzyme activity. Polarized hepatocytes with bile canaliculi-like architecture support unidirectional bile acid transportation. Furthermore, coupled with a high-speed imaging microscope and high through-put image processing, we established an innovative Liver organoid-based Toxicity screen (LoT) system, wherein we tested 4 doses of 238 drugs from an Enzo hepatotoxicity library in 384-microwell plates with multiplexed readouts: cholestasis, mito-tox, and cell viability data simultaneously. The results demonstrated that HLOs can not only respond in a dose dependent manner to known DILI risk drugs but can also positively predict potential DILI risk drugs. Further, selected 10 DILI risk drugs were successfully validated the results in 6 different donors. More intriguingly, we were also able to predicted genomic predisposition (CYP2C9*2) for Bosentan-induced cholestasis. Thus, LoT is a scalable, high-fidelity PSC based model for drug safety with a cost-effective platform, facilitating compound optimization, mechanistic study, and precision medicine as well as drug screening applications.

Funding Source: Cincinnati Children's Research Foundation grant PRESTO grant from Japan Science and Technology Agency

TISSUE ENGINEERING

F-3059

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO TESTICULAR ORGANOIDS FOR STUDYING DE NOVO MUTATIONS IN INFERTIILE MEN

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Human reproduction is vital to our species but worldwide infertility affects 1 in 7 couples, of which one third of the cases are explained by a male factor. Although thousands of genes are known to be involved in spermatogenesis, the genetic causes behind severe spermatogenic failure remain largely unknown. Exome sequencing studies performed in our department will help the investigation of the aetiology of male infertility. With this research we found a prominent role for de novo mutations (DNMs) and identified many potential genes essential for spermatogenesis. Human germ cell induction research is a valuable platform for modelling infertility and congenital anomalies that have been difficult to study in animals. iPS cells were differentiated for one month into germ cells and testicular organoids, in a 2D and 3D culture, respectively. Germ cell type and content were assessed by immunofluorescence staining and quantitative PCR. The analysis performed after one month of culture showed in both methods the increased expression of CXCR4, IFITM3 and VASA, indicating the successful differentiation of iPSCs into spermatogonia. However, a longer culture is needed to obtain further differentiated germ cells. Genetic screening of the testicular organoids will help to link with more confidence the genes affected by a DNM to male infertility. Moreover, the in vitro induction of testicular germ cells and organoids from autologous pluripotent stem cells should lead to a new model for genetic engineering purposes. Putative causative DNM will be mutagenised by Crispr/Cas9 in patient-derived iPSCs, and the effect of the mutation will be assessed in testicular organoids.

F-3061

CELL-FREE THERAPY USING STEM CELL EXOSOMES FOR TREATMENT OF OSTEOARTHRITIS

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Osteoarthritis (OA) is a common degenerative joint disease characterized by cartilage destruction. Despite the steady increase in the incidence of OA, the recent treatments focused on pain management. Driven by the demand of an alternative to overcoming these drawbacks, recent studies focused on the development of effective therapeutic strategies capable of regenerating the damaged cartilage. Stem cells secrete extracellular vesicles composed of microvesicles and exosomes. Particularly, exosomes derived from stem cells contain various proteins, lipids and genetic materials (mRNA, ncRNA and miRNA etc.). Stem cell exosomes are expected to provide biochemical cues for tissue regeneration through cell-to-cell communication. In this study, we hypothesized that exosomes derived from human adipose stem cells (hASCs) could induce the therapeutic effect of degenerative arthritis. Exosomes were isolated by pre-filtration in 0.2 μm, followed by tangential flow filtration (TFF) system (300 kDa MWCO). The isolated exosomes were characterized using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), flow cytometry, cytokine arrays, etc. Exosomal marker proteins such as CD9, CD63 and CD81, were confirmed by western blot (WB). The mRNA expressions of MMP-1, MMP-3, MMP-13, and ADAMTS-5 which activate cartilage degradation were analyzed by real-time polymerase chain reaction (qPCR). A mixture of exosomes (1 ´ 108 particles) and hyaluronic acid hydrogel (1%) were intra-articularly injected 3 times for 3 weeks in MIA-induced subacute OA mouse models. After four weeks, knee joints were harvested and analyzed histologically by safranin O-fast green and hematoxylin and eosin (H&E). Various factors related to anti-inflammatory and cartilage regeneration were found in exosomes. The in vivo studies demonstrated that exosomes prevented proteoglycan degradation and attenuated the cartilage destruction in the damaged articular cartilage. Overall results suggest that exosomes derived from hASCs have great potential for cartilage repair and alleviate the inflammatory reaction caused by OA.

F-3063

EXPANSION OF HAIR FOLLICLE STEM CELLS IN MICROFABRICATED OXYGEN-PERMEABLE CULTURE VESSEL FOR HAIR REGENERATIVE MEDICINE

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Hair regenerative medicine is a promising treatment strategy for hair loss. Considering that the embryonic development of hair follicles is triggered by the formation of a hair follicle germ (HFG), we have proposed an in vitro preparation approach of HFGs and demonstrated efficient hair follicle generation upon transplantation into mice (T. Kageyama, et al, Biomaterials 2018). A challenge is however to expand the number of follicular stem cells (i.e., hair follicle stem cells (HFSCs)) while maintaining stemness prior to the HFG preparation. In this study, we fabricated a microwell array culture vessel where HFSCs formed three-dimensional aggregates and were then encapsulated in Matrigel. The culture vessel was made with oxygen-permeable silicon rubber to improve oxygen supply from the bottom through the culture vessel. The diameter of mouse HFSC aggregates in the culture vessel were uniform and increased from 100 µm to 350 µm during 2 weeks of culture. A quantitative analysis revealed that the number of CD34+ HFSCs increased up to 15 times in 2 weeks of culture. Interestingly, gene expression of trichogenic stem cell markers (e.g., Nfatc1, Tcf3, CD34) was significantly higher than that in a conventional suspension culture in Matrigel. Further, HFSCs grown in this approach were mixed with freshly isolated embryonic mesenchymal cells and formed HFGs. After 18 days of transplantation into the back skin of nude mice, newly generated hair shafts were observed at transplanted site. The hair regeneration efficiency was significantly improved compared to those prepared with a conventional suspension culture in Matrigel. This approach may be useful for large-scale preparation of HFSCs for hair regenerative medicine.

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F-3065

THE EFFECT OF MECHANICAL TRAINING ON HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIAC TISSUE SHEET STACKS

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Cell sheet technology has been previously applied in our lab, with scaffold-free 3D tissues generated by stacking cardiac tissue sheets (CTSs) showing promising therapeutic effects in vivo, as well as using CTSs to develop an in vitro model of cardiac arrhythmia. While functionally promising, these three-dimensional tissues are still structurally immature and fragile to show sufficient force generation. To overcome this, we applied cyclic stretching forces during cell sheet stack culture in order to drive tissue maturation. CTSs were prepared by differentiating cardiomyocytes and mesenchymal cells from 201B6 iPS cells and co-culturing them on temperature-responsive culture plates. Three layers of CTSs were then stacked and allowed to merge with a biocompatible adaptor prior to loading the stacks on a mechanical training device to undergo cyclic stretching or static stretching. CTS stacks were then analysed for cellular viability, protein expression and force generation. Our results showed that cyclic stretching clearly induced cellular alignment in both mural cells and cardiomyocytes, which is the first step towards cardiomyocyte maturation and improved mechanical properties, while keeping cellular viability above 95% (158/164 (96.4%) stretched; 134/137 (99.8%) suspended control; and 120/120 (100%) attached control). To a lesser extent, the static stress derived from tissue suspension also induced cellular alignment. This indicator of tissue maturation was accompanied by an increase in the expression of cardiac troponin I in cardiomyocytes. Additionally, force generation studies showed a tendency for an increase in Young's modulus and active force in the stretched samples (8.4kPa and 0.02mN, respectively) compared to the suspended control (1.0kPa and 0.007mN) and the attached control (7.2kPa and 0.006mN). The application of cyclic mechanical training on CTS stacks drives cellular alignment on cardiomyocytes and mural cells, improves the tissue's mechanical properties and could potentially drive cardiomyocyte maturation, as shown by an increase in cTnI expression. Interestingly, this alignment was perpendicular to the direction of stretching, while the application of static tension resulted in parallel alignment.

F-3067

EVALUATION OF ELECTROSPUN SCAFFOLDS OBTAINED BY CHITOSAN AND POLYCAPROLACTONE BLEND FOR VESSEL TISSUE ENGINEERING

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Currently, there is an absence of vascular grafts suitable for use in replacement surgeries of small diameter vessels. In this context, vascular tissue engineering (VTE) is investigated as a tool for the development of vascular substitutes. In this work, a polymeric blend of PCL and chitosan (CH) was used to produce vascular scaffolds by the electrospinning (ES). Scaffolds with different portions of PCL and CH were evaluated in terms of mechanical properties, cellular adhesion and blood compatibility. PCL and CH solutions with 20 and 2 % (w/v) concentrations were prepared and mixed at different ratios: 4:1, 3:1 and 2:1, and then submitted to ES. A PCL control scaffold was also produced. The morphology of the scaffolds was analyzed by scanning electron

microscopy and analysis of their mechanical properties was made by dynamic mechanical analysis (DMA). For evaluation of cellular adhesion, the mesenchymal stem cells (MSCs) were seeded onto the scaffolds and by the MTT test. The blood compatibility was evaluated by hemolysis assay. All groups of the scaffolds exhibited similar morphology, with well distributed smooth fibers in their structure. The average diameter was 0.73±0.6, 0.81±0.4, 0.80±0.5 and 0.64±0,4 µm for the PCL, PCL: CH 4:1, 3:1 and 2:1 scaffolds, respectively. The DMA tests demonstrated that CH presence did not cause significant changes in the mechanical properties of the scaffolds. The four groups of samples show a similar stress-strain curve. The MTT results showed that the scaffolds produced from blends 4:1 and 3:1 PCL:CH showed higher absorbance than the others. However, their differences were not statistically significant and the average number of adhered cells was 3.0x10-4, 3.7x10-4, 3.2 x10-4 and 2.9 x10-4 for the PCL, PCL: CH 4:1, 3:1 and 2:1 scaffolds, respectively. The hemolysis rates of all groups of the scaffolds were significantly less than the positive control and were similar to the negative control group. PCL is used in VTE due to these desirable mechanical properties. However, PCL shows poor biological properties. Thus, the association of CH with PCL scaffolds could improve their cytocompatibility. Although the cell adhesion results were not conclusive, the addition of CH maintained the mechanical properties and blood compatibility of the scaffolds, which may be interesting for VTE application.

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F-3069

ENGINEERED M13 CADHERIN ASSOCIATED PEPTIDE HAV NANOFIBER ACCELERATES INTERCELLULAR ADHESION STRENGTH AND PROLIFERATION OF FIBROGENIC FIBROBLASTS

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Recently, there has been considerable effort to develop suitable nanofiber for tissue engineering, and nanotechnology about medicine is being developed with evolution of mammalian cells The M13 phage is nano-sized material and capsulated in 2700 copies of the major coat p8 protein, minor coat p3 proteins. The HAV (histidine alanine valine) sequence in the first extracellular domain (ECM) of E-cadherin is crucial for homophilic interactions between cadherins. The extracellular domain of cadherins contains characteristic repeats that regulate homophilic and heterophilic interactions during adhesion and morphogenesis. The relevance of HAV M13 phage in relation to the fibrogenic potential of fibroblast is currently unknown. We have engineered the p8 position of M13 phage using site-directed mutagenesis PCR and target HAV sequence-displaying M13 phage sequencing and analysis. In the previously study, we find that HAV M13 phage accelerates cell-cell adhesion and morphogenesis through HAV tripeptide motif in the most distal EC (EC1). The number of cell and the fibrogenesis related gene level of HAV-M13 phage treatment of cells were increases in a time-dependent manner compared to wild M13 phage. Additionally, activation of morphogenesis during the HAV-M13 phage with co-culture. These results suggest the HAV-M13 phage nanofiber is beneficial to cellular behavior of normal cells and can be applied as a technique for selectively culturing or removing cells having a specific substance

F-3071

ELECTRICAL STIMULATION ENHANCES OSTEOGENESIS OF HUMAN DENTAL PULP-DERIVED STEM CELLS AND ELEVATED THE GENE EXPRESSIONS OF BMP2, BMP3 AND BMP5

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We fabricated an electrical stimulation (ES) device which provide direct current electric field (DCEF) treatment to the cells though conductive polypyrrole (PPy) film. We applied human dental pulp-derived stem cells (hDPSCs) on the PPy film and investigated ES effect on osteo-differentiation of (hDPSCs). ES with electrical field of 0.33 V/cm was applied to treat hDPSCs once for 4 h on different days after the chemical induction of osteogenesis. The alizarin red S staining results suggested that ES could accelerate the mineralization rates of hDPSCs. The calcium quantification analysis results revealed a nearly 3-fold enhancement in calcium deposition of hDPSCs by ES at Day 0, 2, and 4, whereas the promotion effect at later stages was in vain. To determine the ES-mediated signalling pathway, osteogenesis of hDPSCs were induced by osteogenic medium and stimulated immediately by ES, and the expression of genes belongs to the bone morphogenesis protein (BMP) family and related receptors were quantified using qRT-PCR. We found that mRNA levels of BMP2, BMP3 and BMP5 were increased significantly in the ES groups, indicating that these genes involved in the specific signalling routes induced by ES. Other BMPs such as BMP1, BMP4, BMP 6, BMP receptor 1B and BMP receptor 2 showed no statistical difference between control and ES group. The expression levels of BMP7 and BMPR1-A were undetectable in hDPSCs. Our results support that ES treatment facilitate bone formation of hDPSCs, and thus may shorten the healing period in clinical application. We showed here a promising applications of ES on hDPSCs in cell-based bone tissue engineering in the near future.

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F-3073

CIRCADIAN OSCILLATORS DURING HEART TISSUE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Circadian rhythm influences drug effectiveness and toxicity in mammals. It is unknown whether circadian rhythmicity can be established in mouse ES cell-derived heart tissue without a maternal context. It is necessary to synchronize circadian rhythm to all cells in a culture system as well as in animal. Here, we tried to synchronize the circadian rhythm of mES cell-derived heart tissues by the addition of forskolin as a synchronizer for developing a drug test model that exhibit the time dependent cell physiology and drug effectiveness. The expression of the core clock genes, such as Per1 and Bmal1, oscillated in mES cell-derived heart tissues by forskolin, but not in undifferentiated mES cells. This forskolin-synchronized oscillation pattern could be kept at least for three days. Interestingly, the contraction rate had oscillation pattern in this culture system, and also this contraction oscillation could response against β-adrenoreceptor agonist stimulation. In conclusion, these results demonstrate that circadian rhythm develops during cardiomyocyte differentiation process from mES cells. This mES cell-derived heart tissue model having forskolin-synchronized circadian rhythm would be a promising tool for replacing animal experiments for chronotherapy research and circadian rhythms studying in culture.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

F-3075

INTERNATIONAL COMPARISON OF PUBLIC ATTITUDE TOWARD STEM CELL SCIENCE AND REGENERATIVE MEDICINE

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Owing to the rapid progress in stem cell research (SCR) and regenerative medicine (RM), society's expectation and interest in these fields are increasing. For effective communication on issues concerning SCR and RM, surveys for understanding the interests of stakeholders is essential. For this purpose, we conducted an international comparison between six countries: Japan, South Korea, Germany, France, United Kingdom, and United States of America. We collected 100 valid responses through research company monitors from each country. Results showed that the public is generally interested in the post-realization and responsible governance of SCR and RM. Our data indicate that an increased awareness about RM associated social responsibility and regulatory framework is required among scientists, such as those regarding its benefits, potential accidents, abuse, and other social consequences.

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F-3077

PUBLIC SURVEY IN JAPAN ON HUMAN GENOME EDITING FOR CLINICAL PURPOSES

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In Japan, guidelines permitting the genome editing of human embryos only for basic research will soon be established. However, we have already seen genome editing of human embryos for clinical purposes, as illustrated by the claims made by He Jiankui in November 2018, who gene-edited two human embryos that came to term. This announcement highlighted a need for further discussion on whether or to what extent human genome editing for clinical purposes is acceptable. Thus far, the International Society for Stem Cell Research (ISSCR) and the National Academy of Sciences and Medicine in the United States brought attention the importance of including diverse stakeholders in the decision-making on human genome editing. In Japan as well, the opinions of the general public on human genome editing such as the human germline and human somatic cells should be reflected in policy decisions. However, there has been only one survey in Japan about the opinions of the general public and patients that focused on the genome editing of human embryos for therapeutic purposes. In short, the Japanese attitude toward genome editing on the human germline such as gametes and embryos as well as on human somatic cells for clinical purposes remains unclear. Hence, we will conduct an Internet-based questionnaire of about 4,000 general public in Japan. Using an explanation about genome editing techniques and human genome editing, we will present multiple therapeutic and non-therapeutic purposes for the genome editing of human germline and human somatic cells and then ask about their attitudes toward human genome editing. In this presentation,

we will show the survey results. Based on our survey, we will comprehensively grasp the currently unclear public attitude in Japan toward human genome editing for clinical purposes. We believe our survey framework will provide valuable information for policymaker across the globe.

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CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

F-3079

CLINICAL EVALUATION OF MINIMAL CONDITIONING FOR ENGRAFTMENT OF AUTOLOGOUS BLOOD STEM/PROGENITOR CELLS GENETICALLY-MODIFIED WITH A LENTIVIRUS VECTOR ENCODING MULTIPLE ANTI-HIV RNAS

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The lentivirus vector (LV), rHIV7-shI-TAR-CCR5RZ, has been safe when used to transduce hematopoietic stem/progenitor cells (HSPC) that were then transplanted in four patients undergoing complete myeloablative therapy for treatment of AIDS-related lymphoma (ARL) with autologous hematopoietic cell transplantation. To determine if safer and less ablative conditioning regimens could be used as alternative conditioning, we evaluated R-EPOCH [rituximab, etoposide, adriamycin, vincristine, cyclophosphamide, and prednisone] in patients undergoing frontline therapy for ARL (NCT02337985), or low-dose busulfan in HIV-infected subjects in remission from ARL after front-line therapy (NCT01961063). The main goals of these two clinical trials were to demonstrate: a) the safe use of the LV encoding multiple anti-HIV-RNAs in humans, and b) the engraftment of gene-modified progeny cells following minimal conditioning. Long-term follow-up data are still being collected for both clinical trials. In the first study, three subjects with ARL were treated with LV-transduced HSPC, infused two days after they received the last dose of the R-EPOCH regimen. No serious adverse events were attributed to the autologous gene-modified products. With this R-EPOCH regimen, there was some minimal level of marking, tested by ddPCR for WPRE, in the peripheral blood at 1-2 months post infusion, and expression of the shI RNA transgene was detected by RT-loop Q-PCR up to 3 months post infusion. Nevertheless, there was no long-term engraftment of the gene-modified HSPC. Preliminary results showed no apparent effect of treatment on HIV reservoir (cell-associated DNA and RNA and plasma HIV viral load). In the second study, with low-dose busulfan (3.2 mg/kg, intravenous, given once over 4 hours) as conditioning regimen, two research subjects were treated, and as with the other clinical trial subjects, although the therapy was safe, there was negligible vector marking in the peripheral blood. Expression of shI RNA disappeared after Month 2 post infusion. In conclusion, the minimal conditioning regimens chosen here to reduce risk failed to provide adequate preparation for long-term engraftment of LV-modified autologous HSPC patients with ARL.

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F-3081

NAVIGATING HUMAN CELL THERAPY TRIALS: A PLATFORM FOR TRANSLATING NOVEL THERAPIES INTO CLINICAL PRACTICE

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Recent progress in application of human cell and cell-derived therapies around the world demonstrates the rapid pace of discovery and translation of novel approaches to treat unmet patient needs for a number of disease conditions. In order to continue with this ambitious pace, academic, public and private organizations need the requisite tools in place to facilitate further advances in the field. Despite these recent successes, navigating the evolving regulatory and legal environment involves unique challenges in bringing human cell therapy products to market and/or clinical practice. Although common research coordination services exist at many institutions, Mayo Clinic Center for Regenerative Medicine has implemented an integrated system we have titled Translation into Practice Platforms (TIPPs) specifically designed to address the particular challenges within the human cell therapy field. TIPPs is an institution wide program that includes the integral translational components needed to accelerate human cell therapy trials using validated therapies into clinical practice. The objective of the service is to provide partnership with clinicians and research faculty interested in clinical trial research. Components of this partnership include a "Translational Navigator" along with a team consisting of a clinical protocol development specialist, a regulatory support manager, and clinical trial coordinators. We describe how the team connects investigators with resources linking clinical departments and their medical specialty needs with cell therapy manufacturing platforms (cellular, cell-derived, and devices). Collectively, this group provides current and timely education on the scope of the growing human cell therapy field and assists with clinical trial design, IRB approval, regulatory expertise for a given product or device, as well as coordinator support for execution of early clinical trials. As this platform guides clinicians and scientists through education and clinical trial engagement, translational navigation will remain a crucial element ensuring cell therapies progress through the discovery-translation-application continuum via quality clinical trials.

F-3083

TEG-SEQ: A WORKFLOW FOR IN CELLULO MAPPING OF CRISPR SPECIFICITY

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Engineered nucleases, including the CRISPR/Cas9 system, have been widely used for genome editing, and is now being developed to create gene and cell therapies to treat human disease. However, lack of specificity leading to off-target cleavage is still a concern. To measure this, an in cellulo method, genome-wide unbiased identification of double stranded breaks enabled by sequencing (GUIDE-seq) was developed and has been widely used (Tasi Q et.al). However, this method as originally reported was associated with a significant level of non-specific target amplification which reduced sensitivity and increased the cost to detect low-frequency off-target events. In an attempt to improve robustness and sensitivity, we developed a modified method termed Target-Enriched GUIDE-seq (TEG-seq). The modification improves the sensitivity approximately 10 fold compared to GUIDE-seq. In addition to the increased specificity, we developed high-throughput workflow and data analysis tool that led TEG-seq to become more cost-effective. Using TEG-seq, we evaluated a panel of Cas9 mutants to identify potential high-fidelity Cas9 protein that will be a critical for genome editing, especially for gene and cell therapy. We also used TEG-seq to map on- and off-target cleavage events on 22 gRNAs targeting a set of therapeutically relevant SNPs. Finally, TEG-seq was used to evaluate CRISPR off-target profiling for therapeutic applications in different cells including iPSC and CAR-T cells and an animal model. TEG-seq off-target detection with the use of high-fidelity Cas9 proteins will be one of the crucial steps in genome-editing and gene therapy.

F-3085

DEVELOPMENT OF A NEEDLE FOR CELL INJECTION INTO A BRAIN

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We started a clinical trial for a cell transplantation therapy for Parkinson's disease (PD). Previously, we reported successful transplantation of induced pluripotent stem (iPS) cell-derived dopaminergic neuron progenitors into PD model monkeys, and a few issues remain before the clinical application. One of those problems is a development of a device for cell transplantation. In Japan, some cell injecting devices are approved for clinical use by Ministry of Health, Labor and Welfare. However, these devices are not suitable for our cell transplantation therapy, considering a small amount of transplanted cell suspension (about 5 uL). We decided to develop a brand new needle for our clinical trial. We assessed shape, length and diameter of a needle and manufactured a new needle with a corporation. The newly developed needle is comparable with that we used in the previous preclinical trial in terms of survivability of injected cells and injection efficiency, and cells transplanted with the new needle survived in monkey brains. Accuracy of this stereotactic system is estimated to be within ± 2 mm. We successfully performed the first transplantation of iPS cell-derived dopaminergic neuron progenitors into a PD patient. In conclusion, we developed a brand new needle for cell transplantation for Parkinson's disease and this system will be applicable for treatment for other brain disorders in a future.

Funding Source: Japan Agency for Medical Research and Development (AMED)

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

F-3087

EED REPRESSES SOMATIC GENE EXPRESSION DURING MOUSE PRIMORDIAL GERM CELL DEVELOPMENT

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Mammalian Primordial germ cells (PGCs) are specified by induction from the epiblast and serve as the source of all adult germ cells used for reproduction. Following specification, male and female PGCs undergo two-stages of DNA demethylation beginning with global DNA demethylation followed by demethylation of genes responsible for regulating the timing of sex-specific PGC differentiation. As DNA methylation is lost globally from the PGC genome, Histone 3 Lysine 27 trimethylation (H3K27me3) is rapidly enriched. To explore the role of H3K27me3 in PGC development we created a PGC specific conditional knockout of Embryonic Ectoderm Development (EED) in mouse PGCs at the time of induction, which leads to an inability to enrich and maintain H3K27me3 during PGC differentiation. Utilizing immunofluorescence imaging, FACS, and RNA sequencing, we show that H3K27me3 inherited from the epiblast are responsible for regulating somatic cell gene expression. In contrast, H3K27me3 acquired during PGC maturation regulate sex-specific timing of PGC differentiation. Taken together, our data indicate that H3K27me3 plays a complex role in regulating germline cell fate and state in mammals. This work has important consequences for not only elucidating the mechanisms that regulate reproductive health, but also towards developing a fundamental understanding of events that are critical to differentiate germ cells in vitro from stem cells.

F-3089

MITOCHONDRIAL DYNAMICS CONTROLS GERMLINE STEM CELL MAINTENANCE WITH AGE

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Aging is a process which is correlated with inability to maintain tissues' original function which further increase vulnerability of age-related disease. Asymmetric division of stem cells is crucial for life-long tissue homeostasis where one of the daughter cells receive stemness signaling from niche to maintain its stem cell identity; recent studies have shown that mitochondrial dynamics controls stem cell fate decision, suggesting that stem cell fate is coordinated with mitochondrial dynamics to function properly. However, the link between stemness signaling and mitochondrial dynamics in stem cell during aging is obscure. We have previously reported that decrease of maintenance and division in the germline stem cell (GSC) during aging. Here, by analyzing mitochondria labeled by ATP5ase, we found that mitochondrial content is decreased while the number of fragmented mitochondria is increased in aged GSCs. Consistently, disruption of mitochondrial fusion in the GSCs mimics aging phenotype which exhibits decreased division rate, maintenance and stemness signaling. Interestingly, forcing mitochondrial fusion does not cause decreased GSC division and maintenance; rather, it promotes competitiveness of GSCs for niche occupancy. Taken together, these results suggest that, whereas mitochondrial fusion may be serving as a protective mechanism for GSC persistency, mitochondrial fission is detrimental for GSCs during aging. Although the involved mechanisms by which mitochondrial dynamics controls stemness signaling still need to be further investigated, our study has documented the impact of mitochondrial dynamics change on stem cell maintenance and division with age in Drosophila germline.

F-3091

MODELING THE DEVELOPMENT OF NEURAL PLATE BORDER AND EPIDERMIS USING HUMAN PLURIPOTENT STEM CELLS

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Understanding the process of ectoderm development is essential for the prevention and treatment of various birth defects. While the early ectodermal development has been extensively studied using animal models, a model with human genetic background is needed to reveal the human-specific aspect of it. Our previous work has demonstrated that micropatterned culture of human pluripotent stem cells (hPSCs) lead to spatially ordered neuroectoderm and neural plate border differentiation. In this work, we report a fully-defined protocol to induce hPSCs to self-organize into concentric rings of neural plate border cells and epidermis with a clear boundary, mimicking fate patterning in human ectoderm. hPSCs were geometrically confined via micro-contact printing and cultured in E6 medium supplemented with a TGF-β inhibitor, a WNT agonist, and BMP4 protein for five days. We found that cells at the edge of the micropatterned colonies differentiated to trophectoderm (CDX2+ AP2+ SOX10- TP63-). Next to those cells was a ring of cells positive for neural crest markers (AP2+ SOX10+). Inward was another ring of cells positive for epidermis markers (AP2+ TP63+). The cells at the center of the colonies expressed trophectoderm markers. Further studies showed that cell fate patterns depended on the seeding density. Lower seeding density led to patterns without trophectoderm cells at the center while higher seeding density led to patterns without trophectoderm layer at the edge. We further showed that the self-organized ring-shape pattern formation of the neural plate border and epidermis required intermediate WNT and BMP activities. Adding exogenous BMP/WNT Agnoist was required for the formation of complete rings of epidermis and neural plate border, respectively. In addition, a higher dosage of BMP increased the width of trophectoderm and epidermis rings. In summary, by integrating engineering approaches and chemically defined culture conditions, we have developed a highly reproducible, high-throughput in vitro system that can be readily used to quantitatively study the functional role of morphogens and genes in the cell fate patterning of human ectoderm.

F-3093

DECODING PLURIPOTENCY AND TOTIPOTENCY BY NOVEL LONG INTERGENIC NON-CODING RNAS

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Totipotent cell, which is capable of developing into a complete organism, is expected as an important tool in regenerative medicine and disease modelling. Thus, it is foreseeable that in vitro cultured cell model of mammalian totipotent stem cells is of paramount interests to both biomedical and clinical research. Long intergenic non-coding RNAs (lincRNA), which are critical in cell stemness and cell fate, are expected as a novel important tool to induce cell reprogramming. However, to date, biological roles of lincRNA in totipotency has been rarely uncovered. By integrating extensive public RNA-seq datasets of mES, we have discovered ~5000 novel lincRNAs. In addition, from single cell transcriptome we identified a gene cluster comprising protein coding genes annotated lncRNAs as well as novel lincRNAs, which are highly active in mouse 2-cell embryo. In vivo experiments sugguest those genes express specifically in 2-cell mouse embryo. Besides, selected genes and novel lincRNAs can trigger mouse pluripotent stem cells to totipotent-like cells. Our result suggests a novel path to establish the mammalian totipotent stem cell lines in vitro, and eventually promote the totipotent-related stem cell therapy in biomedical and clinical research.

CHROMATIN AND EPIGENETICS

F-3095

ROLE OF CHROMATIN ORGANIZATION IN DOUBLE STRAND BREAK REPAIR IN MOUSE EMBRYONIC STEM CELLS

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The ability of pluripotent cells to differentiate into any embryonic or adult cell type raises requirements to maintain the high genome integrity level. Some stem cell-specific mechanisms to prevent accumulation of mutations and chromosomal aberrations have been discovered. However, study of DNA repair is complicated by the influence of local chromatin structure on its kinetics and the balance among repair pathways. Moreover, some chromatin types like facultative heterochromatin and bivalent chromatin have been less studied than the others in this context. Double strand breaks (DSBs) are the most dangerous DNA lesion type as the second strand is not available as a template for repair. Embryonic stem cells (ESCs) have been described to amend this kind of damage in a more error-free way than somatic cells. However, the underlying mechanisms are not clear yet. We aimed to assess them by following the repair outcome in ESCs in comparison with differentiated cells. To this end, we developed a mouse ESC line stably expressing Cas9 and designed guide RNAs to induce DSBs in different chromatin types: euchromatin, facultative heterochromatin and bivalent regions. We used 3T3 fibroblasts as a differentiated cell model for comparison. We assessed repair fidelity by TIDE, a sequencing-based method that render the readout of particular insertions and deletions as well as total proportion of mutated sequences. To evaluate the efficiency of homologous recombination (HR), we co-transfected guide RNAs and homologous templates with a small insert at the place of expected DNA break. Predictably we observed that ESCs perform higher rate of HR than the differentiated cells. However, to our surprise, this was the case in both active and inactive genes, implying that facultative heterochromatin is HR permissive. Additionally, we can also conclude that bivalent regions are repaired the same way as euchromatin. Interestingly, preliminary results from blocking cell cycle suggest

that shortened G1 phase in ESCs compare to differentiated cells might be one of the main reasons of the low rate of erroneous repair. To conclude, our work improves the understanding of the interplay between chromatin and DNA repair in ESCs and provides insights to potential mechanisms of genome integrity maintenance.

F-3097

THE EFFECT OF MATERNAL DIET AND NUTRITIONAL STATUS ON MAMMALIAN SEX DETERMINATION

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Mammalian sex is determined by the expression of Sry in the fetal gonadal somatic cells (pre-Sertoli cells). We previously found that epigenetic regulation plays an important role on Sry regulation. The H3K9 demethylase Jmjd1a directly targets Sry and removes H3K9 methylation mark from this locus to activate Sry. In XY Jmjd1a-deficient mice, male-to-female sex reversal was observed at certain frequency. The discovery of the fundamental role of epigenetic regulation of mammalian sex-determining gene Sry led to a new research area, namely, on the mechanistic link between environmental cues and sex determination. Embryonic development may be influenced by the maternal nutritional and metabolic state as fetal environment. Interestingly, we found that the maternal diet affected the frequency of sex-reversal of XY Jmjd1a-deficient mice. Since the maternal diet may directly affect the embryonic metabolism, we speculate that the metabolism and nutritional status of fetal pre-Sertoli cells influence their sex determination process. We first compared the gene expression profile between pre-Sertoli cells and the other gonadal somatic cells at the sex-determining period. Gene ontology analysis showed that metabolism-related process is clearly enriched in pre-Sertoli cells. To investigate the role of metabolic genes on sex determination that were highly expressed in pre-Sertoli cells, we generated knockout mice of these genes using CRISPR-Cas9 system. At this conference, we will report on the progress of phenotype analysis of several knockout lines. Some metabolites play as the substrates or co-factors for certain epigenetic enzymes. Therefore, it is considered that metabolites can regulate gene expression epigenetically. Our study lead to identification of novel pathway that links metabolites and mammalian sex determination.

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F-3099

FOLATE SUPPLEMENTS NEURAL TUBE DEFECTS BY TRANSCRIPTIONAL ACTIVATION OF NEURAL LINEAGE GENES VIA SUPPRESSING MDM2-MEDIATED H2A UBIQUITYLATION (MOUSE EMBRYO AND HUMAN EMBRYO)

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Folate has been widely used in clinics to prevent neural tube defects (NTDs) during the pregnancy. However, the mechanisms by which folate regulates the neural crest lineage cell differentiation is unknown. In this study, we studied methotrexate (MTX)-induced differential gene expression and histone modifications in mouse embryonic stem cells (mESCs), brain/spine tissues from mouse and human NTD fetuses by advanced biochemical and molecular approaches. We found that MTX suppressed embryonic cell differentiation and caused NTDs by repressing neural lineage gene expression in mESCs. Treatment of mESCs with MTX caused an increase in MDM2 expression and binding to lineage genes, leading to H2AK119 monoubiquitylation (H2AK119ub1) and transcriptional repression. Knockdown of MDM expression abolished MTX induction of H2AK119 ubiquitylation, and re-activate lineage gene expression. We also demonstrated that MTX treatment induced H2A ubiquitylation at DSB target sites by a mechanism dependent on ATM activation. Treatment of mESCs with Folate suppress MTX-induced MDM2 expression and H2AK119 ubiquitylation in the neural precursor markers genes, restored the transcriptional activation of these genes, and prevented NTDs in mice. In agreement with the data from mouse studies, we observed a dramatic down-regulation of neural precursor genes in fetal NTD brain tissue that was associated with low levels of folate in maternal sera, and enhanced levels of H2AK119ub1 in NTD tissue. Our studies indicate that folate prevents NTD by maintaining normal lineage cell differentiation via repressing MDM2 expression, reducing MDM2-mediated H2AK119ub1 and transactional repression of the neural precursor marker genes.

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F-3101

TARGETED GENE ACTIVATION DIRECTS TROPHOBLAST TRANS-DIFFERENTIATION USING THE NOVEL DESIGNED EPIGENETIC INHIBITOR, EEDBINDER-DCAS9

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Bifurcations in cell fates are controlled through epigenetic modifications, however the key loci regulated by PRC2 dependent H3K27me3 repressive marks are not known. To dissect the functional loci regulated by PRC2 in trophoblast to ICM bifurcation we fused a computationally designed protein, EED binder (EB) that tightly binds EED and disrupts PRC2 function, to dCas9 to direct PRC2 inhibition at precise loci using gRNA. Free of DNA manipulations or chemical inducers, EBdCas9 is able to transdifferentiate human induced pluripotent stem cells (iPSC) to human trophoblast fate using gRNA specific to two key transcription factors CDX2 and GATA3. Co-transfection of gRNA targeting GATA3 and CDX2 resulted in 40-80 fold increase of these transcripts, as well as the trophoblast markers. ChIPseq analysis showed H3K27me3 reduction in GATA3 TSS and gene body compared to untransfected samples. RNAseq analysis revealed that our EBdCas9 gRNA transfected, but not control hPSC produced gene expression signature that corresponds to human trophoectoderm and alignment against early cynomolgus monkey single cell transcriptome showed enhanced advancement towards trophectoderm. Epigenetic memory tracing of the newly trans-differentiated trophoectoderm cells confirms a transcript upregulation threshold that is maintained for at least 21 days following initial gRNA transfection. These data reveal for the first time that the first human fate bifurcation between trophoblast and ICM is solely controlled by PRC2 dependent epigenetic H3K27me3 marks in precise loci upstream of Gata3 and CDX2 TSSs. We also tested the other general applicability of EBdCas9, by using tiling to identify the regions where gRNAs induce transcription in the following bivalent genes: TBX18, p16, and Klf4. In total, we have targeted 40 sites upstream of five different genes, and observed significant transcriptional derepression in all genes, all together in 17 loci. EBdCas9 tool is broadly applicable to questions in epigenetic regulation of single locus to pinpoint critical marks for control of gene expression by PRC2.

PLURIPOTENCY

F-3105

CULTURE OF HIGH-QUALITY HUMAN PLURIPOTENT STEM CELLS WITH VERSATILE WORKFLOWS USING MTESR PLUS, A NEW STABILIZED TESR MAINTENANCE MEDIUM

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Specialized culture media is required to maintain the self-renewal and pluripotent properties of human pluripotent stem cells (hPSC). To date, the majority of culture systems require daily medium changes in order to replenish levels of critical components and eliminate accumulated metabolic waste. This is time-consuming when maintaining multiple cell lines, especially in hPSC core facilities, and typically requires operators to change medium over the weekend. mTeSR™ Plus, based on the mTeSR™1 formulation, was specifically developed to ensure truly versatile feeding schedules while maintaining high quality hPSC cultures. The stabilization of FGF2 levels over 72 hours at 37°C (83.6% ± 7.3%, n=3) combined with an enhanced buffering capacity that maintains pH ≥7.0 for up to 72 hours without feeding, supports flexibility for every other day or weekend-free schedules. We investigated key cell quality parameters of hPSCs cultured for ≥10 passages in mTeSR™ Plus with reduced feeding compared with cells in mTeSR™1 with daily feeding. hPSC marker expression was assessed by flow cytometry every 5 passages and hPSCs cultured in mTeSR™ Plus maintained an average of 98.2 ± 2.1% OCT4 and 93.5 ± 3.1% TRA-1-60 for up to 20 passages (n=4 cell lines). Likewise, hPSCs maintained in mTeSR™ Plus with reduced feeding were capable of directed differentiation to all three germ layers using the STEMdiff™ Trilineage Kit (n=4 cell lines). hPSCs maintained in mTeSR™ Plus (>10p) were karyotypically normal by G-banding and no common chromosomal abnormalities were detected by the hPSC Genetic Analysis Kit every 5 passages (n=4 cell lines). Transcriptome analysis of 19,665 genes by RNA sequencing of hPSCs maintained in mTeSR™ Plus resulted in a gene expression profile indistinguishable from cultures maintained in mTeSR™1 (n=2 cell lines). Furthermore, mTeSR™ Plus demonstrated 33.9 ± 1.4% gene knockout efficiency using the ArciTect™ CRISPR/Cas9 system (n=2) and 26.0 ± 4.4% cloning efficiency with CloneR™ supplement (n =3), which is similar to or better than hPSCs cultured in mTeSR™1. In summary, mTeSR™ Plus is an improved medium that promotes a more consistent cell culture environment enabling versatile workflows while maintaining high quality hPSCs that are fully compatible with established genome editing and differentiation protocols.

F-3107

DERIVATION OF PIG EMBRYONIC STEM CELLS USING CHEMICALLY-DEFINED MEDIA.

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Pig embryonic stem cells (ESCs) have been considered an important candidate for preclinical research on human therapies. However, the lack of understanding of pig pluripotent networks has hampered the establishment of authentic pig ESCs. Here, we report that FGF2, ACTVIN, and WNT signaling are essential to sustain pig pluripotency in vitro. Pig ESC lines derived by stimulating three signalings formed colonies with a flattened monolayer morphology. Newly derived ESCs were stably maintained over an extended period and were capable of forming teratomas that contained three germ layers. Furthermore, the pig stem cells have the ability of direct differentiation into specific cells including mature neuron, pancreas progenitor and cardiac muscle cells. Immunostaining showed that the stem cells expressed pluripotency markers including OCT4, SOX2, NANOG, SSEA1, and SSEA4. Interestingly, the pig ESCs had distinct features such as coexpression of SSEA1 and SSEA4, two active X chromosomes, and a unique transcriptional pattern. In conclusion, we derived authentic pig ESCs using novel cell culture conditions. Our findings will facilitate both the development of large animal models for human stem cell therapy and the generation of pluripotent stem cells from other domestic animals for agricultural use.

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F-3109

A NOVEL GPCR FAMILY GENE HECAT5 MODULATES CELL CYCLE PROGRESSION

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Embryonic stem (ES) cells are originated from the pluripotent inner cell mass of the blastocyst stage. They have two important properties: self-renewal and pluripotency. For these reasons, ES cells can be used in potential therapeutic applications in regenerative medicine. But, there are ethical problems and adverse effects regarding the use of ES cells. To investigate the mechanism regulating stemness, especially cell cycle, of ES cells, we identified HECAT5, the gene co-expressed in both ES and tumors, but not normal tissues, using Digital Differential Display (DDD). Overexpression of HECAT5 dramatically increased S phase population and cell proliferation in vitro in hNSC and HEK293, but not NIH3T3 cells. In addition, it enhanced tumorigenicity and growth in soft agar assay and in xenograft tumor assay. HECAT5 had effects on those through interaction with M3R because M3R-specific antagonists specifically inhibit cell proliferation induced by HECAT5 overexpression. All our results demosntrated that novel HECAT5 gene may play a role in the regulation of stemness in hES cells.

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F-3111

EIF4A1-MEDIATED SPECIFIC PROTEIN TRANSLATION IN HUMAN EMBRYONIC STEM CELL

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Protein translation is critical for numerous cellular functions such as transcription, metabolism, stress response and cellular proliferation. However, it is unclear how translation is regulated in human embryonic stem cells(hESC), and how it affects specific cellular functions. In this report, we show that eIF4A1-dependent translation initiation plays critical roles in hESC maintenance. EIF4A1 is essential for Cap-dependent translation, and its inhibition by small chemical silverstrol leads to severe cell type specific cell death in hESC, but not in other somatic cells. Genome-wide ribosome profiling analysis reveals that silvestrol treatment specifically suppresses pathways involved in TGFβ signaling, MAPK signaling, apoptosis and so on. Further study shows that silvestrol specifically suppressed NANOG and CDH1; but not OCT4 and SOX2. These data show eIF4A1-mediated translation differentially control pluripotency related proteins, and influence stem cell survival and cell fate determination.

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F-3113

HLA DR GENOME EDITING WITH TALENS IN HUMAN IPSCS PRODUCED IMMUNE-TOLERANT DENDRITIC CELLS

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Although human induced pluripotent stem cells (iPSCs) can serve as a universal cell source for regenerative medicine, the use of iPSCs in clinical applications is limited by high costs and long time required for generation. Moreover, allogeneic iPSC transplantation requires preclusion of mismatches between the donor and recipient human leukocyte antigen (HLA). We, therefore, generated universally compatible immune non-responsive human iPSCs by gene editing. Transcription activator-like effector nucleases (TALENs) were designed for selective elimination of HLA DR expression. The engineered nucleases completely disrupted the expression of HLA DR on human dermal fibroblast cells (HDF) that did not express HLA DR even after stimulation with IFN-γ. Teratomas formed by HLA DR knockout iPSCs did not express HLA DR, and dendritic cells differentiated from HLA DR knockout iPSCs reduced CD4+ T cell activation. These engineered iPSCs might provide a novel translational approach to treat multiple recipients from a limited number of cell donors.

F-3115

THE IMPACT OF DOUBLE X-DOSAGE ON SIGNALING PATHWAYS IMPLICATED IN PLURIPOTENCY

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For a short time window during early development of mammalian embryos, both X chromosomes in females are active, before dosage-compensation is ensured through X-chromosome inactivation (XCI) during the exit from the pluripotent ground state. The double dose of X-linked genes compared to male cells, in female mouse embryonic stem cells (mESC) that still have both X chromosome active, has been found to increase expression of pluripotency factors, attenuate differentiation and decrease global DNA methylation. To identify X-dosage dependent effects on the signaling network in we combine systematic perturbation experiments with mathematical modeling. mESCs with either one (XO) or two active X chromosomes (XX) were treated with exogenous stimulants or inhibitors targeting 5 different pathways (Fgf/Mapk, Lif/Stat3, Pi3k/Akt, Bmp4/Smad1, Activin/Smad2), either individually or in combinations (53 treatments per cell line). Signaling intermediates were measured using a bead-based multiplexed phosphorylation assay and western blotting. We then apply a semi-quantitative modeling approach based on Modular Response Analysis to this perturbation data set to reconstruct the signaling networks in XX and XO mESCs. Specifically, a literature-derived starting network comprised of only the canonical linear cascades is optimized, by adding or removing links, to best fit the perturbation data. In this way several previously reported interactions were identified, such as a negative feedback loop in the Mapk pathway and cross talk from Lif pathway towards Mapk and Akt activation. Additionally, not yet described cross talk between the Bmp4, Activin and Mapk pathways was identified, which is currently being validated in experiments. Through comparison of the networks reconstructed for XX and XO cells we can now identify X-dosage dependent differences in their signaling networks. We find that cells having a single active X are more sensitive to the differentiation promoting Activin stimulation, than cells with two active X chromosomes. In summary, a data-driven systems biology approach allows us to identify novel cross-talk between different signaling pathways and to narrow down the putative point(s) in the network via which a double dose of active X-chromosomes might mediate its global effect on the pluripotent state.

PLURIPOTENT STEM CELL DIFFERENTIATION

F-3119

3D MICRO-CULTURE PLATFORM ENABLES ADVANCED, HIGH-THROUGHPUT SCREENING FOR DIFFERENTIATION OF HUMAN PSC-DERIVED CELL THERAPIES

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The promising outlook for human Pluripotent Stem Cell (hPSC) derived cell therapies motivates the development of manufacturing processes to meet the patient demand for such therapeutics. Toward this aim, 3D culture systems for hPSC differentiation are emerging because of their potential for higher expansion and yield of target cell types compared to 2D culture systems. Therefore, the ability to screen through a multifactorial parameter space of exogenous biochemical cues for 3D hPSC cultures would greatly accelerate the pace of discovery and development of efficient in vitro differentiation protocols for target cell types of interest. Here, we demonstrate the advanced capabilities of a 3D micro-culture platform to screen dosage, duration, dynamics, and combinations of 12 culture parameters, totaling more than 1000 unique 3D culture environments, to derive Olig2+Nkx2.2+ oligodendrocyte progenitor cells (OPCs) from hPSCs with 0.2% of the reagent volumes used in 96-well plates. Additionally, we leverage novel fluorescent hPSC reporter cell lines, engineered using a Cas9 knock-in strategy, to monitor proliferation and differentiation in situ for over 80 days in the 3D micro-culture system. We identified several early culture parameters that could be tuned to increase the Olig2 expression 10-fold. In addition, we observed different sensitivities to signaling pathways across time. Finally, a holistic analysis using statistical models uncovered and ranked parameters and combinations thereof according to their positive effect on OPC differentiation to prioritize them in future optimizations. To show the generalizability of the platform, we then applied it to simultaneously assay 90 unique differentiation protocols to derive TH+ midbrain dopaminergic neurons from hPSCs. Overall, we demonstrate a strong methodology for upstream microscale screening/optimization to inform downstream scale-up processes to improve 3D production strategies of hPSC-derived cell replacement therapies.

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F-3121

DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TOWARDS MESENCHYMAL STROMAL CELLS IS TRIGGERED BY FLAT SUBSTRATES

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Differentiation of induced pluripotent stem cells (iPSCs) towards mesenchymal stromal cells (MSCs) remains a major challenge in regenerative medicine. On conventional tissue culture plastic, this differentiation process is incomplete, at least on epigenetic level. However, there is evidence, that differentiation of iPSCs can be guided by mechanical cues and therefore, we investigated if this process can be triggered by differentiation in a 3D matrix. iPSCs were embedded into fibrin hydrogels to enable a one-step differentiation procedure towards MSCs within a scaffold. Differentiation of iPSCs on tissue culture plastic or on top of fibrin hydrogels resulted in a typical MSC-like morphology and immunophenotype. In contrast, iPSCs embedded into fibrin gel gave rise to much smaller cells with heterogeneous growth patterns, absence of fibronectin, and faint expression of CD73 and CD105. Global gene expression profiles of differentiated iPSCs demonstrated that MSC-specific genes were only up-regulated on flat substrates, whereas genes of neural development were up-regulated in 3D culture. Furthermore, global DNA methylation profiles were very similar if iPSCs were differentiated towards MSCs either on tissue culture plastic or on top of hydrogels. In contrast, there were marked epigenetic differences if iPSCs were differentiated within fibrin gels, pointing towards neural differentiation. Taken together, the 3D culture conditions within fibrin hydrogel supported growth and differentiation of iPSCs, but they hampered differentiation towards MSCs. These results indicate that the typical fibroblastoid and adhesive growth pattern on flat substrates might not only be a cellular characteristic of MSCs, but it might also trigger differentiation towards this cell type.

F-3123

TRANSPLANTATION OF IPSC-DERIVED RETINAL PIGMENT EPITHELIAL (RPE) CELLS RESCUE OF PHOTORECEPTOR CELL DEATH IN RETINAL DEGENERATIVE RCS RATS

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The physiological basis of vision is associated with the interaction between retinal pigment epithelium (RPE) and photoreceptor cells. Thus, RPE transplantation is extensively used as cell-based therapy to restore the subretinal anatomy in age-

related macular degeneration (AMD). Here, we aim to use the suspension of hiPSC-derived RPE cells to inject into subretinal space of RCS rats to study the biological behavior in the host retina. For this experiment, RPE cells were generated from human epidermal keratinocytes using non-integrating CytoTune 2.0 Sendai reprogramming kit. The primary hiPSC colonies were observed in 12 days that showed the expression of pluripotency markers like Oct4, Sox2, Nanog, Tra1-60 and SSEA4 analyzed by immunohistochemistry and reverse transcription-PCR. Then, the neuroectodermal differentiation of RPE was performed by monolayer culture embryoid bodies in vitronectin coated plates containing retinal differentiation medium supplemented with B27. RPE cells were characterized using immunostaining, Western blot, and PCR. The differentiated RPE showed characteristic flat hexagonal morphology that expressed markers for tight junctions (ZO1), and RPE cells (MITF, CRALBP, Bestrophin, and RPE65). After eight weeks of differentiation, the pigmentation was observed in RPE cells analyzed using a transmission electron microscope (TEM). In a long-term culture, the formation of RPE fluid-filled sac was observed and a layer of sac expressed marker, such as Bestrophin. The phagocytic activity of RPE was evident as phagocytose of latex beads. Furthermore, suspension of GFP labeled hiPSC-RPE cells were injected in the subretinal spaces of 7 RCS rats. In vivo study of transplantation showed the improvement of visual function apparent by electroretinogram (ERG) analysis. This indicates the rescue of photoreceptor loss in retinal degenerative rats, but validation with histological assessments are required to confirm the improvement. To conclude, hiPSC was generated from keratinocytes were differentiated into functional RPE cells. Also, the implantation of hiPSC-RPE cells showed transient improvement of visual function in RCS rats.

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F-3125

IMPROVED HEPATIC MATURATION OF CGMP-COMPLIANT HUMAN PLURIPOTENT STEM CELLS USING BIOMIMETIC PEG SUBSTRATES OF PHYSIOLOGICAL STIFFNESS

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cGMP-compliant human pluripotent stem cells (hPSCs), which are suitable for cell therapy, can be successfully differentiated into hepatocytes (hPSC-Heps); however, hPSC-Heps often fail to reproduce the transcriptomic and functional profile of freshly isolated hepatocytes. During development, hepatic cell fate is impacted by the local microenvironment. However, the hepatic 3D milieu is complex and the biological and physical factors that drive hepatic maturation remain unidentified. Using 2D and 3D biomaterial culture platforms, extracellular cues, such as stiffness and adhesive ligand concentration can be dissected and their impact on hepatic maturation studied. hPSC-Heps were differentiated using standard chemical cocktails on a modifiable 4-armed polyethylene glycol (PEG) hydrogel system conjugated with RGD sequence-containing peptides. Hydrogels with two different solid content concentrations (2.5% and 10%) with Young's moduli mimicking healthy adult (1kPa) and fibrotic liver (7kPa) were examined. Hepatic phenotype was measured by ELISA (albumin secretion), luciferin-IPA luminescence (CYP3A4 activity) and qPCR (gene expression). When comparing hPSC-Heps cultured for 3 weeks on soft 1kPa PEG substrates to those on 7kPa substrates, significantly greater cytochrome p450 CYP3A4 enzyme activity, albumin secretion, and expression of key hepatic genes were measured. Whereas, for those on 7kPa PEG, expression of genes associated with liver fibrosis (TGFB, IHH/SHH, VEGF, PDGFB) were elevated, along with the expression of Yes-associated protein (YAP) and WWTR1 (TAZ) target genes. Notably, the mRNA levels for RGD binding integrin subunits ITGB1 and ITGB3 were significantly greater in hPSC-Heps matured on 7kPa rather than 1kPa substrates, despite ligand concentration remaining constant. Moreover, when encapsulated within 1kPa PEG, further phenotypic improvement to hPSC-Heps (increased albumin secretion) was measured compared to 2D cultures. This data implies that stem cell culture platforms can be optimised through tuning of their biomechanical properties. Understanding the interplay between mechanical and biochemical factors in cell fate determination will help the development of patient-specific research tools, as well as provide hPSC-Heps better suited for clinical therapies.

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F-3127

RELIABILITY OF HUMAN CORTICAL ORGANOID GENERATION

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The differentiation of pluripotent stem cells in three-dimensional (3D) neural cultures can recapitulate key aspects of brain development in vitro, but protocols are prone to variable results. Here, we differentiated multiple human pluripotent stem cell (hPSC) lines for over 100 days to generate brain-region specific organoids called human cortical spheroids (hCS), which are 3D floating cultures that resemble the cerebral cortex. Human induced pluripotent stem cells (hiPSC) were cultured in feeder-free and xeno-free conditions on human recombinant vitronectin, aggregated in AggreWell-800 plates to obtain uniform 3D spheroids, and moved to ultra-low attachments plates for neural differentiation. We differentiated multiple hiPSC lines for 4 to 11 times each in 100 day-long experiments. As we have previously shown, hCS showed an internal cytoarchitecture that included proliferative zones and contained neurons resembling deep and superficial cortical layers as well as non-reactive astrocytes. Neurons were electrophysiologically mature and displayed synaptic activity. Single-cell RNA-seq at day 105 showed a similar distribution of the cell types across multiple lines, and bulk transcriptional profiling showed high consistency across hiPSC lines and experiments. We anticipate that this reliable and scalable brain-region specific organoid can be used for large scale differentiation experiments and disease modeling.

F-3129

BIOCHEMICAL AND PHYSICAL CUES COMBINE TO AUGMENT IPSC-DERIVED SKELETAL MUSCLE DIFFERENTIATION AND MATURATION FOR IMPROVED DISEASE MODELING

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Inherited myopathies encompass a wide range of debilitating diseases and affect millions of people worldwide. These diseases can impact mobility, strength, speech, and respiration and are ultimately fatal in many cases. Human induced pluripotent stem cells (hiPSCs) present a feasible and exciting model to study inherited myopathies and their potential remedies in vitro. This model would allow for comprehensive drug testing on diseased cells derived from affected individuals, thereby eliminating the need for invasive, time-consuming, and often costly therapeutic trials in vivo. Attempts to recapitulate myogenesis in vitro using hiPSCs, however, have been challenging and often result in heterogeneous cell cultures, immature neonatal myofibers, and disorganized fiber arrangements with asynchronous myotube contractions. This could limit the usefulness of these cells for modeling mature or late onset disease phenotypes. Here, we report biochemical and physical cues that augment myogenic differentiation and maturation. Surface patterning and nanoscale topographical cues have been shown to control cell alignment. hiPSC-derived myoblasts cultured on nanopatterned plates with ridges and grooves align and fuse to form myotubes that are structurally ordered, creating anisotropic muscular tissue in both normal and diseased cell types. Furthermore, addition of Dexamethasone, a synthetic glucocorticoid that stimulates myoblast differentiation and fusion into myotubes, promotes enhanced sarcomeric assembly and increased myotube width compared with untreated controls. The cytokine interleukin-4, which acts as a myoblast recruitment factor, promotes myoblast fusion resulting in longer, multinucleated myotubes. Our combinatorial differentiation strategy produces robust skeletal muscle myofibers which may display more clinically relevant phenotypes as muscle matures in vitro. Single cell RNA-sequencing shows that normal iPS cells and cells derived from a patient with Duchenne muscular dystrophy diverge in their developmental trajectories soon after dystrophin is expected to be expressed during myogenesis. This validates that our differentiation protocol yields myogenic cells that manifest disease phenotypes which can be used for more accurate disease modeling and drug discovery.

F-3131

MICROENVIRONMENT OPTIMISATION FOR THE DIFFERENTIATION OF INNER EAR ORGANOIDS FROM MOUSE EMBRYONIC STEM CELLS

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Hearing loss is one of the most common forms of sensory impairment in humans, affecting > 5% of the world's population. To date, our understanding of the mechanisms of hearing loss and the generation of therapies have been hampered by the lack of a human in vitro model. Previous work showed that it is

possible to generate inner ear organoids containing functional hair cells from mouse (mPSCs) or human pluripotent stem cells (hPSCs) through a three-dimensional (3D) culture system; however, their translation into an in vitro model for drug screening or developmental modelling is limited by low differentiation yield, lack of reproducibility and standardisation of the differentiation protocol. This study aims to generate an engineered stem cell niche in which pluripotent stem cells could be cultured and differentiated into inner ear organoids and ultimately combined with microfluidic systems for translational research. We obtained inner ear organoids containing hair cells, supporting cells and neurons with a mouse embryonic stem cell (mESC) Atoh1/nGFP line (a gift from Stefan Heller). We then examined how micro-environment and size control may impact mESC otic differentiation, varying culture chamber size (96-well plate v AggreWellTM) and extracellular matrix (ECM). Results show that microwells allow the control of organoid size and also increase expression of Atoh1/nGFP. However, we did not observe the morphological and phenotypical changes expected during otic differentiation. Preliminary data on organoids embedded in ECM revealed that they are able to grow and undergo the morphological changes observed during the first seven days of the standard differentiation protocol. Our work provides a step forward in the development of a stem cell niche to sustain PSCs in vitro and regulate differentiation into inner ear organoids. The translation of this niche in a microfluidic device would, for example, allow the generation of human Inner-Ear-On-A-Chip for personalised therapies for hearing loss and also for drug screening. The model will be important for understanding the mechanisms underlying inner ear development and hearing loss.

F-3133

TRANSCRIPTOME ANALYSIS OF HUMAN SOMATIC CELL NUCLEAR TRANSFER-EMBRYONIC STEM CELL DERIVED RETINAL PIGMENT EPITHELIAL CELLS

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Dysfunction of retinal pigment epithelial cells (RPE) in retina causes serious visual impairments and retinal degenerative disease such as age-related macular degeneration (AMD) and retinitis pigmentosa. The defects in RPE function can affect the integrity and viability of photoreceptors, so RPE become the main target for treating many retinal degenerative diseases. One of the most promising treatments for RPE related disorders is cell replacement of the dysfunctional RPE. Because of proliferation and differentiation abilities of human embryonic stem cells (hES), hES derived RPE is proposed as a potentially suitable resource of cell replacement therapy for AMD. Recently, we produced RPE lines using somatic cell nuclear transfer(SCNT)-hES derived from a normal healthy donor (CHA-hES NT4) and AMD patients (CHA-hES NT5 and NT8). Then, we have analyzed the characteristics and functional efficacy of the RPE lines compared to hES derived RPE (MA09-RPE), which have been used in clinical trials, for their future applications. In the present study, we conducted the microarray-based analysis comparing SCNT-hES derived RPE lines with MA09-RPE. All of the SCNT-hES derived RPE lines showed high similarity to MA09-RPE in typical RPE characteristics; pigmented polygonal shape, expression of RPE-related markers, epithelial polarization and phagocytosis activity. The pattern of gene expression related in cell proliferation, migration, and immune response was very similar among all RPE lines. Regarding the expression of genes related to the functions of retinal cells, most of them were similar among all RPE lines, but there was a difference in the expression of several genes between the patient group (CHA-hES NT5-RPE and NT8-RPE) and the normal group (CHA-hES NT4-RPE and MA09-RPE). Forward, it should be studied whether the difference in some gene expression affects the actual RPE function. Since there is no difference between healthy donor derived SCNT-hES-RPE and MA09-RPE, HLA-matched allogenic SCNT-hES derived RPE might be one of the effective solutions for the treatment of retinal disease.

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F-3135

OFF THE SHELF BLOOD PRODUCTS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Manufacture of red blood cells (RBC) as an “off the shelf” cell therapy is an extremely challenging goal, as a packed unit of blood has 10e12 cells in 500mls. A renewable source of cells such as human pluripotent stem cells must be identified and the process optimized over 4 stages to generate this dose of RBC. We began this journey by optimizing the first stage of hemangioblast differentiation by creating a cell microcarrier aggregate differentiation process that increased the yields 60 fold over an embryoid body method. Next optimizing the Wnt activation pathway with CHR99021 and BMP4 resulted in a further 5 fold improvement in yields of haemopoietic precursors at the second stage of differentiation. Thereafter at the third step, erythroblasts (CD 235a+) were expanded another 1000 fold from these precursors. Six human pluripotent stem cell lines successfully differentiated to erythroblasts following this protocol. Cumulatively, one of the lines X13 achieved 12,605

fold expansion reaching a cell density of 15x10e6 cells/ml. Enucleation efficiency was effected with OP9 co-cultures to 79% and oxygen carrying capacity was validated for all 6 cell lines. Finally, this 4 stage bioprocess was reproduced in 125ml bioreactors in a fully integrated suspension mode as proof of concept for small scale manufacturing. Each step of improvement has led to a 4 stage process which can be intensified in a bioreactor that can concentrate erythroblast cell production by a factor of 5 times to 10e8 cells/ml which will bring the cost of manufacturing to the $1000s/unit of blood. Further optimization through media replacement of growth factors with alternatives, combined with process engineering will bring the RBC production costs to even lower levels.

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F-3137

INDUCING HALLMARKS OF INTERNEURON MATURATION

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Human pluripotent stem cells (hPSCs) provide the unique opportunity to derive all somatic cell types from an unlimited source in a patient specific manner. Since the derivation of hPSCs, many somatic cell types have been successfully generated, including neurons, which have been shown to represent cells of the central nervous system (CNS). A huge step forward in better understanding how the CNS develops, and consequently, how diseases arise in the brain, the neurons that are generated from hPSCs in vitro nevertheless do not fully resemble the mature neurons that are present in the human brain. This therefore limits the use of these hPSC-derived neurons for applications such as drug discovery and disease modelling for adult diseases, including Alzheimer's, Autism, Epilepsy, amongst others. By using high-throughput single cell techniques, we have interrogated the gene expression patterns of hPSC-derived, fetal and adult interneurons and cataloged key differences between their development. We have identified key genes that are absent in hPSC-derived interneurons and by over-expressing these genes we have been able to push these cells to a more adult, mature like state. We hope this work will offer a platform for more effective treatments of CNS diseases.

F-3139

DISTINCT REGULATORY MECHANISMS OF TUMOR SUPRESSOR PROTEIN P16/INK4A IN SELF-RENEWING HUMAN PLURIPOTENT AND NEURAL STEM CELLS

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Human pluripotent stem cells (hPSCs) have the ability to differentiate and to unlimitedly self-renew. This is partially ensured by rapid cell division and specific cell cycle regulatory mechanisms. Importantly, length of G1 phase, and activity of specific cell cycle regulators determine the cell fate decision and differentiation. Here we aimed to study the cell cycle inhibitor p16/INK4A (p16), an important regulator of G1 phase transition that has been associated with cellular senescence and is frequently deregulated in human glioblastoma. Initially, we noticed that expression of p16 in hPSCs is low in early passages (<40) and increases with high number of passages (>60). Surprisingly, the elevated level of p16 in cells of high passage does not affect their proliferation. We analysed the cellular localisation of p16 in hPSCs expressing p16 and found that, in addition to nucleus, p16 also localizes to cytoplasm, a phenotype previously found in numerous tumours.. We further studied possible regulations of p16 expression in hPSCs and in hPSC-derived neural stem cells (NSCs) and our functional experiments suggest that regulations of p16 change upon differentiation of hPSCs into NSCs. While p16 protein level increased in hPSCs after inhibition of miRNA biogenesis, suggesting regulation by miRNA, in NSCs p16 level is modulated by proteasomal degradation. Finally, we also investigated the relationship between p16 and its known regulators Ets1, p53, and Bmi1. In undifferentiated hPSCs, our results point to an interesting regulatory loop between p16 and p53. Ets1, although elevated in high passages of hPSCs does not seem to directly regulate p16. Upon differentiation, in NSCs, the expression of p16 is partly inhibited by polycomb protein Bmi1, oncogene necessary for self-renewal. Altogether, our results reveal novel expression patterns of protein p16 during early human development in vitro and point to several molecular pathways by which its expression is being regulated.

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F-3141

THE LONG NON-CODING RNA SNHG16 REGULATES NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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The human genome encodes many thousands of non-coding RNAs, but little is known about lncRNAs regulating neurogenesis. Here we identified SNHG16 as a lncRNA whose stable expression in human embryonic stem cells and derived neural stem cells (NSCs) decreases along with neural differentiation. Silencing of SNHG16 potentiates neuronal differentiation and depletes the NSCs population, whereas SNHG16 overexpression induces NSCs self-renewal. We further explored the underlying mechanism of SNHG16 in neurogenesis and found that SNHG16 directly binds miR-124 and inhibits its action in neural differentiation. In addition, SNHG16 reversed the effect of miR-124 by targeting the transcription factor SOX8. Our data highlight the pivotal role of SNHG16 as a neurogenic lncRNA that acts as a microRNA sponge and transcription factor regulator in human NSCs.

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F-3143

THE EFFECTS OF METFORMIN ON HUMAN CORTICAL DEVELOPMENT

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Metformin is widely used as an antidiabetic drug to treat patients who have been diagnosed with type 2 diabetes mellitus. Although it is known to cross the placenta, it is one of only two oral medications that can be prescribed for pregnant diabetic women. To date, the effects of metformin on the development of human neurons, particularly cortical neurons in the fetus, have not been evaluated in any study. Human pluripotent stem cells (hPSCs) are considered to be invaluable in the modulation of normal development and drug testing in vitro. Thus, the effects of metformin on cortical neurons derived from hPSCs were evaluated in the current study. Metformin has been added to cortical differentiation cultures established from hPSCs to examine whether metformin has an effect on this early developmental process. The expression of several genes and transcription factors were evaluated at different times points throughout the differentiation. Metformin was shown to delay the differentiation of PAX6-positive early cortical progenitors into TBR2-positive intermediate progenitors ($p = < 0.050$). During maturation, a reduction in the number of CTIP2-positive neurons, which normally reside in the deep layers of the cerebral cortex, was evident in the metformin-treated differentiation culture compared to the control differentiation culture ($p = < 0.050$). By contrast, a difference was not observed in the number of TBR1-positive neurons identified in the two differentiation cultures. The delay in differentiation in the metformin-treated culture was found to be due to the activation of the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway on further analysis. By inhibiting the AMPK signaling pathway in the metformin-treated cultures, differentiation of the human cortical neurons therein was similar to that in the control group. To the best of our knowledge, this is the first study to have demonstrated the effect of metformin on human cortical development in vitro. Further studies are warranted to examine the effects of metformin on the morphogenesis and synaptogenesis of cortical neurons.

F-3145

THE ROLE OF SOX1 IN DETERMINING REGIONAL IDENTITY OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL PROGENITOR CELLS

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During human embryogenesis, primitive neural cells start to be generated at the time of gastrulation and gradually acquire regional identities, which is a process called neural patterning and is precisely controlled. However, how intrinsic factors of the primitive neural cells respond to exogenous patterning signals remains poorly understood. Human embryonic stem cells (hESCs) provide a useful model to recapitulate this process. Through exogenous manipulation of canonical Wnt signaling during neural differentiation of hESCs, dose-dependent specification

of regionally defined neural progenitor cells (NPCs) ranging from the telencephalon to spinal cord could be rapidly and efficiently induced. Unexpectedly, we find that SOX1, generally referred as a pan-neural gene, displays a regional specific distribution in the neural patterning process. To investigate the expression and function of SOX1 efficiently, we generate the SOX1-EGFP reporter and SOX1-knockout (KO) hESCs lines using the CRISPR/Cas9 system. We find that SOX1 expression peaks in the anterior hindbrain NPCs at the early stage. Its depletion leads to the up-regulation of midbrain markers and down-regulation of anterior hindbrain markers. Our in-depth analysis of SOX1 ChIP-sequencing and transcriptomic data indicate that SOX1 may bind to the distal enhancer of GBX2 and activate its expression. Taken together, this study identifies SOX1 as one of the intrinsic factors key for the patterning establishment in NPCs, particularly for defining the midbrain and hindbrain identity.

F-3147

DIFFERENTIATION AND FUNCTIONAL COMPARISON OF MONOCYTES AND MACROPHAGES FROM HIPSCS WITH PERIPHERAL BLOOD DERIVATIVES

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A renewable source of human monocytes and macrophages would be a valuable alternative to primary cells from peripheral blood (PB) in many biomedical applications. Here we developed a fully defined, efficient protocol to derive monocytes and macrophages subtypes from human induced pluripotent stem cells (hiPSCs). They were phenotypically and functionally similar to PB-derived cells but also showed important differences. First, hiPSC-derived monocytes were functional after cryopreservation and exhibited comparable gene expression profiles as PB-derived monocytes. Notably, hiPSC-derived monocytes were more activated and showed greater adhesion to endothelial cells under physiological flow. Second, hiPSC-derived monocytes could be polarized to pro- or anti-inflammatory macrophage subtypes. hiPSC-derived macrophages showed similar pan- and subtype-specific gene and surface protein expression and cytokine secretion to PB-derived macrophages. Transcriptomic analysis of hiPSC-derived macrophages indicated a clear subtype specific signature and functional pathways expressed in these cells. Third, functionally, hiPSC-derived macrophages exhibited similar bacterial phagocytosis but higher AcLDL endocytosis and efferocytosis (phagocytic clearance of dead cells) compared to PB-derived macrophages. Last, hiPSC-derived macrophages showed similar ability to phagocytose tumour cells as PB-derived macrophages, indicating their potential value in cancer immunotherapy. In summary, we developed a robust protocol to generate hiPSC-monocytes and macrophages from independent hiPSC lines that showed aspects of functional maturity comparable with those from PB.

F-3149

EFFECTS OF COMPOUND A IN THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO MATURE CARDIOMYOCYTES

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Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) can be used for a wide range of applications such as cardiac drug toxicity screening, modeling and therapy of cardiac diseases. However, increasing evidence demonstrates that currently available hESC-CMs represent immature embryonic or fetal stage and structurally and functionally different from mature human cardiomyocytes. Therefore, the application of hESC-CMs is largely limited by their immature phenotypes. In order to overcome the immature problem of hESC-CMs, it is needed to stimulate maturation of hESC-CMs. In this study, for the first time, we identified compound A as a novel factor stimulating maturation of hESC-CMs. Treatment of the hESC with compound A during cardiomyocyte differentiation stimulated expression of several cardiomyocyte-specific markers, including cTnT, α-sarcomeric actinin, and myosin light chain isoforms. Moreover, Compound A treatment increased the density of T-tubule and the expression of T-tubule-associated proteins, BIN1 and JPH2. In addition, the contents of mitochondria in hESC-CMs was also increased after treatment with compound A. Consistently, Compound A-treated hESC-CMs exhibited increased mitochondrial membrane potential compared with the immature hESC-CMs. Compound A not only improved the activity of mitochondrial activity, but also made the whole structure of mitochondrial and cristae elongate and clear. This is also functionally increased in mitochondrial oxygen consumption measurements, such as maximum respiratory capacity and ATP production. However, by using a patch clamp analysis, we observed that the activity and expression of cardiomyocyte-specific ion channels were not change in compound A-treated hESC-CMs compared with the control cells. These results suggest that compound A promotes maturation of hESC-CMs through mitochondrial maturation and healthy condition, which can be used for cardiac toxicity screening and cell therapy.

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F-3151

NON-DISRUPTIVE EVALUATION OF DIFFERENTIATION EFFICIENCY FROM HUMAN PLURIPOTENT STEM CELLS TO DIFFERENTIATED CELLS BY MONITORING THE AMOUNT OF MIRNAS IN CULTURE SUPERNATANTS

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In recent years, several types of therapeutic products derived from human iPS cells (hiPSCs) are being developed for use in regenerative medicine. One of the important problems is that differentiation efficiency is unstable in order to use these products clinically. Therefore, to realize regenerative medicine using hiPSCs in clinical practice, quality control of differentiated cells and differentiation processes are necessary. Although various methods for evaluating differentiated cells and differentiation processes have been used, most of them are disruptive methods that involve irreversibly lysing or dying a portion of the hiPSCs-derived products. In this study, we report, in order to monitor differentiation processes and evaluate hiPSCs-derived products non-disruptively and high-sensitively, identification of specific miRNAs for hiPSCs or differentiated cells in the culture supernatants and evaluation of variations in the amount of their miRNAs during differentiation. First, the culture supernatants were collected for each cell 1 day after medium change to obtain culture supernatants of hiPSCs and cardiomyocytes as differentiated cells, and cell debris were removed by centrifuging the culture supernatant at 1,500 g for 10 minutes. Thereafter, RNAs were purified from 50 µl of the culture supernatant, and miRNAs copy numbers were mesured by RT-PCR method. As a result, we were able to find specific miRNAs for hiPSCs or cardiomyocytes in the culture supernatants and verify that copy numbers of specific miRNAs for hiPSCs decreased, while copy numbers of specific miRNAs for cardiomyocytes increased, as the differentiation progresses. In addition, this method could estimate differentiation efficiency non-disruptively at an early stage of differentiation. Our method has high potential to contribute the clinical applications of hiPSCs, especially in terms of monitoring the differentiation processes and quality assessment of hiPSCs-derived products.

F-3153

A NEW CULTURE MEDIUM THAT REDUCES PHOTOTOXICITY AND AUTOFLUORESCENCE WHILE SUPPORTING ACTIVITY IN LONG-TERM PRIMARY TISSUE- AND HPSC-DERIVED NEURONS

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The development of imaging technologies coupled with fluorescent sensors has dramatically increased the number of live-cell imaging applications. However, autofluorescence of culture media reduces signal-to-noise and repeated light exposure can result in accumulation of toxic byproducts. We have developed a new medium that overcomes these issues, while maintaining functional neuronal activity, called BrainPhys™ Imaging Optimized Medium (BrainPhys™ IO) based on the original BrainPhys™ formulation (Bardy et al. 2015). For experiments using primary tissues, E18 rat cortices were dissociated into single cell suspensions and plated in NeuroCult™ Neuronal Plating Medium supplemented with NeuroCult™ SM1 Neuronal Supplement (SM1). After 5 days, cultures were transitioned to BrainPhys™ (control) or BrainPhys™ IO both supplemented with SM1 for up to 21 days. Neurons (βIII tubulin positive) were quantified in 25 random fields per well, in triplicate. BrainPhys™ IO supported equivalent survival relative to BrainPhys™ (8293±642 vs. 7582±655 neurons per well; mean ± SE; n = 11) at 21 days in culture. To assess phototoxicity, neurons at 14 days were exposed to blue LED light for 12 hours. Neurons in BrainPhys™ showed disintegrated neurites and cell bodies post-light exposure while BrainPhys™ IO-cultured neurons remained healthy. To assess autofluorescence of the culture media, BrainPhys™ or BrainPhys™ IO was exposed to 450-490 nm light and mean emission at 525 nm across three fields of view was recorded. Autofluorescence in BrainPhys™ IO was reduced by 41.1% ± 3.6% (mean ± SE; n = 3) relative to BrainPhys™. We also tested BrainPhys™ IO for the culture of hPSC-derived neurons. Neural progenitor cells derived from the iPS cell line XCL-1 were differentiated in BrainPhys™ plus growth factors and cultured for 8 weeks in multielectrode array plates. Subsequently, for half the wells, the media was exchanged with BrainPhys™ IO rather than BrainPhys™ (control) for 3 weeks and activity was recorded twice weekly. In BrainPhys™ IO, neurons had an average mean firing rate of 0.63±0.10 Hz compared to control neurons with

0.51±0.06 Hz (n=1; mean±SE 6 recordings). Together, these data demonstrate that BrainPhys™ IO is an effective medium for fluorescent imaging applications while supporting survival and functional neuronal activity.

F-3155

INSULIN SUPPLEMENT IS ESSENTIAL IN DIFFERENTIATING HUMAN INDUCED PLURIPOTENT STEM CELLS INTO PANCREATIC BETA CELLS

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Insulin (INS) and insulin-like growth factor 1 (IGF1) are ubiquitous hormones that play important roles in regulating important metabolic and developmental processes, including pancreatic islets. Instead of IGF1 receptors, INS receptors are critical in mediating pancreatic beta cell growth response, which was reported by Ueki K. et. al., 2006, using beta cell specific INS or IGF1 receptor knockout mice. However, there is still less known about the specificity of INS versus IGF1 in human pancreatic development. In this research, we aim to examine this specificity in human cells by focusing on the INS or IGF1 supplements used in cell culture media. Using a five-step rotating culture system established in our lab, we attempted to generate pancreatic beta cells from human induced pluripotent stem cells (hiPSCs) *in vitro under cell culture media supplemented with INS or IGF1 conditions. The differentiated cells in each step were collected and assayed for protein and gene expression levels to examine cell maturation. Here, we first report that both INS and IGF1 are capable of maintaining cell culture of pluripotent hiPSCs. Next, our results demonstrated that either INS or IGF1 can be used as medium supplements in differentiating definitive endoderm cells derived from hiPSCs. However, during pancreatic progenitor cell differentiation, instead of IGF1, INS signaling is necessary for the induction of a larger population of pancreatic progenitor, PDX1-expressing cells. We also found that endocrine cells that were cultured under INS supplemented medium condition generated a higher ratio of insulin and NKX6.1-double positive cells. While cells cultured in IGF1 supplemented medium did not turned into insulin-expressing cells. In pancreatic beta cells, both insulin and NKX6.1 are expressed. Therefore, our results were consistent with those reported in mice, proposing INS as an essential component in pancreatic development in comparison to IGF1. This in vitro cell culture model we used precisely demonstrated the effect of INS or IGF1 supplemented cell culture media in pancreatic development. Using this differentiation model, we can find key pancreatic development stages and signalling molecules that might lead to beta cell destruction and dysfunction in both type 1 and type 2 diabetes.*

PLURIPOTENT STEM CELL: DISEASE MODELING

F-3159

HUMAN IPS CELL-DERIVED ARTIFICIAL SKELETAL MUSCLES FOR MODELING MUSCULAR DYSTROPHY AND DEVELOPING THERAPIES

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Skeletal muscle morphology and function is impaired in severe genetic disorders named muscular dystrophies. Most muscular dystrophies are rare, which makes relying on patient biopsies for research a major hurdle towards the development of therapies; moreover, various animal models poorly recapitulate the phenotype. Recent advances in experimental therapies for muscular dystrophies, such as exon-skipping, gene-editing and read-through technologies promise to ameliorate disease phenotype. However, the development of these therapies is limited by the lack of a temporally, economically and ethically viable platform. Therefore, generating human skeletal muscle models able to faithfully recapitulate disease-specific features is instrumental for investigating pathology and developing therapies. However, several muscle engineering platforms

are challenged by the limited proliferation and differentiation of primary myogenic cells. To this aim, we developed three-dimensional (3D) artificial skeletal muscles from human induced pluripotent stem cells (iPSCs) derived from patients with Duchenne (DMD), limb-girdle and congenital muscular dystrophies. Artificial muscles recapitulated key characteristics of skeletal muscle tissue, contained up to four isogenic lineages and could be implanted into immunodeficient mice. Notably, we used this novel platform to model features of muscular dystrophy in vitro in order to develop possible treatments. In the case of limb-girdle and congenital muscular dystrophies caused by mutations in the LMNA gene, we showed that pathological cellular hallmarks can be modeled with higher fidelity using this 3D platform than standard 2D cultures. Notably, we have identified nuclear length as a robust and objective mutation-specific outcome to measure response to treatments. In parallel, to facilitate the development of therapies for DMD we exploited CRISPR/Cas9 gene editing to develop dystrophin-detectable iPSCs and artificial muscles by inserting a novel reporter cassette able to track dystrophin expression levels temporally and spatially, in real time and in fixed cells. These results lay the foundation for next-generation complex disease modeling of muscle diseases, accelerating development of personalized therapies for incurable neuromuscular disorders.

F-3161

FUNCTIONAL STUDY OF NON-CODING VARIANTS IN HIRSCHSPRUNG DISEASE USING HUMAN PLURIPOTENT STEM CELL-BASED MODEL

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Hirschsprung disease (HSCR) is a complex multigenic disorder which is caused by incomplete colonization of enteric neural crest cells (ENCCs) in the gut. The aganglionic gut of HSCR patients is functionally obstructed due to uncoordinated muscular peristalsis. Previously, our whole genome sequencing study has identified thousands of HSCR-associated genetic variants residing in the non-coding regions of the human genome. Intriguingly, many of these variants are spanning throughout the non-coding regions of the RET gene and they are highly associated with HSCR susceptibility. rs2435357 (C>T), in particular, has been suggested to increase the risks of HSCR by >4-fold, especially in Chinese patients. In this study, we established an experimental paradigm to interconnect the HSCR associated non-coding variants with the disease phenotypes and severity using human pluripotent stem cell (hiPSC)-based model. By CRISPR-Cas9 genome editing technology, C allele of rs2435357 was changed to T allele in the control hiPSCs or vice versa in the patient-derived hiPSCs. The hiPSCs were directed to ENCC lineage through inhibition of BMP and TGFβ pathways, followed by activation of the WNT pathway and caudalized with retinoic acid. The ENCCs would then be differentiated into neurons by addition of various neurotrophic factors. We examined the RET expression in different developmental stages of cells and found that rs2435357 (C>T) alone is not sufficient to alter RET expression level in either ENCCs or their neuronal derivatives. Based on in silico analysis, we have identified more HSCR susceptible variants in the putative enhancers of RET gene. In further study, multiplex approach will be used to examine the accumulative effect of multiple variants in the development of enteric neurons.

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F-3163

DRUG SCREENING PLATFORM DEVELOPMENT USING A HIGHLY RESPONSE NADH/NAD+ SENSOR IN MELAS CARDIOMYOCYTES

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Nicotinamide adenine dinucleotide (NAD+) is a central metabolic cofactor in eukaryotic cells that plays a critical role in regulating cellular metabolism and energy homeostasis. The reduced form, NADH, serves as the primary electron donor in the mitochondrial respiratory chain, essential for generating adenosine triphosphate (ATP) by oxidative phosphorylation. Here, we present an assay capable of monitoring in real time the level of NADH/NAD+ redox state in MELAS cardiomyocytes, using the highly responsive, genetically encoded fluorescence sensor SoNar (sensor of NAD (H) redox). We initially determined the dynamic range of SoNar by measuring the level of NADH/NAD+ in the presence of different concentration of lactate and pyruvate in our system. Next, we used SoNar sensor to determine whether there is a difference in the level of NADH/ NAD+ ratio in both MELAS cardiomyocytes with high (disease) and low (healthy) heteroplasmy. Our data demonstrated a positive correlation between high levels of heteroplasmy (94%) and an increased

NADH/NAD+ ratio compared to a clone exhibiting no detectable heteroplasmy. This study demonstrates genetically encoded SoNar as an efficient platform based metabolic screening could serve as a valuable approach for drug discovery.

F-3165

SINGLE-CELL RECONSTRUCTION OF HUMAN HINDBRAIN AND SPINAL CORD IN IPSC MODELS OF AMYOTROPHIC LATERAL SCLEROSIS REVEALS CELL TYPE-SPECIFIC TRANSCRIPTIONAL SIGNATURES OF DISEASE

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder defined by cortical and spinal motor neuron (MN) death, typically presented in adulthood as a spreading paralysis of various motor circuits throughout the hindbrain and spinal cord, ultimately leading to asphyxiation. ALS patients have an average life expectancy of three years after diagnosis. Of ALS cases, ten percent are attributed to genetic mutations, among which hexanucleotide repeat expansions in C9orf72 are the most common, and 90 percent are of unknown genetic cause (sporadic ALS). While several pathways and cell types have been associated with ALS etiology, there are currently no cures available due to the lack of definitive mechanisms and thus therapeutic targets. Given the heterogeneous genotypes and clinical and molecular phenotypes associated with ALS, iPSC models, which can capture the genetic architecture of ALS patients in differentiated MNs as well as accessory cell types, are amenable to study the underlying causes of ALS in search of early biomarkers or candidate drug targets. Here we expression profile iPSC-differentiated neuronal cultures from C9orf72 ALS, sporadic ALS, and unaffected patients at the single cell level to characterize the developmental, anatomical, and functional identity of each cell. Using a rostro-caudal developmental HOX gene expression code, we observe that iPSC-differentiated neural cultures resemble hindbrain and rostral rather than caudal spinal cord segments. Using a combination of developmental marker genes with global clustering algorithms, we define ventral MN, V1, and V2 interneuron populations in cultures. We subsequently detect transcriptional dysregulation by ALS within each cell type. Despite resembling immature fetal tissue, iPSC-derived MNs specifically exhibit transcriptional changes that are detectable in and concordant with post-mortem, laser captured MNs from sporadic ALS patients. Overall, our analysis enables the resolution of cell identities (e.g. rostral vs. caudal, hindbrain vs. spinal cord, dorsal vs. ventral regions, and progenitors vs. postmitotic neurons) and disrupted physiologies relevant to individual ALS patients. Furthermore, our results illustrate that early signatures and potential therapeutic targets of ALS can be observed in iPSC models.

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F-3167

IPSC DERIVED BRAIN CHIP SYSTEMS TO STUDY HUMAN NEURODEGENERATIVE DISEASE

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The physiological, molecular and cellular changes that underlie amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) are complex. Rare monogenetic forms of these diseases, while informative, do not reflect the approximately 90% of ALS and PD cases with no known genetic mutations (termed sporadic). The greater prevalence of sporadic ALS and PD cases prompts the immediate need to develop sporadic disease models. Working with the Cedars-Sinai's Induced Pluripotent Stem Cell (iPSC) Core to generate large cohorts of ALS and PD lines, we have developed advanced differentiation techniques for cell types relevant to central nervous system function. Among these are robust methods to derive spinal motor neurons (spMNs) and dopaminergic neurons (DANs) that are uniquely susceptible in ALS and PD, respectively. From iPSCs, we can derive other cell types that are also affected in disease, including astrocytes and microglia, as well as brain microvascular endothelial cells (BMECs) that replicate blood brain barrier (BBB) function. Here we have combined these iPSC-derived cells with scalable microphysiological systems (MPS), also known as Organ-Chips (Emulate Inc.). Through co-culture of either spMNs or DANs with additional supportive cell types, we have developed highly physiological models of ALS and PD on Organ-Chips referred to as ALS-Chip and PD-Chip. These neurodegenerative Chip models are perfuseable culture systems capable of sustained flow of media and even human blood. To uncover disease-specific pathophysiology in the ALS-Chip or PD-Chip and to generate novel biomarkers for both diseases, we have developed a comprehensive array of genomic, proteome, metabolomic, and electrophysiological assays. The primary outcome of this project is the establishment of reproducible disease-specific phenotypes of both sporadic ALS and PD that will then be used as physiological biomarkers to screen for novel pathological-mitigating drugs.

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F-3169

INFLAMMATION AND PHAGOCYTIC DYSREGULATION IN A MODEL OF HIV NEUROPATHOGENESIS

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HIV-Associated Neurocognitive Disorders (HAND) affect 55% of HIV-infected individuals worldwide. While antiretroviral treatments have reduced the severity of HAND, the prevalence has increased due to increased life expectancy. In addition, little progress has been made in developing therapeutics to reduce the prevalence of HAND. While the major pathological manifestation of HAND is synaptodendritic damage, the full, underlying mechanism is unknown partly since there is no in vitro model to study the direct interactions between HIV-infected macrophages/microglia and neurons. In order to address this problem, we have developed a human-induced pluripotent stem cell (HiPSC) based model; whereby, we separately differentiate HiPSCs into forebrain, glutamatergic-like neurons, astrocytes, and microglia and create a co-culture of the three cell types with or without HIV-infection. As expected, we found increased production of inflammatory cytokines by HIV-infected microglia. However, we discovered a reduction in synaptic phagocytosis by the infected microglia, suggesting phagocytosis is not the mechanism behind synaptodendritic damage in HAND.

F-3171

AN INDUCED PLURIPOTENT STEM CELL RESOURCE FROM A POPULATION ISOLATE INTEGRATES GENETICS WITH BIOLOGY FOR STUDIES OF BIPOLAR AND RELATED NEUROPSYCHIATRIC DISORDERS

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Multiple risk-associated variants revealed through genome-wide association studies (GWAS) highlight the polygenic architecture of bipolar disorder (BD), major depressive disorder (MDD) and schizophrenia (SCZ). These findings have fueled intensive investigations on the biological impact of common alleles on the pathogenesis of these complex neuropsychiatric disorders. Common low-risk alleles may be complemented by variants of higher impact that are enriched in genetically isolated populations. To identify such variants, we are carrying out a genetic study of Amish and Mennonite individuals whose common ancestors originated from central and northern Europe. These communities convey the advantages of large families, relatively homogeneous lifestyles, well-documented genealogies, and increased frequencies of certain otherwise rare alleles. Cases of BD and their relatives undergo a mental health evaluation, neurocognitive testing, and provide blood samples. Whole genome SNP array analysis has revealed rare copy number variants, such as a 16p11.2 duplication previously associated with schizophrenia and bipolar disorder. Exome sequencing in the first 600 participants has detected numerous otherwise rare variants, some of which are predicted to be damaging, within genes implicated in BD or SCZ by GWAS. Probands and one unaffected relative per family have provided skin biopsies, over 50 of which have been reprogrammed to iPSC. Neural derivatives of these clones constitute cellular reagents to model biological impact of risk alleles and to screen for novel therapeutics. We have performed initial characterization of selected clones, which are available to collaborating laboratories at no cost. Expansion of the resource continues in earnest. Comprehensive genetic and phenotype analyses on iPSC-derived neural cells may help explain how polygenic variants and rare alleles contribute to the neurobiological changes that put carriers at increased risk for debilitating mental disorders.

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F-3173

THE TREM2 R47H RARE VARIANT AND ALZHEIMER DISEASE: AN IPSC-BASED PLATFORM FOR DISEASE MODELING

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Recently, genes associated with immune response and inflammation have been identified as genetic risk factors for late-onset Alzheimer disease (LOAD). One of them is the rare p.Arg47His (R47H) variant within the gene encoding triggering receptor expressed on myeloid cells 2 (TREM2), which has been shown to increase the risk of developing AD by 2-3-fold. TREM2 is a cell surface receptor of the immunoglobulin superfamily that initiates a signalling cascade modulating cell proliferation and differentiation, survival, chemotaxis and inflammation. Importantly, TREM2 is required for microglial phagocytosis of a variety of substrates, including apoptotic neurons and Aβ and thus plays a prominent role in driving microgliosis. Although the role of TREM2 in AD has been a focus of study using post-mortem brains, mouse models and heterologous cell lines, no concluding agreement has been made probably due to the inability to model this complex disease. Here, we report the generation and characterization of a model of LOAD using lymphoblast-derived iPSCs from patients harbouring the R47H mutation in TREM2, as well as from control individuals without dementia. iPSCs efficiently differentiated into mature neuronal networks composed of neurons and glia cells. Comparative global transcriptome analysis identified a distinct gene expression profile in AD TREM2 neuronal networks suggesting that these lines exhibit alteration in key signaling pathways related to metabolism and immune system in comparison to control. Although the neuronal networks derived from AD TREM2 lines secreted Aβ with a similar Aβ42 ratio compared to controls, manipulation with an Aβ 42-S8C peptide dimer revealed metabolic dysregulation, impaired phagocytosis-related pathway and failed to induce an inflammatory response. In conclusion, our study has shown that our AD-iPSCs based model can be used for indepth studies to reveal putative molecular mechanisms underlying the onset of Alzheimer disease and for screening of potential therapeutic targets.

Funding Source: Medical faculty of Heinrich Heine University Düsseldorf

F-3175

ELUCIDATION OF NON-CELL AUTONOMOUS NEURODEGENERATION IN SPINAL AND BULBAR MUSCULAR ATROPHY USING IPSC-DERIVED NEUROMUSCULAR MODELS

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Spinal bulbar muscular atrophy (SBMA) is an adult onset lower motor neuron disease caused by the abnormal expansion of polyglutamine tract (CAG repeat) in Androgen receptor (AR). So far, SBMA have been considered to be caused by cell-autonomous motor neuron degeneration. However, recent analyses have shown non-cell autonomous neurodegeneration by skeletal muscles, though detailed molecular mechanisms have not been fully elucidated. In this study, we established a human induced pluripotent stem cells (iPSCs) based neuro-muscular co-culture system to elucidate neuro-muscular pathology of SBMA. We first established human myoblast cell lines (Hu5/E18) stably expressing wild-type (Hu5/E18-AR24Q) or mutant human AR (Hu5/E18-AR55Q, and 97Q), and found that mutant AR expressing myoblasts exhibited poor differentiation into myotubes compared with those expressing wild type AR. Then, to examine the roles of mutant AR in neuromuscular interaction, motor neurons derived from control iPSCs (201B7) were co-cultured with myotubes expressing wild-type or mutant AR. As expected, iPSC-derived motor neurons formed significantly less numbers of αBTX-positive neuromuscular junctions (NMJs) with myotubes expressing mutant AR than with myotubes expressing wild-type AR, suggesting that mutant AR expressed in myotubes suppresses NMJ formation even with healthy motor neurons. Moreover, motor neurons co-cultured with mutant AR expressing myotubes exhibited significantly higher numbers of cleaved Caspase-3 positive apoptotic cells than those co-cultured with myotubes expressing wild-type AR. These results suggest that mutant AR expressed in skeletal muscles may induce neuronal cell death through NMJs. To further elucidate actual pathogenesis of non-cell autonomous neurodegeneration caused by mutant AR in myotubes, we are currently undergoing co-cultures of patient or control iPSC-derived skeletal muscles and motor neurons in various combinations. In our preliminary data, co-culture of iPSC-derived motor neurons (Control) and skeletal muscles (SBMA) showed dying back-like neurodegeneration by time-lapse imaging. In the future, we would investigate underlying mechanisms of non-cell autonomous motor neuron degeneration to explore common or unique therapeutic targets for motor neuron disease.

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F-3177

HEPATITIS B VIRUS INFECTION IN METABOLICALLY IMPROVED STEM CELL DERIVED HEPATOCYTE-LIKE CELLS

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Worldwide, an estimated 260 million people are chronically infected with the hepatitis B virus (HBV) and at high risk of developing liver cirrhosis and hepatocellular carcinoma. Although the currently available nucleoside viral polymerase inhibitors are highly efficient in lowering viral load, they are unable to eradicate the virus. Consequently, life-long treatment is mostly necessary as the covalently closed circular DNA (cccDNA) is retained in the hepatocyte nucleus. Hence, there is an urgent need for therapeutics that allow to cure patients from their infection. Primary human hepatocytes (PHH) are currently the standard for HBV studies. However, because of shortage of donor organs and the labour-intensive work with PHH, alternatives are needed. Therefore, the use of human pluripotent stem cells (hPSC) derived hepatocyte-like cells (HLCs) in HBV infection models is being explored, which also offer the advantage of diverse genetic backgrounds. Although protocols have been developed to generate HLCs from hPSC, all hPSC-HLCs remain immature, lacking major drug metabolizing enzymes such as CYP3A4. To address this maturation problem, the Verfaillie lab has generated a hPSC line that overexpresses three liver-specific transcription factors (termed HC3x) in a doxycycline inducible manner as well as optimised medium conditions for these HC3x-HLCs. Differentiation of HC3x-HLCs in the optimised medium resulted in a more mature hepatocyte progeny, with increased production of albumin and functional CYP3A4 in comparison to control hPSC-HLCs. HC3x-HLCs could efficiently be infected with HBV, as demonstrated by staining for HBV core and surface antigen (infection efficiency between 9 and 20%). We are now further validating the culture system with various known anti-HBV antiviral drugs. The culture system can also be downscaled to 96 and 384-well format culture plates, which will enable us to perform large scale small molecule screens to identify novel and selective anti-HBV drugs to improve and expand the existing therapeutic regimens.

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F-3179

MODELING PCDH19-RELATED EPILEPSY IN HUMAN EMBRYONIC STEM CELL DERIVED NEURONS AND CEREBRAL ORGANOIDS

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PCDH19-Related Epilepsy (PRE) is caused by mutations of the PCDH19 gene on the X-chromosome and exclusively affects females and mosaic males while male carriers are spared. Mosaic expression of PCDH19 due to random X-inactivation is thought to cause impaired cell-cell interactions between mutant and wild type PCDH19-expressing cell populations to produce the disease phenotype. However the precise function of PCDH19 during human cortical development and how the mosaic expression of PCDH19 leads to PRE remain unclear. Our goal is to use genome editing and stem cell approaches, including human cerebral organoids (hCOs), to interrogate the function of PCDH19 and how its mutations lead to seizure-like activity in developing human brain in vitro. To this end, we used CRIPSR/Cas9 to generate in-frame epitope tagged PCDH19 H9 female hESCs that allow us to use a standard antibody against the epitope to detect PCDH19 expression as most antibodies to date have shown non-specific labeling. We found that PCDH19, along with N-Cadherin, is localized to the apical lumens of neural rosettes in both 2D adherent cultures and 3D hCOs that are derived from the tagged hESCs, consistent with its high mRNA expression level at this stage. Using CRISPR/Cas9, we also established homozygous PCDH19 knockout H9 hESCs and generated a “virtual PRE patient” model in which isogenic H9 lines with a HA-FLAG-tagged PCDH19 allele are mixed with knockout cells, providing a reliable system to model the mosaic expression of PCDH19 that occurs in vivo due to random X-inactivation. Lastly, we cultured hCOs to enable a detailed examination of early human cortical development, preserving structural aspects and temporal-spatial relations critical for understanding epileptogenesis. We observed altered N-Cadherin+ apical lumens and abnormal cell segregation in hCOs derived from a “virtual PRE patient” model. Our results suggest that PCDH19 acts as a critical cell-cell adhesion molecule through the interactions with other cadherin proteins in the developing human brain. Further analyses including gene expression and electrophysiology in hCOs will help to identify PRE-related abnormalities in human brain development and seizure mechanisms that should lead to novel therapies.

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F-3181

ROLE OF TBX3 IN HUMAN STEM CELL-DERIVED HYPOTHALAMIC NEURONS

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The leptin-melanocortin pathway of the hypothalamus is a master regulator of body weight. Thus, it is no surprise that mutations in genes expressed in hypothalamic pro-opiomelanocortin (POMC) neurons have been identified as monogenic causes of human obesity. So far, the functional relevance of novel variants identified in sequencing studies of obese humans has been tested mostly in non-human models and human non-neuronal cell lines. Here, we use human stem cell-derived hypothalamic neurons as a model to dissect the role of novel variants associated with human obesity. Mutations in the transcription factor T-box 3 (TBX3) have been associated with obesity in humans, but the molecular mechanism underlying this association remains unknown. We have recently shown that loss-of-function of TBX3 homolog in Drosophila causes excessive fat accumulation, while hypothalamic loss-of-function of Tbx3 in mice disrupts peptidergic identity and maturation of POMC-expressing neurons. Based on these findings from non-human models, we further investigated the role of TBX3 in the differentiation of human stem cells into hypothalamic neurons to establish a functional and causal link between TBX3 mutations and the development of obesity in humans. Loss-of-function models were created using CRISPR/Cas9 and their cellular and molecular phenotypes were obtained at several time points during the course of differentiation from stem cells into

hypothalamic neurons. These studies revealed the critical role of TBX3 in the maturation of hypothalamic progenitors into POMC-expressing neurons. These results also suggest that the role of TBX3 in the regulation of energy homeostasis is conserved across species.

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F-3183

DISSECTING THE MECHANISMS OF SCHIZOPHRENIA USING PATIENT-DERIVED STEM CELLS

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Schizophrenia affects about 1% of the world's general population. Although widely accepted as having a strong genetic basis and a root in neurodevelopment, the exact mechanisms of the disease remain unknown. We recruited a group of patients (n=17) with adolescent-onset schizophrenia who suffer from severe symptoms and were free from prolonged medications. Whole exome sequencing using DNA from patients and their parents revealed both inherited and de novo candidate variants. Olfactory stem cells, as a unique source of adult stem cells in the nasal cavity, when isolated from patients showed a significantly reduced cell-to-matrix adhesion. This was coupled with significantly disturbed global protein synthesis, stress responses and actin dynamics in patient olfactory stem cells based on proteomics analysis. These results highly agreed with previous studies addressing adult-onset schizophrenia, suggesting that the adolescent-onset group and adult-onset group share very similar mechanisms. Induced pluripotent stem cells were derived from three patients with family histories of schizophrenia, as well as age- and gender-matched controls. Organoids that resemble the fetal forebrains, and microglia that were incorporated into the organoids, were generated and the key components in neurodevelopment are currently being checked longitudinally. Induced neurons resembling glutamatergic layer 2/3 neurons in frontal lobes are being derived from a patient with variants in SYNGAP1 and SHANK2 –two important members of the post-synaptic density. Electrophysiological studies will be performed on induced neurons from this patient and isogenic controls. Together, this study used a group of patients enriched for clinically severity, and aims to understand the possible mechanisms of schizophrenia from the cellular and molecular perspectives.

F-3185

SHP2 MUTATIONS CAUSE NEURODEVELOPMENTAL ABNORMALITIES IN NOONAN SYNDROME DERIVED IPSCS

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Noonan syndrome (NS) is a genetic disorder caused by gain-of-function mutations in SHP2 (Src homology 2 domain-containing protein tyrosine phosphatase 2). Although approximately 30-50% of NS patients have the cognitive deficits including lower intelligence and neuropsychological complications, how SHP2 mutations are associated with neural development in NS patients remains elusive. In this study, induced pluripotent stem cells generated from NS-patient dermal fibroblasts (NS-iPSCs)

differentiated into embryoid bodies (EBs), neural rosettes (NRs), neural precursor cells (NPCs), and neural cells in vitro. NS-EBs showed abnormal morphologies and defective development to NRs. Inhibition of both BMP and TGF-β signaling pathways rescued impaired early neuroectodermal development of NS-iPSCs. Recued NS-EBs normally differentiated into NRs and NPCs. NS-neural cells developed from NS-NPCs exhibited phenotypic abnormalities such as increment of glial cells and shortened neurites of neuronal cells as compared with wild-type (WT)-neural cells. They also decreased in extracellular spontaneous firing in NS-neural cells at 12 weeks during neural differentiation of NS-NPCs. SHP2 inhibition helps partially restore defective phenotypes and dysfunctional electrophysiology of NS-neural cells. Recently, cerebral organoids developed from NS-iPSCs recapitulated biased differentiation into glial cells. Our results provide a possibility that imbalanced neural development may contribute to cognitive deficits in NS patients.

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F-3187

DEVELOPMENT OF DRUG SCREENING PLATFORM THE ALPHA-1 ANTITRYPSIN DEFICIENCY IN PATIENT DERIVED HUMAN INDUCED PLURIPOTENT STEM CELL

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The liver genetic diseases associated with alpha-1 antitrypsin deficiency (A1ATD) is a gain-of-toxic function mechanism. The misfolded insoluble globular proteins accumulate in the endoplasmic reticulum, leading to hepatic fibrosis and even hepatocellular carcinoma. However, efforts to identify pharmaceuticals to treat heritable liver diseases have been hamper by the lack of human model systems. Recently, the rapidly development of human induced pluripotent stem cells (iPSCs) technology has ushered in new era for the fields of disease modelling and drug discovery. The advantages of iPSCs used drug development are their expandability, easy accessibility, human origin, avoidance of ethical concerns associated with human embryonic stem cells (ESCs) and the potential to develop personalized medicine using patient-specific iPSCs. In this study, we reprogrammed the A1ATD patient fibroblasts into iPSCs using Yamanaka factor (Oct3/4, Sox2, Klf4, c-Myc). From these patient-specific hiPSCs, we have successfully differentiated into hepatocyte-like cells (HLCs) using various growth factors and chemicals. The percentage of hepatocytes differentiated from iPSCs using FACS analysis and immunocytochemistry (ICC) was confirmed more than 90% of hepatocyte specific marker albumin and HNF4α positive cells. The liver function of glycogen storage was also observed in A1ATD-iPSC-derived HCLs by the periodic acid Schiff (PAS) staining. Furthermore, these HCLs showed the aggregation of alpha-1 antitrypsin (A1AT) similar to the hepatocytes of the A1ATD patient. Based on these results, we are going to establish drug screening platform targeting A1ATD in liver genetic diseases.

REPROGRAMMING

F-3191

COMBINED RNA-BASED GENE EDITING AND REPROGRAMMING OF HUMAN IPSCS: A CLINICALLY RELEVANT APPROACH

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Induced pluripotent stem cells (iPSCs) hold great promise as a therapeutic for many currently incurable genetic diseases. The successful development of autologous therapeutics derived from iPSCs depends on several critical steps: (1) reprogramming patient cells into iPSCs, (2) correction or replacement of a disease-causing gene in iPSCs, and (3) differentiation of the corrected iPSCs into functional replacement tissues. Usually these steps are performed sequentially, leading to a lengthy, complicated, and expensive manufacturing process. To reduce the number of steps associated with the generation of genetically corrected iPSCs, we combined our previously reported high-efficiency RNA-based reprogramming protocol together with CRISPR/Cas9-mediated correction into a one-step procedure which can be performed within 5-6 weeks. As proof-of-principle for our one-step correction/reprogramming approach, we generated corrected iPSCs from multiple patients with recessive dystrophyic epidermolysis bullosa (RDEB) resulting from various COL7A1 mutations (c.7485+5G>A, IVS26-3T>G, c.6781C>T). We observed correction efficiencies as high as 5% in reprogrammed iPSCs which were recoverable in clonally expanded colonies. Keratinocytes differentiated from corrected iPSCs express Col7 protein detected by immuno-staining, indicating functional recovery of the corrected gene. Due to its high efficiency and reproducibility, our combined RNA-based gene editing and reprogramming approach

provides an opportunity to shorten the time between patient biopsy and the generation of gene edited iPSC lines, simplifying the manufacturing process of iPSC-based therapies for clinical applications.

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F-3193

MOUSE ADULT NEURAL CREST-DERIVED STEM CELL REPROGRAMMING TO PLURIPOTENT STATE AND THE SEROTONIN SYSTEM

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The discovery of the phenomenon of somatic cell reprogramming into a pluripotent state was a scientific breakthrough. In addition to new fundamental data, it enables obtaining any cell types for use in regenerative medicine. Despite intensive studies of the reprogramming and the pluripotent states, many issues remain unresolved: the choice of the starting cell type, the appropriate cocktail of transcription factors, the type of transfer vector, the use of small molecules etc. Adult neural crest-derived stem cells (NCSCs) are a promising cell type, both for studying the reprogramming and for potential clinical applications. The NCSCs express a number of genes associated with pluripotency: Sox2, Oct3/4, Nanog, Klf4, Lin28, cMyc (albeit at a lower level than ESCs and iPSCs). Thus, probably fewer factors may be used to reprogram adult NCSCs to iPSCs than the classic "magic of four" of Yamanaka. Small molecules are widely used in reprogramming to increase efficiency. There is evidence that serotonin, or molecules that affect the serotonin system (agonists and antagonists of 5-HT receptors, or inhibitors of the Slc6a4 transporter or the two rate-limiting enzymes TPH 1 and 2 may be used for this purpose. Accordingly, it was recently discovered that the 5-HT3 agonist, 2-methyl-serotonin, can replace the Oct3/4 in this process. In our study, we compared the reprogramming efficiency of mouse adult NCSCs using: the classic combination of single lentiviruses with the four Yamanaka's factors (Oct3/4, Sox2, Klf4 and cMyc); a combination of three single lentiviruses without Sox2 due to its high expression at the protein level in NCSCs, as well as two polycistronic vectors expressing all four factors, OSKM and OKSM. The reprogramming efficiency of NCSCs from WT and 2KO mice, lacking both TPH isoforms (Tph1/Tph2), was also compared. When using the classic combination of Yamanaka's factors as single vectors, the reprogramming efficiency was 0.071 ± 0.013 %. When using a cocktail of three single vectors (without Sox2): 0.011 ± 0.003 %. In case the OSKM vector, adult NCSCs were not reprogrammed unlike MEFs. When using the OKSM vector, the reprogramming efficiency was 0.015 ± 0.006 % for WT and 0.087 ± 0.016 % for 2KO. NCSCs. The NCSCs are a promising tool for the study of reprogramming and the association of this process with the serotonin system.

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F-3195

A NOBLE FINDING OF MIRNAS IN NEUROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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MicroRNAs (miRNAs) are small noncoding RNAs that emerge as regulators of stem cell lineage such as proliferation, development, differentiation, and apoptosis. We hypothesized that miRNA was involved in the neurogenic differentiation of mesenchymal stem cells. Here, the role of miRNAs in neurogenic differentiation of human mesenchymal stem cells (MSCs) is investigated. By performing a miRNA-mRNA paired microarray screening, we identified miR-4650-5p and miR-3146 among the most upregulated miRNAs during neurogenic differentiation. After selection of the miRNAs, we investigated the ability of neurogenic differentiation of miRNAs in human adipose tissue-derived MSCs (hADSCs). We found that miR-4650-5p or miR-3146 was increased the most of neuronal gene expressions by a quantitative PCR. Using bioinformatics and functional assay, we confirmed that miR-4650-5p and miR-3146 potentially targeted

on JNK and GSK3β to regulate Wnt signaling pathway. Overall comparative analysis revealed that Wnt signaling was enhanced more potently and played a more important role in neurogenic differentiation of hADSCs. These findings suggest that the miR-4650-5p and miR-3146 expression contributes the neurogenic differentiation of MSCs by increasing the neuronal genes and Wnt signaling pathway. The miRNAs regulation and downstream pathway network suggested the important role of miRNAs and Wnt signaling in the neurogenic differentiation of MSCs.

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F-3197

ABCG2-MEDIATED TRANSPORTATION OF N-METHYL-PROTOPORPHYRIN IX TRIGGERED HEPATOCYTE REPROGRAMMING IN LIVER INJURY

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Liver transplantation can aid to improve survival of patients with liver failure. However, the shortage of donor liver and poor liver graft survival limit the potential use of liver transplantation. Since the liver has been renowned for its remarkable regenerative capacity, we tried to address the potential of reprogramming mature hepatocytes to bipotential progenitors for the purpose of repairing liver injury. Initial efforts had demonstrated that periportal hepatocytes could be reprogrammed into Sox9-expressing progenitor cells in mice treated 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC). In order to reveal insight into the cellular and molecular mechanism of the liver regeneration and to develop strategies to trigger hepatocyte reprogramming toward bipotential progenitor fate via activating the genetic routes that mimicking natural regeneration machineries, we generated mice that were knockout of ATP binding cassette transporter ABCG2 and discovered ABCG2 deficiency affected reprogramming-mediated liver repairing. The further analysis revealed, in DDC-treated mice, N-methyl-protoporphyrin IX(N-methyl-PPIX) produced from the breakdown of Cytochrome P450 and transported through ABCG2 could trigger induction of Sox-9 expression in hepatocyte reprogramming. We found depletion ABCG2 possibly restricted N-methyl-PPIX transportation thus inhibiting Sox9 induction and hepatocyte reprogramming. In contrast, ductular reaction and hepatocyte reprogramming toward Sox9-expressing progenitor cells could be stored either by p53 knockout or by rescuing ABCG2 expression via hydrodynamic injection. Immunohistochemical staining further identified induction of SOX9 was found to associated hepatocyte dedifferentiation together with upregulation of Yap1 and NFATc1. Importantly, we found that treatment of human and mouse primary hepatocytes with N-methyl-PPIX was sufficient to be converted into Sox9-expressing cells. And transplantation of reprogrammed Sox9-expressing cells into to the mice treated with carbon tetrachloride (CCL4) could replenish damaged hepatocytes. Hopefully, the findings from the work can lead to understanding the insight of liver regeneration and to development of cell therapeutic strategies for patients suffering from liver injury.

F-3199

SWITCHING FATES TO UNDERSTAND LINEAGE DECISIONS AND THE RISE OF PLURIPOTENCY

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The blastocyst, a developmental stage shared among all mammals, is the source of a bona fide in vivo pluripotent population, the epiblast (EPI). Three lineages comprise the mature blastocyst – trophectoderm (TE), primitive (extra-embryonic) endoderm (PrE), and epiblast – which can also be captured ex vivo as trophoblast (TS), eXtraembryonic ENdoderm (XEN) and embryonic (ES) stem cells, respectively. A progenitor population, the inner cell mass (ICM), makes a bi-lineage choice to generate the EPI versus PrE. The EPI and ES cells give rise to all somatic tissues of adult mammals. Thus, determining how pluripotency is established in vivo holds far-reaching implications for stem cell biology, and regenerative medicine. While genetic and ex utero experiments have helped explain how these two lineages are segregated, we do not yet understand the mechanisms underlying individual cell-fate decisions, and factors maintaining a developmental barrier between the sister lineages. Much has been gleaned about the establishment of pluripotency by transcription factor-based reprogramming of somatic cells. Ectopic expression of Oct3/4, Klf4 and Sox2 induces pluripotency in somatic and TS cells, but XEN cells have never been tested for their potential to acquire a pluripotent identity despite being developmentally closer than TS cells. Conversely, ES cells acquire a XEN-like state with Gata4 misexpression. We are probing the mechanisms underlying the EPI-vs-PrE fate decision by inducing pluripotency within the PrE in vivo and in XEN cells in vitro. This in parallel with a Gata4-induced ES-to-XEN conversion will serve as an ex vivo model of ICM lineage specification.

F-3201

GENERATION, GENETIC MANIPULATION, AND AIRWAY DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM PRIMARY HUMAN AIRWAY EPITHELIAL CELLS

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Air-liquid interface (ALI) differentiation of human airway epithelial cells (AEC) is a well-established model to study the airway epithelium in vitro. The replicative airway basal epithelial cell used for ALI culture lacks the ability to be passaged indefinitely and is not easily genetically manipulated. Therefore, alternative approaches for epithelial basal cell regeneration and gene editing are needed to further model airway development and disease, as well as for regenerative therapeutics. Recently, various human cell types have been reprogrammed into induced pluripotent stem cells (iPSCs) using integration-free methods like mRNAs. Here we demonstrate for the first time the successful generation of iPSCs using readily accessible epithelial cells from the upper airways as a source, with a clinically-relevant non-modified RNA based reprogramming technology that combines a cocktail of synthetic reprogramming and immune evasion mRNAs with reprogramming-enhancing mature, double-stranded microRNAs. We collected human primary AECs from nasal and bronchial brushings, and generated iPSCs with reprogramming efficiencies of up to 0.17%. These iPSCs expressed pluripotency markers (OCT4, NANOG, SSEA4, and ALP) and effectively formed tissues representing all three germ layers under both targeted and spontaneous differentiation conditions. We successfully differentiated these iPSCs to Keratin 5-expressing basal-like airway epithelial cells through stage specific culture conditions. Further, we successfully expanded and maintained these iPSC-derived basal-like cells for multiple passages (>3) without losing the basal cell markers Keratin 5 and TP63. Moreover, using a CRISPR/Cas9 gene editing approach, we knocked out the epithelial cell marker Epithelial Cell Adhesion Molecule (EPCAM) in these iPSCs with >66% efficiency as measured by flow cytometry. We were also able to utilize CRISPR/Cas9 homology-directed repair (HDR) to incorporate full-length eGFP-tagged B-actin protein with 1.17% HDR efficiency. In conclusion, we found airway brushings can serve as a minimally invasive source of cells capable of reprogramming to iPSCs, gene manipulation, and redifferentiation to airway cells. This work highlights a potential strategy for gene correction and regeneration of the human airway.

F-3203

MITOCHONDRIAL ACTIVE NAIVE-LIKE PLUIPOTENT STEM CELL INDUCED BY PRIMITIVE GROWTH FACTOR, NME7AB

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Naïve pluripotent stem cells (PSCs) display a distinctive phenotype when compared to their "primed" counterparts. This phenotype includes, but is not limited to, increased potency to differentiate and more robust mitochondrial respiration. The cultivation and maintenance of naïve PSCs has been notoriously challenging, requiring the use of complex cytokine cocktails. NME7AB is a newly discovered embryonic stem cell growth factor that is expressed exclusively in the first few days of human blastocyst development. It has been previously reported that growing primed state iPSCs in bFGF depleted medium with NME7AB as the only added growth factor facilitates the regression of these cells to the naïve state. Here, we confirmed this regression by demonstrating the reactivation of mitochondrial function in the naïve state PSCs and their increased ATP production, as compared to that in primed state iPSCs.

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F-3205

INTERCELLULAR RNA TRANSFER-DRIVEN EPIGENETIC REPROGRAMMING OF HUMAN PLURIPOTENT STEM CELLS INTO NAÏVE-LIKE STATE

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Understanding the molecular basis of intercellular coordination will facilitate our ability to control collective cell behaviors. Herein we found the unexpectedly high incidence of bidirectional mRNA exchanges between human and mouse pluripotent stem cells in the xenogeneic coculture. Transferred mRNA accounts for

0.5-3.0% of the transcripts in the cells, that can be inhibited by the toll-like receptor 4 (TLR4) pathway. The mRNA transfer was primarily driven by direct cell-cell contact rather than by secretion mechanisms. Remarkably, human primed induced pluripotent stem cells (iPSCs) were converted from flat into domed colony morphology when cocultured with mouse naïve embryonic stem cells (ESCs). These human iPSCs expressed the naïve-specific markers including KLF4, TFCP2L1, and CD130, indicating the conversion from primed into naïve-like pluripotency in human iPSCs. The naïve-like conversion of human iPSCs was inhibited by the TLR4 ligand treatment. Furthermore, transcriptome and epigenome analyses revealed that during the conversion some mRNAs coding transcription factors were transferred from mouse ESCs into human iPSCs accompanied by naïve-specific enhancer openings in hiPSCs, strongly suggesting the potential of the intercellular mRNA transfer in cell fate conversion. Taking these results together, we propose that this intercellular mRNA transfer-based reprogramming phenomenon is a potential strategy to fine-tune a variable intercellular epigenomic identity, that can be leveraged for a novel reprogramming method for human cells without the use of conventional reprogramming factors.

F-3207

INDUCING HEPATOCYTE-LIKE CELLS FROM FIBROBLASTS BY PURE CHEMICAL APPROACH

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Recently, pure chemical methods are reported to reprogram fibroblasts into a series of cell types, such as pluripotent stem cells, functional neurons, neural progenitor cells, astrocytes, skeletal muscle cells and cardiomyocytes, which may provide a promising approach to obtain stem/progenitor cells or functional cell types for tissue engineering and regenerative medicine, and bypass the concerns of potential genetic alterations by transgenic approach. Here in our study, we find that the expression of hepatocyte-associated transcription factors (TFs) is stimulated in the first 4 days of 40-day chemical reprogramming process from mouse embryonic fibroblasts (MEFs) into chemically-induced pluripotent stem cells (CiPSCs), suggesting that these cells may be primed to hepatic lineage. Thus, we optimize the combination of chemical cocktails after day 4 of chemical reprogramming while using hepatocyte culture medium (HCM), and we find pure chemically-induced hepatocyte-like cells (CiHeps) emerged 12 days later. The CiHeps are epithelioid and colony-forming, co-expressing hepatic markers Albumin and Hnf4a, detected by immunostaining. The upregulated mRNA levels of other hepatic genes, such as Hnf1a, Foxa2, Ttr, Tdo2, Cps1, and Cyp2d10 are also detected by real-time PCR. Moreover, we find few CiHeps are obtained without the chemical treatment of the stage I medium of CiPSC induction for the first 4 days, supporting our hypothesis that an early cell plasticization process mediates chemical reprogramming into multiple cell lineages. Furthermore, we find the cells induced during hepatic reprogramming process do not express Sall4, a master gene of the extraembryonic endoderm (XEN), suggesting that the generation of those hepatocyte-like cells are dispensable of the generation of XEN-like cells, an intermediate state during the process of CiPSC induction. Overall, our findings not only pave a new way for obtaining hepatocytes with pure chemicals, but also suggest a general approach in developing chemical reprogramming methods through a multiple-lineage priming state.

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TECHNOLOGIES FOR STEM CELL RESEARCH

F-3209

ESTABLISHING A CLINICALLY COMPLIANT GMP HUMAN EMBRYONIC CELL LINE DERIVED UNDER XENO-FREE AND DEFINED CONDITIONS IN A GMP FACILITY

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Human embryonic stem cells (hESCs), are a promising cell source for therapies in regenerative medicine since they can differentiate in vitro to many different cell types. Consequently cell transplantation has a great potential as treatment for many diseases. Crucial for clinical translation is high-quality hESC derived under GMP conditions. We have established a clinically compliant GMP hESC line derived under these conditions and we now present the protocol. We have previously published xeno-free and defined protocols for derivation and culture but now all the components in the original protocol are reevaluated and this resulted in a new media formulation and a GMP compatible laminin 521 culture matrix. All reagents have also been functionally tested and the protocol showed good derivation efficiency. The derivations are done using frozen surplus human

embryos, donated with informed consent and with ethical approval from the Regional Ethics Board in Stockholm. We are now expanding the first lines at Vecura, a GMP facility within Karolinska University hospital. The first line has been analyzed for pluripotency by immunostaining and FACS, has been karyotyped, analyzed by iCS digital pluritest, and tested for the absence of human viruses. Our facility will produce cell lines and provide clinical grade GMP hESCs for experimental research and for clinical applications in regenerative medicine.

F-3211

AUTOMATED PASSAGING, FEEDING AND PLATING ON GLASS OF GENE-EDITED HUMAN INDUCED PLURIPOTENT STEM CELLS FOR HIGH-THROUGHPUT 3D LIVE CELL MICROSCOPY PIPELINE

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The Allen Institute for Cell Science combines genomics, gene editing, and 3D live cell imaging of cell organization to understand what defines different stem cell states and to unlock the underlying mechanisms for their production. We use human induced pluripotent stem cell (hiPSC) lines expressing green fluorescent protein tagged to proteins identifying specific cellular organelles and structures. To produce large numbers of standardized images, we have developed an automated hiPSC culture procedure. We use the Hamilton Star robotic platform to generate imaging plates with uniform and reproducible cellular confluency and morphology. Here we provide specific values on parameters such as the movements of the plates across the deck, the angle and speed of the aspiration and dispensing of media, the seeding strategies and the timing of every step that we optimized. This approach was developed and applied to coating, seeding, passaging and feeding procedures for cell expansion in 6-well plastic plates and 96-well glass bottom imaging plates. The overall optimization procedure aimed to preserve the undifferentiated state, maintain pluripotency, and prevent karyotyping abnormality of our gene-edited hiPSC lines for up to 10 passages. We present a side by side comparison of quality control results obtained from manual and automated operations. We also developed an automated image-based colony segmentation pipeline to measure and track colony growth characteristics over time. The colony feature measurements (number, size, textures, etc.) are used to rank 96-well plates and ensure consistent and adequate quality control. We have also implemented cell passaging based on image-based confluency calculations to eliminate the need for manual cell counting steps. In addition to providing higher uniformity, reproducibility, and overall cell quality for imaging samples, the standardized automation protocol ensures consistency by removing operator-to-operator variability and potential error or bias introduced by manual repetitive tasks and fatigue of the operators.

F-3213

HIGH-PRECISION AND SCALABLE FABRICATION OF BIOMIMETIC CULTURE ENVIRONMENTS FOR ENHANCING STEM CELL MATURATION

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Differentiated stem cells when kept in culture can lose cell-type specific phenotypes or fail to express many mature phenotypes found in vivo. Traditional cell culture environments—typically composed of glass or plastic—are partially responsible for this effect. Considerable effort has been directed at generating biomimetic cell culture environments to maintain or promote mature in vivo phenotypes. However, fabrication of biomimetic substrates capable of mimicking different aspects of the extracellular matrix (ECM) in vivo typically involves costly or hard-to-reproduce techniques that are often incompatible with many standard assays. Here, we will present a novel method of generating surfaces that mimic mechanical, nanoscale shape and structural cues of the collagen ECM. The fabrication scheme described is highly reproducible, scalable, and amenable to integration with most industry-standard endpoint assays, including high-NA optical microscopy. We present techniques to fabricate biomimetic culture surfaces out of elastomers that can be stretched in order to reproduce mechanical cues that are critical in the development and function of certain tissues. Various cell types were tested and were amenable to this approach. For example, hiPSC-derived cardiomyocytes (CMs) showed more in vivo-like myofibril alignment, sarcomere spacing and width, and expression of CM-specific proteins that are present in mature myocytes. Furthermore, higher-ordered 2D anisotropic myocyte tissues also showed adult-like structure and electrophysiological responses to drugs in vitro when compared to traditional unordered 2D isotropic constructs. Examples of phenotype enhancement of other mammalian cell types will be presented, further demonstrating the utility of the approach for fabrication of highly scalable and precise biomimetic surfaces.

F-3215

ROLE OF IMPELLER DESIGN FOR THE CULTIVATION OF STEM CELLS IN STIRRED-TANK BIOREACTORS

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The routine use of stem cells and stem cell-derived cells in drug research and regenerative medicine requires the constant supply of high cell numbers in consistent quality. Stirred-tank bioreactors have emerged as promising cultivation systems, which facilitate close control of critical process parameters and have proven their value for efficient process scaling. Cell cultivation on microcarriers or as cell aggregates are two widely used methods for expanding anchorage-dependent cells in stirred-tank bioreactors. Suitable agitation conditions have to be established to keep cell aggregates or microcarriers in suspension while avoiding damaging shear forces.We have developed a novel 8-blade impeller to improve the cultivation of stem cells as aggregates and on microcarriers in the DASbox Mini Bioreactor System. Use of this impeller supported the formation and growth of stem cell aggregates in the bioreactor. Cell aggregate formation was more efficient than when using a conventional pitched-blade or Rushton-type impeller. Furthermore, microcarriers could be efficiently brought to and kept in suspension. Our results suggest that the impeller shape is an important parameter to consider when optimizing the agitation conditions in stirred-tank bioreactors.

F-3217

NOVEL SOLUTIONS FOR CARDIAC DRUG SAFETY AND TOXICOLOGY RESEARCH

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Human induced pluripotent stem cells (hiPSCs) have been proven instrumental for cardiac safety and toxicology testing due to their validated predictivity (e.g. CiPA study). We combined impedance and extracellular field potential (EFP) measurements with solid-supported membrane (SSM) based electrophysiology to study drug safety and toxicology in hiPSC-CMs. We have performed dual impedance and EFP-recordings to monitor cell proliferation and contractility, over prolonged time periods, contrary to standard mostly endpoint cytotoxicity assays. As the emerging field of cardio-oncology aims to find a balance between oncologic efficacy and reducing adverse cardiovascular effects, we tested the same treatment used in breast cancer chemotherapy, on hiPSC derived cardiomyocytes (iPSC-CMs). One of the standard clinical regimens for breast cancer is a combination of cyclophosphamide, adriamycin (doxorubicin) and 5-fluorouracil (CAF) administered for 4 months. We investigated putative cardiovascular side effects of CAF mix and paclitaxel and their long-and short-term implications on iPSC-CMs viability. We show a dose dependent negative effect of paclitaxel on iPSC-CMs viability (base impedance reduction). This was also observed for doxorubicin alone, but not the rest of the CAF compound mix. Paclitaxel and CAF also induced negative changes in cell contraction properties. We also investigated the role of the Na+/Ca2+- exchanger (NCX) in cellular Ca2+ homeostasis of iPSC-CMs. NCX is important for Ca2+ homeostasis, and it can contribute to cell damage by Ca2+ overloading or induce an anti-arrhythmic effect, when inhibited or when direction reversed. By developing a suitable sensor-based method (SSM based electrophysiology) we recorded specific NCX current responses of high amplitude. These currents showed similar Ca2+ affinity compared to NCX1 expressed in HEK cells and were sensitive to nickel, KB-R7943 and SEA0400. Using the impedance-based system, we also observed a significant increase in beating rate, as a long-term effect, when inhibiting NCX with SEA0400, in contrast to previous studies using non-human systems and focusing on short-term effects. In summary, we demonstrate the importance of new combination of tools, which help generate new insights into the pharmacology and toxicology of iPSC-CMs.

F-3219

LAMININ-FUNCTIONALIZED 3D SILK SCAFFOLD MAINTAINS EXPANSION, STEMNESS AND DIFFERENTIATION POTENTIAL OF HUMAN PLURIPOTENT STEM CELLS

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Tissues are 3D formations of cells integrated in an extracellular matrix (ECM) with specific sites for cell anchorage offering positional and instructive information, regulating cell behavior. A vast number of publications in the last decades have proven the reciprocal interaction between the ECM, cytoskeleton and nuclear matrix, showing that these structures exert a physical and chemical influence over gene expression. Thus, to culture authentic cells from which real biological questions

can be answered, environmental context is pivotal. Laminins comprise a family of 16 unique heterotrimeric glycoproteins naturally present in the body. Laminins are tissue specific, supply a natural environment for all cell types, and influence cell adhesion, differentiation, migration, phenotypic stability, and cell functionality. As such, recombinant laminins are biologically relevant ECM protein substrates that can be used to mimic the in vivo cell niche in vitro. The laminin 521 isoform is a critical factor supporting pluripotent stem cells in culture, and given the right context, this isoform also provides support for other differentiated cell types. Silk 521 is a biomaterial made of recombinant silk, which is functionalized with human recombinant laminin 521 protein (Biolamininâ„¢ 521). This material supports integration and proliferation of human pluripotent stem cells (PSCs) in vitro and serves as a viable base for the development of subsequent lineage-specific differentiation in a 3D format. Human PSCs seeded in the Silk 521 scaffold integrate, migrate, and form small colonies at day 1 post seeding and retain pluripotency thereafter. Human PSCs in the Silk 521 scaffold could be cultured using different types of medium, expressed pluripotent markers, spontaneously differentiated to all three tissues germ layers, and directly to neural fates. On the contrary, cells seeded in silk only (control), could hardly integrate into the foam and the cells did not amplify at all, regardless of media or cell line used, and regardless of the presence of ROCKi. Together these data highlight the biological effects of the laminin substrate within the Silk 521 biomaterial. In sum, Silk 521 is a biorelevant 3D system that is biodegradable and non-immunogenic, and thus ideal for cell culturing and many other biomedical applications.

F-3221

ASSOCIATION OF HIF-1A AND ALIX IN ENHANCED BIOGENESIS AND SECRETION OF EXOSOMES FROM HUMAN MESENCHYMAL STEM CELLS UNDER HYPOXIA

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With the advancements in research, it is now well-known that Mesenchymal Stem Cells (MSCs) exhibit their therapeutic effect via a paracrine mechanism where MSCs produce membrane-bound nanovesicles called exosomes. These exosomes contain biologically active proteins and miRNAs. Due to their role in cell free therapy, they are considered as ideal candidates for regenerative medicine. However, the yield of secreted exosomes is a limiting factor for obtaining sufficient amounts of exosomes for cell-free therapies. To this end, we cultured MSCs isolated from adipose tissue (ADSC) and bone marrow (BMSC) in different culture condition (hypoxia -2.5% Oxygen and normoxia-21% oxygen) for different time points, i.e., 24h and 48h. The cells were assessed for HIF-1α expression through qPCR, and conditioned media was used for exosome secretion using Nanoparticle Tracking Analysis (NTA).We further studied differential expression of genes involved in exosomes biogenesis and secretion with primary focus on ESCRT dependent pathway genes (ALIX, Tsg101) and secretion genes (Rab27a and Rab27b). It was found that tissue specific MSCs showed differential response to hypoxia treatment with 2 fold up regulation for HIF-1α expression 48h in ADSC while it was at 24h in BMSC. Also, NTA showed that secreted exosomes size range from 30nm to 150nm. Exosome secretion for ADSC was 1.4 X 106 at 48h in hypoxia while it was 1.6 X 106 for BMSC at 24h. This suggested that there is a direct co-relation between HIF-1α and exosome secretion. Also, with respect to exosome biogenesis and secretion gene expression across these tissue specific MSCs in hypoxia, there was specifically up regulation of ALIX expression. BMSC exhibits 2 fold increase in ALIX expression at 24h whereas it was a 6 fold increase in ADSC at 48h. Hence, we found that the expression of ALIX was directly related to HIF-1α expression. This study suggests that in MSCs there is ALIX dependent release of exosomes. Further studies need to be done for understanding the role of ALIX in MSCs derived exosome secretion.ALIX role in other cell type like Hela, DC cells is well known, however, it is still in its preliminary stage for MSCs.

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F-3223

REFERENCE CELL LINES FOR THE ANALYSIS OF ONCOGENIC TRANSFORMATION OF HUMAN PLURIPOTENT STEM CELLS

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The potential for tumor formation is a major safety concern for the use of cellular therapeutics derived from human pluripotent stem cells (hPSC). Low level product contamination with genetically abnormal cells with oncogenic potential poses particular challenges to safety assessment. At present there are no in vitro surrogate assays for oncogenic transformation of hPSC. While a few reports suggest that teratoma assays in mice can identify abnormal hPSC with malignant potential, there is limited evidence to validate this approach. Embryonal carcinoma (EC) cell lines provide a positive control for tumor formation, but often differ markedly from hPSC in their limited differentiation capacity and growth requirements in vitro. We describe two clonal cell lines derived from a primary teratocarcinoma of the testis which encompass a broad range of biological behavior. Both cell lines express pluripotency-associated cell surface markers and transcription factors, and both show karyotypic abnormalities

common to germ cell tumors and genetically abnormal hPSC. Cell line GCT27C4 shows high cloning efficiency in standard culture medium without feeder cell support, but its sibling GCT27D1, like normal hPSC, clones poorly even in the presence of a mouse embryo fibroblast feeder cell layer (cloning efficiency <0.1%). GCT27C4 shows little capacity for differentiation in vitro, but GCT27D1 can differentiate spontaneously into a range of cell types. The two cell lines form xenograft tumors in immunodeprived mice with similar efficiency. Tumors derived from GCT27C4 consist of undifferentiated EC cells only. Tumors derived from GCT27X1 contain derivatives of all three embryonic germ layers, but unlike tumors derived from normal hPSC, they also contain undifferentiated EC cells, sometimes accompanied by large blocks of primitive neuroectodermal tissue and yolk sac elements, features seen in malignant pediatric germ cell tumors. RNA-seq analysis reveals significant differences in gene expression between each of the two EC cell clones and normal hPSC cell lines. These two EC cell lines should prove useful in the refinement of teratoma assays and for the development of in vitro assays for oncogenic transformation of hPSC. We will distribute these cell lines through a central cell bank.

F-3225

COMPUTATIONAL MODELING OF NEURAL STEM CELL MIGRATION ROUTES IN THE BRAIN

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Neural stem cells (NSCs) are inherently patho-tropic and have been shown to be effective for tissue regeneration and delivery of therapeutics in cases of brain injuries and tumors. The delivery of NSCs in the brain can be either via intracranial, intraventricular or intranasal routes. The therapeutic efficiency of NSCs depends both on the distance between the injection site and target, as well as the route characteristics. Prediction of NSC delivery efficiency and routes of migration based on the injection site is instrumental for optimizing NSC dosage. We have recently shown the migration of NSCs along white matter in naïve mice brain. Here, we present a computational model for simulation of NSC migration within mice and human brain. Fractional anisotropy atlas of the mouse brain was obtained using Diffusion Tensor Magnetic Resonance Imaging (DT-MRI). The Fiber Assignment with Continuous Tracking (FACT) algorithm which is typically used in tractography, is used to predict migration routes from potential injection sites. The FACT algorithm generates the migration direction based on the direction of dominant eigenvector (from DT-MRI atlas) at the tissue voxel. An injection site was simulated as a specific region from which a number of paths would be stochastically simulated to originate from. Migration paths in mice brain are simulated with potential injection sites in the corpus callosum (CC injection) and olfactory bulb (OB injection). Results predicted NSC migration routes to be along the corpus callosum and towards the frontal cortex for the CC injection and towards the optic chiasm for the OB injection. The model is applied to human brain DT-MRI atlas to simulate NSC migration paths with putamen as the potential injection site. The model predicts the migration of NSCs to the frontal cortex and along the subventricular zones. The model is well poised to incorporate directed cues for simulation of NSC migration in the presence of tumors or injuries. A successful modeling framework verified against experimental data will be useful for optimizing stem cell therapeutic doses and modes of delivery.

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F-3227

A ROBUST METHOD FOR TAGGING ENDOGENOUS GENES THROUGH PROMOTER TRAPPING AND SHORT HOMOLOGY ARMS

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Precise genome editing via homology-directed repair (HDR) pathway holds great promise for gene and stem cell therapy. However, the efficiency of integrating large DNA molecules into mammalian genome via HDR is inherently low. Recently, we showed that the use of short homology arms (~35nt) was sufficient to introduce small changes in mammalian genome. Now, we take a step further and develop a novel method for tagging endogenous genes through promoter trapping and short homology arms, which dramatically increases the efficiency and specificity of integration. The efficiency of tagging endogenous genes with a 1.4 kb promoterless GFP reporter ranges from 50% to 100% upon antibiotic selection with higher level of specificity occuring at the C-terminus than at the N-terminus. The method has been validated using multiple targets in many different cell lines, including human induced pluripotent stem cells and hematopoietic stem cells. The basal expression levels of various fluorescent fusion proteins and their subcellular locations could be visualized by fluorescence microscopy or detected by western blotting. This method has broad applications in general genome engineering, DNA cloning, protein production and immune cell therapy.

Funding Source: Thermo Fisher scientific

F-3229

AN IMAGE PROCESSING METHOD FOR CREATING HIGH QUALITY IPS CELL STOCKS

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Cells used in research are generally stored as a certain amount of stockpile for maintaining cell performance. Regarding induced pluripotent stem (iPS) cells, we have previously reported that it is important to set an appropriate timing of cryopreservation after cell seeding for increasing the cell stock quality. In fact, iPS cells cryopreserved in the late stage of cell growth had less ability to attach to culture dishes despite high cell yield. We also assumed that morphological features such as colony size, colony-colony contact, and colony circularity before freezing also affect the iPS cell stock quality. This study aims to reveal which morphological parameters of iPS colonies would be effective to estimate cell stock quality and quantity prior to freeze-thawing. In the first step, we cultured iPS cells (line 201B7 and 1210B2) under multiple culture conditions (multiple seeding density, culture period, and the passage number), and obtained colony images by a common phase contrast microscope. We next freeze-thawed and cultured the iPS cells to confirm the stock conditions. In the second step, we applied an image analysis technique to evaluate multiple colony morphological parameters by a computational software, and then statistically analyzed the data. The result indicates that a proper combination of the threshold values for each parameter is effective for preventing cell death and greater stability in long-term cell culture after thawing. In summary, this method allows us to evaluate cells non-invasively and quantitatively for cryopreservation, and establish valuable iPS cell resources.

F-3231

A SAFEGUARD SYSTEM FOR HUMAN PLURIPOTENT STEM CELL-BASED THERAPIES

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Human pluripotent stem cells (hPSCs) hold enormous promise for cell-based therapy. However, the risk of generating malignancy from contaminating undifferentiated hPSCs in the final cell product for therapeutics is still one major concern for clinical applications. A suicide protein, iC9, containing a fusion between human Caspase 9 and FK506-binding protein, was developed previously. Dimerization of iC9 with AP1903, a small molecule which is well tolerated in culture cells and in clinical studies, activates one of the last steps in the apoptotic cascade to induce rapid cell death. To selectively eradicate undifferentiated hPSCs, we inserted a suicide gene in-frame into the stem cell-specific SOX2 locus in human embryonic stem cell line H1 (H1-iC9) by Crispr-Cas9. The inserted iC9 gene was under the direct transcription control of SOX2 while the endogenous SOX2 expression was not disrupted. This strategy restricted iC9 expression to undifferentiated cells. Administration of AP1903 dimerized iC9 and induced apoptosis of undifferentiated H1-iC9 cells, while the differentiation products derived from H1-iC9 cells, including hematopoietic cells, beta-like cells and neurons, were not affected. Our results showed the feasibility of using suicide gene to remove undifferentiated stem cells without affecting the viability of differentiated cell products. This strategy therefore provides a layer of safety control to reduce the risk of teratoma when applying hPSC-derived cell products in therapies.

Funding Source: Wanek Innovation Program

F-3233

STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS OF YM155 FOR INDUCING SELECTIVE CELL DEATH OF HUMAN PLURIPOTENT STEM CELLS

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Despite great potential for regenerative medicine, the high tumorigenic potential of human pluripotent stem cells (hPSCs) to form undesirable teratoma is an important technical hurdle preventing safe cell therapy. Various small molecules that induce the complete elimination of undifferentiated hPSCs, referred to as ‘stem-toxics’, have been developed to facilitate tumor-free cell therapy, including the Survivin inhibitor YM155. In the present work, based on the chemical structure of YM155, total 26 analogs were synthesized and tested for stem-toxic activity toward human embryonic stem cells (hESCs) and induced PSCs (iPSCs). We found that a hydrogen bond acceptor in the pyrazine ring of YM155 derivatives is critical for stem-toxic activity, which is completely lost in hESCs lacking SLC35F2 encoding a solute

carrier protein. These results suggest that hydrogen bonding interactions between the nitrogens of the pyrazine ring and the SLC35F2 protein are critical for entry of YM155 into hPSCs, and hence stem-toxic activity.

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F-3235

LEVERAGING ADVANCES IN BIOMEDICINE TOWARD DEVELOPMENT OF THE CELL-BASED MEAT INDUSTRY

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The utilization of animal stem cells to grow muscle and fat tissues in vitro for consumption, dubbed “cell-based meat,” offers an unprecedented opportunity to transform animal agriculture and produce meat in a humane and sustainable way. Here, we provide an industry snapshot and highlight advances in regenerative medicine, genetic engineering, bioengineering, and large-scale bioprocessing methods for their applicability to this fast-growing nascent industry. We discuss the current challenges in scaling cell-based meat production and opportunities for development of new tools, resources, or optimizations required to reach price parity with traditional animal meat.

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F-3239

EFFICIENT GMP-COMPLIANT EXPANSION OF MESENCHYMAL STROMAL CELLS (MSCS) IN A CLOSED CULTIVATION SYSTEM USING XENO-FREE MSC-BREW GMP MEDIUM

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Human mesenchymal stem cells (MSCs) hold great promise for clinical use and cell therapy applications and can be isolated from multiple tissue, e.g. bone marrow (BM), umbilical cord (UC) or adipose tissue (AT). As there is a high number of clinical trials and applications using MSCs, quality and safety of the resulting cellular products are indispensable. The xeno-free MSC-Brew GMP Medium developed and manufactured following the recommendations of USP <1043> on ancillary materials enables a standardized expansion and culture of MSC from different tissue sources. To increase the level of process standardization and product safety we developed the CliniMACS Prodigy® Adherent Cell Culture System for GMP compliant isolation and cultivation of adherent cells using a closed single used tubing set. The process includes the flexible combination of the following modules: 1. Density Gradient Centrifugation (DGC) 2. Surface coating 3. Inoculation 4. Culture 5. Media Change 6. Harvest. Here we show that MSCs from different tissue sources (AT, BM, UC) can be expanded and passaged using the CliniMACS Prodigy® Adherent Cell Culture System from primary tissue or single-cell suspensions combining the process modules in a flexible way. BM-MSCs were isolated via DGC and subsequent adherence to plastic using semi-automatic feeding, harvesting and reseeding procedures. AT-MSCs as well as UC-MSCs were isolated and expanded from single-cell suspensions obtained after enzymatic digestion. UC was digested using an optimized and automated procedure using the gentle MACSTM Dissociator. Subsequently, MSCs were isolated from single cell suspension using the flexible cultivation procedure of the CliniMACS Prodigy® Adherent Cell Culture System. Resulting cells displayed an MSC specific phenotype as defined by the ISCT consortium. Furthermore MSCs revealed their immunomodulatory potential as assessed by in vitro assay and multi-color flow cytometric analysis. With the CliniMACS Prodigy®, MSCs can be expanded in large scales, thus providing high numbers of cells for consecutive clinical applications.

F-3241

HUMAN IPS CELL-DERIVED PERIPHERAL NEURONS MODULATE TARGET ORGAN TISSUE FUNCTIONS BY CO-CULTURING IN MICROTUNNEL-EMBEDDED MICROFABRICATED DEVICE

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The peripheral nervous system (PNS) is connected to the central nervous system (CNS) in our body, and the PNS governs the homeostasis of various tissues and organs. Understanding the molecular mechanism of peripheral neuropathy progression and the interaction of the PNS with target organs might contribute to the development of novel therapeutic methods for a complete cure. Thus, induction of peripheral neurons in vitro and construction of co-culture model system of peripheral neurons and target organ tissues are useful for clarifying the progressive mechanism of PNS-related diseases. In this study, we have constructed co-culture networks using human PNS and target organs cells. First, we fabricated a poly(dimethylsiloxane) (PDMS) -based co-culture chamber, which consisted of two culture compartments connected with 20 microtunnels, and we cultured induced PNS and CNS neurons differentiated from human iPS (induced pluripotent stem) cells. Development of their functional

connections was evaluated with microscopic observations, immunochemical analysis, and calcium imaging. In particular, we confirmed that CNS neurons showed an increase in calcium signals during electrical stimulation of networked bundles from PNS neurons, which demonstrated the formation of functional cell-cell interactions. Furthermore, we prepared a co-culture system using PNS neurons and cardiomyocytes, both derived from human iPS cells, or insulin-secreting cells, to confirm that our microfabricated device can be used with various cell types. These co-culture systems would be a promising tool to form networks of PNS neurons and target organs, and might help to understand functional mechanisms under normal and pathological conditions.

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LATE-BREAKING ABSTRACTS

F-4001

HUMAN NAÏVE PLURIPOTENCY: VARIATIONS ON A THEME

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Although naïve mouse embryonic stem cells (mESC) are generally derived and maintained at normoxia (O2 > 19%), many protocols for the derivation or reversion of human pluripotent cells to a naïve state are performed under hypoxia (< 5% O2). We previously used RSeT medium under normoxia to evaluate differences between the naïve and primed pluripotent states described in the literature. We observed some results contrary to those published but it was unclear if these discrepancies were due to cells used, normoxic conditions or lab-to-lab variation. We have now compared 3 hESC lines in primed and 'naïve' culture conditions under normoxia and hypoxia with and without the use of the ROCK inhibitor, Y27632. Morphologically, naïve cells should be domed and exhibit rapid cell division, but we found that all 3 cell lines behaved differently in RSeT medium with H9 performing best and H1 performing worst. With the removal of Y27632 after passage 4, H1 cells exhibited extremely poor growth, which was exacerbated by hypoxia (3% O2). Hypoxia appeared to reduce the mitotic index of all lines under both culture conditions but did not otherwise affect morphology. H9 also outperformed H1 and H7 with respect to other indicators of the naive pluripotent state. For example, a reduction in global methylation was only observed in 'naïve' H9 cells under both hypoxia and normoxia, although 'naïve' H7 cells did show decreased methylation levels under hypoxia. In addition, when immunostained for H3K27me, only primed H9 cells demonstrated a punctate nuclear pattern, indicative of an inactive X chromosome, which was lacking in the 'naïve' cells. These data suggest that cell line choice strongly affects how successful naïve culture will be. In contrast, we found that discrepancies between our previous data and the published literature were not due to normoxia. We found agreement in upregulated RNA expression of several genes such as TFCP2L1, KLF2 and KLF4 under 'naïve' conditions but pluripotency markers such as POU5F1 and NANOG were still downregulated in our hands in both hypoxia and normoxia. Interestingly, these differences appeared more pronounced in cultures grown without Y27632. We will present further gene expression data regarding changes specific to hypoxia/normoxia, naïve/primed and +/- Y27632 as well as protein levels and metabolic analyses.

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F-4003

HSA-MIR-302S ARE ESSENTIAL FOR SELF-RENEW OF HUMAN PLURIPOTENT STEM CELLS

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Recent studies have revealed that human pluripotent stem cell (PSC)-specific microRNAs (miRs), including hsa-miR-302/367 cluster, are important for self-renewal, differentiation, and cellular reprogramming, however, their functional roles are largely unknown. Here, we showed that hsa-miR-302s are essential for self-renew of hPSCs by targeting hsa-miR-302/367 region via Cas9 nuclease complex with guide RNA and replacement that region to fluorescent protein. Using homologous donor with green or red florescence protein, we confirmed the deletion of the hsa-miR-302/367 region under microscope, but no homozygous knockout colony was detected, suggesting that hsa-miR-302/367 homozygous knock out cells were negatively selected. Next we analyzed the comprehensive expression dynamics of both miRs and mRNAs and identify 22 candidate targets of human PSC-specific miRs that were moderately expressed in undifferentiated hPSCs and more than 2-fold up-regulated during differentiation. The deleted in azoospermia-associated protein 2 (DAZAP2), one of those targets, was directly repressed by hsa-miR-302a/b/c/d, but not by hsa-miR-367. We found moderate expression of DAZAP2 mRNAs but hardly found expression of DAZAP2 proteins in undifferentiated iPSCs,

indicating that their expression was strictly regulated by hsa-miR-302s. Overexpression of dazap2 caused cell proliferation decreased in undifferentiated hPSCs, although morphology and undifferentiated marker gene expressions was not affected. In addition, neural differentiation was suppressed in DAZAP2 overexpressing hPSCs. Taken together, our study revealed that hsa-miR-302s control self-renew and also differentiation via repressing DAZAP2, suggest that overexpression of target genes could be a solution for dissecting functions of miR.

F-4005

CONCERNING THE UNCERTAINTY OF HUMAN NAÏVE PLURIPOTENCY

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To achieve precision use of desired human pluripotent stem cells (hPSCs) for medical and pharmaceutical applications, it is essential to have a thorough understanding of all fundamental properties of these starting cell sources. One of the most important properties is related to the ground or naïve pluripotent state that is primarily established in mouse embryonic stem cells (mESCs). Thus far, more than five well-established research groups have reported the existence of human naïve pluripotency, which is believed to have major benefits for hPSC growth, genetic engineering, disease modeling, and drug discovery. In this study, we have derived and characterized naïve-like hPSCs (NLPs) using five initially reported human naïve protocols under normoxic growth conditions. Our data indicated that there is a significant heterogeneity regarding various pluripotent states in NLPs using these protocols. In general, current derived NLPs lack some hallmarks of naïve pluripotency as described in mESCs. Briefly, these NLPs exhibit much lower single-cell plating efficiency and growth rates, commonly lack unique mouse naïve cell surface marker (e.g., SSEA-1) expression, and have altered or abrogated cell growth under normoxia. Moreover, some examined NLPs are independent of BMP4 signaling and sensitive to the inhibitor of the Janus kinase (JAK). The genome-wide meta-analysis also revealed various fundamental inconsistencies between NLPs and their human and mouse counterparts. Thus, our data suggest the existence of dynamic pluripotent states in these derived NLPs, arguing about the rationale of genuine naïve pluripotency in these cells under normoxic growth conditions. To further recapitulate physiologically relevant human naïve pluripotency in hPSCs under hypoxic growth conditions would warrant their future broad applications.

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F-4009

OVEREXPRESSION OF BCL-2 AND BDNF AS EFFECTIVE PROTECTORS AGAINST INDUCED CELL DEATH AND EFFICIENT INDUCERS OF THE DIFFERENTIATION OF HUMAN WHARTONS JELLY MESENCHYMAL STEM CELLS INTO DOPAMINERGIC NEURONS

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Dopamine (DA) neurons, which are derived from MSCs, are a valuable source for cell replacement therapy in Parkinson's disease. Although there have been numerous studies to develop a protocol for differentiating MSCs into DAergic neurons in recent years, there is still no efficient method that enables a relatively homogeneous population of functional DAergic neurons with an increased resistance to cell-death factors that could be used for transplantation to be obtained. We think that cells that have all of the above-mentioned features will survive after transplantation and will create a network of functional neurons. We hypothesize that this can be done because of the synergistic overexpression of Bcl-2 and BDNF. A stable overexpression of Bcl-2 in MSCs protects cells from apoptotic factors and a stable overexpression of BDNF enables MSCs to differentiate into functional DAergic neurons. We determined that the synergistic overexpression of Bcl-2 and BDNF in MSCs is stable and lasts at least 60 days. As a result, the level of Bcl-2 and BDNF proteins significantly exaggerated the level that was observed in MSCs without an overexperssion. These results suggest that the Bcl-2 and BDNF proteins that are produced are functional because the transfected MSCs are more resistant to the apoptotic factors that induce cell death via intrinsic and extrinsic pathways. Overexpressed MSCs with a high efficiency differentiated into DAergic cells (thyrosine hydroxylase possitive). The trace expression of the genes that are typical for neurons with different phenotypes was also observed. The new cells produced dopamine, and after depolarization, their quantity increased sharply. These results suggest that the new population of dopaminergic neurons was homogeneous and functional. The results, which are based on the synergistic overexpression of genes, may indicate a new pathway for experiments to obtain homogeneous populations of functional neurons with an increased survival capacity. In this model, the overexpressed genes can be changed to obtain

a homogenous population of different neurons that can be investigated. In the near future, I plan to study the functionality of the obtained DAergic neuron population in a rat model of Parkinson's disease.

F-4011

SALL3 EXPRESSION BALANCE UNDERLIES LINEAGE BIASES IN HUMAN IPS CELL DIFFERENTIATION

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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. For efficient selection of hiPSC lines suitable for differentiation into desired cell lineages, we tried identifying the marker genes that potentially predict the differentiation propensity of hiPSCs into three germ layers. Our approach for identifying differentiation propensity markers is essentially based on the statistical comparison of the geneexpression profiles of undifferentiated hiPSCs with each cell line's in vitro differentiation potential using the rank correlation method. In this study, we identified the SALL3 gene as predicting differentiation propensity using the rank correlation method and analysis of 10 hiPSC lines. We show that SALL3 expression correlates positively with ectoderm differentiation and negatively with mesoderm/endoderm differentiation during embryoid body (EB) formation and that SALL3 inversely regulates the capacities of cardiac and neural differentiation in hiPSCs. Mechanistically, SALL3 is found to repress gene body methylation in hiPSCs, leading to their epigenetic changes. Our findings provide a practical method for selecting appropriate hPSC lines in clinical-grade cell banks, allowing for the prediction of differentiation capacity toward a desired cell lineage.

F-4013

EFFICIENT GENERATION OF CLINICAL-GRADE MIDBRAIN DOPAMINERGIC NEURAL PROGENITORS FROM HUMAN EMBRYONIC STEM CELLS

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Differentiation of human pluripotent stem cells (hPSCs) into midbrain dopaminergic neural progenitors (mDA) promises a cell-based therapy for Parkinson's disease. Here, we report the generation of clinical-grade mDA from embryonic stem cells (ESCs) under defined xeno-free conditions. By optimizing the treatment time of SHH and the concentration of CHIR99021, we could efficiently generate EN1+ mDA and developed a chemical recipe and a streamlined protocol. Using this protocol, the percentage of EN1 positive cells increased from 20% to about 50%. The cells also expressed appropriate mDA markers, such as FOXA2, LMX1A and NURR1. Upon transplantation into brains of rat model of Parkinson's disease, they exhibited sufficient safety and reasonable efficacy. Therefore, this protocol enables advancement of stem cell-based therapies towards neural regeneration.

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F-4015

NFIA IS A GLIOGENIC SWITCH ENABLING RAPID DERIVATION OF FUNCTIONAL HUMAN ASTROCYTES FROM PLURIPOTENT STEM CELLS

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Astrocytes are the most abundant glial cell type in the human brain, and their dysfunction is a driver in the pathogenesis of both neurodevelopmental and neurodegenerative disorders. During early development, neural stem cells (NSCs) are fate-restricted to exclusively produce neurons, while at later stages, they undergo a switch from neurogenic to gliogenic competency resulting in progressive production of astrocytes and oligodendrocytes. The molecular nature of the gliogenic switch has remained elusive, and its timing varies dramatically across species from 7 days in the mouse to 6-9 months during human development. Those species-specific timing differences similarly apply to NSCs derived from human pluripotent stem cells (hPSCs). The highly protracted timing of acquiring glial competency in hPSCs presents a major roadblock in the quest for deriving human astrocytes for basic and translational applications. Here, we identify Nuclear Factor IA (NFIA) as the molecular switch for inducing human glial competency. Transient expression of NFIA for 5 days, in the presence of factors promoting glial differentiation, is sufficient to trigger glial competency and generate hPSC-derived astrocytes as compared to 3-6 months of differentiation using current protocols. NFIA-induced astrocytes promote synaptogenesis, exhibit neuroprotective properties, display calcium transients in response to appropriate stimuli, and engraft in the adult brain. Finally, NFIA-induced astrocytes can be induced to express features of region-specific and of reactive astrocytes. The underlying mechanism of NFIA-induced glial competency involves rapid but reversible chromatin remodeling, GFAP promoter demethylation, and a striking lengthening of the G1 phase in the cell cycle. Genetic or pharmacological manipulation of G1 length partially mimics NFIA function in glial competency. Our study addresses a significant roadblock in hPSC and glial biology by defining key mechanistic features of the gliogenic switch and by enabling the rapid production of human astrocytes for disease modeling and regenerative medicine.

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F-4017

WNT INHIBITION AND BMP4 REGULATE OCULAR SURFACE EPITHELIAL FATE IN HUMAN PLURIPOTENT STEM CELLS

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The corneal epithelium is derived from the ocular surface ectoderm (OSE) via the non-neural ectoderm. How and when the OSE commits to presumptive corneal epithelial cells, however, is not well defined. What is well-known, is that BMP and WNT signalling pathways have crucial roles within cells during various stages of development. BMP4 is expressed during the beginning of spontaneous human induced pluripotent stem cells (hiPSC) differentiation, as well as gastrulation, and cells exposed to BMP4 are directed towards an ectodermal fate. Simultaneously, WNT signalling is precisely controlled for optic cup formation in eye development. Standard hiPSC differentiation in vitro, involves a cultivation period of 10 to 12 weeks, before isolation of presumptive corneal epithelial cells that express p63 and PAX6, markers for epithelial stem cells and OSE, respectively. Interestingly, we found that p63 and PAX6 double positive (p63+/PAX6+) cells were detected by immunofluorescence at 10 days of differentiation, and this finding is mimicked in vivo by p63+/PAX6+ cells within mouse embryonic eye at E8.5. Based on these findings, hiPSCs were treated with a combination of WNT inhibitor (IWP2) and BMP4, during the initial 4 days of differentiation, to observe effects of OSE formation at 10 days and 6 weeks of differentiation. A p63 knock-in hiPSC-line was used in order to trace epithelial stem cells via EGFP expression. To determine the OSE differentiation efficiency, p63-EGFP+ cells were isolated by FACS. p63-EGFP+ cells were then stained with immunofluorescent-PAX6, in order to quantify the p63+/PAX6+ cell ratio. Results showed 1.66-fold increase on 10 days of differentiation and 3.24-fold increase on 6 weeks of differentiation, of p63+/PAX6+ cells compared to non-treated cells. As such, combined treatment of IWP2 and exogenous BMP4, greatly enhanced OSE differentiation efficiency. Furthermore, the data indicates simultaneous WNT inhibition and BMP4 were promoted not only p63+ cells, but considerably maintained p63+/PAX6+ cells within the OSE. Therefore, surface ectodermal cell lineage is likely determined during early eye development. This study has great future potential for research to clarify the intricate mechanisms of epithelial development, as well as OSE cell lineage determination.

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F-4019

REPROGRAMMING IPSCS INTO GLUTAMATERGIC NEURONS - RECONSTRUCTION OF TRANSCRIPTIONAL EVENTS AND CELLULAR STATES USING AN INTEGRATED GENOMIC APPROACH

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Direct cell reprogramming is a rapidly growing field that challenges traditional concepts of cellular identity. Expression of Neurogenin 2 (NGN2) has been used to efficiently reprogram human pluripotent stem cells (PSCs) into functional cortical glutamatergic neurons. This approach combines the advantage of the highly proliferative and epigenetically malleable PSC stage with the efficiency of direct reprogramming. We have previously demonstrated that gene targeting the components of a Tet-On system into two separate safe harbour sites overcomes gene silencing, results in optimised transgene expression in hiPSCs (OptiOx), and yields highly homogenous cultures of pure glutamatergic neurons within less than four days. The mechanisms that mediate this remarkable cellular metamorphosis remain poorly understood. To study the transcriptional events occurring as a result of NGN2 expression, three independent biological replicates were harvested at the iPSC stage (day 0), and 6h, 12h, 24h, 36h, day 2, day 3, day 4, day 14, and day 21 post induction. Cell extracts were subsequently processed for bulk RNA sequencing. This demonstrated distinct and highly reproducible transcriptional changes across each of the individual time points. To gain a more detailed insight into the cellular states during reprogramming, we complemented our bulk-RNA-Seq approach with single cell RNA sequencing of cells harvested at the iPSC stage (day 0), as well as 12h, 24h, day 2, day 3, day 4, day 14, and day 21 post induction. Louvain network analysis demonstrated distinct transient populations through which cells synchronously progressed with little heterogeneity until attaining a distinct neuronal phenotype. In order to differentiate direct from indirect NGN2 down-stream effectors, an inducible hiPSC cell line in which NGN2 tagged with HA was constructed. CHiP-Seq data acquired on day 2 after induction was subsequently overlaid with bulk seq data and single cell seq data. This enabled identification of direct and indirect effectors of NGN2 with regards to gene regulatory networks as well as peripheral genes. In conclusion, we provide a detailed analysis of transcriptional events that govern the transition of iPSCs into mature glutamatergic neurons following NGN2 expression.

F-4021

MEDIAL INDUCTION ACCELERATES NEUROEPITHELIAL CONVERSION OF HUMAN PLURIPOTENT STEM CELLS

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Differentiation of human pluripotent stem cells (hPSCs) into ectoderm provides neurons and glia useful for research, disease modeling, drug discovery, and potential cell therapies. In current protocols, hPSCs are traditionally differentiated into ectoderm after 6 to 10 days in vitro when protected from mesendoderm inducers. This protracted timing has made ectoderm a difficult germ layer to access and manipulate, hindering development of efficient differentiation protocols for ectoderm-derived cell types. Here we report efficient and serum-free differentiation of hPSCs into ectoderm within 24 hours using a novel combination of chemical inhibitors. This method is greater than 70% efficient, is broadly applicable to a panel of five independent hPSC lines, and accelerates the emergence of downstream intermediate and terminal neurodevelopmental landmarks. Given its rapid and flexible nature, we expect this method to democratize the development and execution of significantly more efficient protocols for the differentiation of ectoderm-derived cell types from hPSCs.

F-4023

DEVELOPMENT OF COMMERCIALLY VIABLE MANUFACTURING PROCESSES FOR CELL THERAPY PRODUCTS DERIVED FROM IPSCS

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Human induced pluripotent stem cells (iPSCs) reprogrammed from different type of starting materials are able to renew indefinitely and differentiate into specialized cell types of the body. These characteristics give iPSCs very attractive potential as unlimited source of cells for a wide range of cell therapy applications. While current processes for derivation, expansion, and directed differentiation of iPSCs are significantly improved and demonstrate potentials of these cells, they face significant manufacturing challenges because they are open, manual, and carried out under uncontrolled 2D environment. To address these challenges and allow manufacturing of iPSC derived CT products under commercial setting, it is important to develop robust and reproducible processes by implementing appropriate process optimization and process control strategies along with incorporating innovative technologies during the development phase. We previously reported manufacturing of human iPSC master cell banks (MCB) under current Good Manufacturing Practices (cGMP). We have also shown that these cGMP compliant iPSCs can be differentiated into specialized cell types from all three embryonic lineages with morphological and cellular characteristics of cardiomyocytes, definitive endoderm (DE), and neural stem cells (NSCs) in 2D. Here we report our approach for development of a robust and reproducible expansion and directed differentiation of iPSCs under controlled condition in 3D in computer controlled bioreactors. In particular, we demonstrate that these fully characterized human iPSC lines can readily differentiate into cardiomyocytes, and the directed differentiation process can be further optimized to establish a robust and reproducible process. This is a major step forward in the development of a salable and commercially viable cGMP-compliant manufacturing possess to generate clinical quantities of iPSC derived cell therapy products.

F-4025

INTENSIVE GENETIC QUALITY TESTS OF GMP COMPLIANT IPSC AND THEIR DERIVATIVES

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Most hiPSC lines are karyotypically normal during long-term maintenance however, still possess mutations in risk, therefore, it is imperative to perform intensive genetic quality controls in clinical grade iPSC, better in cost-effective manner. As a conceptual approach, we performed an intensive genetic quality test with a clinical grade homozygous HLA iPSC and their derivatives in post-distributing condition using a single RNA sequencing data. We found that degree of accumulated SNPs by prolonged culture and differentiation were differ by institutes. However, CNVs and mutations in HLA molecular types were not detected in any of the samples. The RNA expression levels of cancerous and immunogenic genes were not different between the early and late passaged iPSC and their derivatives. Advanced time-course analysis techniques identified 5 clusters showing different patterns in prolonged -culture condition and 40 clusters remaining in similar pattern. Importantly, we were able to access theses genetic quality testing results using a single RNA sequencing data. We believe the benefit of theses analysis approaches and the post-distribution monitoring would facilitate identification of a right iPSC seed stock for the clinical application.

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F-4027

TIMED INDUCTION OF MIXL1 PLAYS A ROLE IN ENDODERM BIAS IN MOUSE EPIBLAST STEM CELLS

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In early mouse embryos, gastrulation forms the three germ layers from which all cells of the mouse will derive. The signalling factors and gene networks involved in the formation of the mouse endoderm have not been fully elucidated. We have focused on Mix1 homeobox-like 1, encoded by Mixl1, which is expressed in the primitive streak during gastrulation and is vital for the formation of the endoderm. Previous in vitro studies have shown that Mixl1 expression is necessary for both haematopoietic and endoderm differentiation. Furthermore, a previous study within the lab found a correlation between the timing of Mixl1 expression and germ layer specification. However, the role of Mixl1 expression timing as well as the direct targets of Mixl1 had not been fully explored. In attempting to determine how Mixl1 could be responsible for both mesoderm and endoderm differentiation, we used epiblast stem cells (EpiSC) that are inducible for Mixl1. Using timed doxycycline inductions of Mixl1, we have performed the first ChIP-seq analysis of Mixl1 in a proper gastrulation model. We have built a comprehensive gene regulatory network centred on Mixl1 by combining ATAC-seq and RNA-seq in the same cell conditions and adding on published data on promoter capture Hi-C in EpiSC and histone methylation marker ChIP-seq in human embryonic stem cells. We found that the early induction of Mixl1 during gastrulation results in the expression of the anterior primitive streak markers, Gsc and Lhx1, from where the endoderm progenitors emerge. Mixl1 also activates WNT signalling pathway genes and results in reduced accessibility in genes involved in stem cell maintenance. Moreover, we have found Mixl1 timing to be instrumental in endoderm differentiation. Together, these results suggest that early Mixl1 activation pushes cells towards endodermal progenitors within the anterior primitive streak.

F-4029

MODELING THE CARDIAC ELECTRICAL PHENOTYPE OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE USING PATIENT IPS CELL-DERIVED CARDIOMYOCYTES

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Mutations in PKD1 or PKD2 gene lead to autosomal dominant polycystic kidney disease (ADPKD). The mechanism of ADPKD progression and its link to increased cardiovascular mortality is still elusive. In this study, we applied patient-specific induced pluripotent stem cells (iPSCs) to establish a human cell-based, in vitro model for studying ADPKD-associated cardiac manifestations. We efficiently generated iPSC-derived cardiomyocytes (CMs) with predominantly ventricular-like cells that exhibited PKD1 and PKD2 expression. The ADPKD patient-specific iPSC-CMs had decreased sarcoplasmic reticulum calcium content compared with control-CMs. Spontaneous action potential of the PKD2 mutation line-derived CMs demonstrated lower beating rate and longer action potential duration. The PKD1 mutation line-derived CMs showed a comparable dose-dependent shortening of phase II repolarization with the control-CMs, but a significant increase in beating frequency in response to L-type calcium channel blocker. The PKD1-mutant iPSC-CMs also showed a relatively unstable baseline as a greater percentage of cells exhibited delayed afterdepolarizations (DADs). Both the ADPKD patient-specific iPSC-CMs showed more β-adrenergic agonist-elicited DADs compared with control-CMs suggesting a possible common proarrythmogenic phenotype of ADPKD. The close mimicry of the electrophysiological characteristics of patient-specific iPSC-CMs with their clinical cardiovascular phenotype demonstrated that iPSC-CMs may be invaluable in disease modeling and as a drug screening platform for ADPKD-associated cardiovascular complications.

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F-4031

HOMOLOGY-MEDIATED END JOINING (HMEJ)-BASED STRATEGY IN GENOME EDITING AND GENE THERAPIES IN MOUSE AND MONKEY EMBRYO

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As a promising genome editing platform, the CRISPR/Cas9 system has great potential for efficient genetic manipulation, especially for targeted integration of transgenes. However, due to the low efficiency of homologous recombination (HR) and various indel mutations of non-homologous end joining (NHEJ)-based strategies in non-dividing cells, in vivo genome editing remains a great challenge. Here, we describe a homology mediated end joining (HMEJ)-based CRISPR/Cas9 system for efficient in vivo precise targeted integration. In this system, the targeted genome and the donor vector containing homology arms (~800 bp) flanked by single guide RNA (sgRNA) target sequences are cleaved by CRISPR/Cas9. This HMEJ-based strategy achieves efficient transgene integration in mouse and monkey zygotes,as well as in hepatocytes and neurons in vivo. Moreover, this HMEJ-based strategy offers an efficient approach for correction of fumarylacetoacetate hydrolase (Fah) mutation in the hepatocytes and rescues Fah-deficiency

induced liver failure mice.Taken together, focusing on targeted integration, this HMEJ-based strategy provides a promising tool for a variety of applications, including generation of genetically modified animal models and targeted gene therapies.

F-4033

DEVELOPING STEM CELL-BASED ASSAYS FOR NEUROLOGICAL DISEASE DRUG DISCOVERY

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A major challenge in neurological drug discovery has been establishing translatability between use of heterologous cell lines in preclinical studies and predictive responses in patients. The application of human induced pluripotent stem cells (iPSCs) represents a promising avenue for drug discovery where patient-specific cellular phenotypes that model aspects of neurological diseases can translate into clinically relevant outcomes. Our goal was to develop iPSC-based assays using patient-derived iPSCs and iPSC-derived cells to support neurological drug discovery efforts. We are interested in evaluating assay performance in self renewing iPSC vs iPSC-motor neurons as the latter is the most relevant to phenotypes that manifest in patients. Here, we present iPSC-based assays for two phenotypic discovery projects. The assays are evaluating protein readouts in response to treatment with well characterized tool compounds and antisense oligonucleotides (ASO). The first assay measures downregulation of a protein in response to treatment with small molecules or knockdown ASO in wild type iPSCs and iPSC-motor neurons using electrochemiluminescence. The second assay uses undifferentiated patient-derived iPSCs and patient iPSC-motor neurons to determine an increase in expression of a protein by Homogenous Time-Resolved Fluorescence (HTRF). For the second assay, we are using two patient-derived iPSC lines and their respective iPSC-motor neurons to assess assay performance across different iPSC lines. We will present assay performance characteristics including signal window, assay sensitivity, specificity and consistency across runs. Preliminary data for the second assay indicate a robust performance with a high signal to background ratio and low inter-assay variability. Differences between iPSC lines were subtle. This assay was very scalable for HTS and was adapted to automation without compromising assay metrics. We ran pilot studies using informer compound sets to thoroughly assess these assays prior to HTS. The HTS and follow up experiments using iPSC-derived motor neurons are in progress and will be presented.

F-4035

PATIENT-SPECIFIC IPSC-DERIVED KERATINOCYTES RECAPITULATE THE GENETIC DEFECTS IN PSORIASIS DISEASE

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Psoriasis is a chronic inflammatory disease of the skin characterized by keratinocytes hyperproliferation, affecting 2-3% of world population. The etiology of psoriasis is complex and is supposed to result from the interplay between genetic predisposition and environmental factors. A major limitation in understanding the pathogenesis of psoriasis and subsequent development of novel therapies is the lack of suitable in vitro models mimicking the disease. Recent progress in the generation of induced pluripotent stem cells (iPSCs) has provided unique opportunities for obtaining patient specific cells for modeling skin diseases and future clinical applications. In the present work, we showed the successful generation of iPSCs from patients having familial history of psoriasis as well as from healthy controls. The generated iPSC lines were characterized for the pluripotency and self-renewal properties. All iPSCs lines showed normal karyotype, expressed pluripotency markers and differentiated into all three germ layers upon spontaneous and direct differentiation. The pluripotent ability of the generated iPSC lines was further validated by the TaqMan hPSC Scorecard assay in vitro. Patient-specific iPSCs and healthy controls were efficiently differentiated into keratinocyte progenitors and mature keratinocytes, which expressed the key markers, including p63,

KRT18, KRT14, LORICRIN, KRT1, INVOLCURIN, and LAMININ. High throughput sequencing analysis showed dysregulated expression of psoriasis-associated genes in patient specific iPSC-derived keratinocytes. Those genes are mainly involved in epithelial proliferation, keratinocyte differentiation, and in the immune system. These include members of the solute carriers family, interleukins, Human leukocytes antigen (HLA) and Keratins (KRT) as well as filaggrin (FLG), involucrin (IVL), TGM5, PSORSICI genes shown to be implicated in the pathogenesis of psoriasis. Our results demonstrate successful modeling of psoriasis from patient derived iPSCs that could be used to understand the pathogenesis of psoriasis and screening of novel therapeutics.

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F-4037

OPTIMIZED PROTOCOL FOR GENERATING SAFE SPINAL CORD NEURAL STEM CELLS FROM HUMAN EMBRYONIC STEM CELLS

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Recently, we reported the generation of spinal cord neural stem cells(NSCs) from human pluripotent stem cells (hPSCs) (Kumamaru et al., 2018, Nat Methods). However, the prolonged culture of NSCs with high doses of small molecules, such as a SMAD inhibitor, SB431542 and GSK-3 inhibitor, CHIR99021, may cause chromosomal instability. To reduce the potential negative effect of small molecules used in the neural induction and maintenance of spinal cord neural stem cells, we reduced the concentration of CHIR99021 and SB431542. In addition, we carefully assessed the effects of the concentration of FGF proteins and reduced their concentration as well. We successfully developed a modified protocol with reduced doses of small molecules and FGF proteins while retaining high efficiency in generating and maintaining spinal cord NSCs. In optimum culture conditions, the spinal cord NSCs show an equivalent identity to the original protocol and can be maintained for prolonged time periods, up to twenty passages. Cluster analysis of RNAseq data shows that hPSC-derived spinal cord NSCs are closely grouped with human fetal spinal cord tissue, and karyotype analysis shows that 8 out of 10 batches exhibited normal karyotype. Two-three months after transplantation into injured spinal cords of rats and monkeys, the grafted human NSCs survive and integrated well without evident expansion. In addition, grafted human spinal cord NSCs differentiate into both neurons and glia and extended very large numbers of axons into the host spinal cord. Long-term studies are underway to assess the safety of human spinal cord NSCs for potential human translation.

F-4039

EFFECT OF TRISOMY 21 ON THE DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TO NEURAL PROGENITOR CELLS

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Down syndrome (DS) is characterized by a complex phenotype, including intellectual disability, developmental complications, and chronic health conditions. The ubiquity of cognitive deficits in DS has made structural and cellular changes in the brain the focus of much research effort. However, no prior studies have utilized DS induced pluripotent stem cells (iPSC) to assess the effect of trisomy 21 on neurogenesis. The main aim of the current study was to define, evaluate, and compare neural differentiation in DS and euploid iPSCs through the formation and maturation of embryoid bodies (EB) to neural progenitor cells (NPC). Embryoid body formation is an important initial step and common platform in directed differentiation protocols that is often overlooked and under-researched. Here we show that DS iPSCs produce larger and more EBs compared to their euploid counterparts. We found that several neural differentiation markers, such as TuJ1 (neurons) and GFAP (glial cells), are upregulated in DS EBs. Furthermore, DS EBs showed increased expression of several genes involved in the TGF-β pathway – a critical regulator of pluripotency in embryonic stem cells and lineage determination in progenitor cells. In particular, DS EBs showed an increased gene expression of several bone morphogenetic proteins (BMP) which have been shown to switch progenitors to an astrocytic fate. These observations and the lack of proper neural rosette formation in DS iPSCs suggest accelerated neural and glial differentiation and reduced progenitor cell development. Irregular cell fate specification in DS iPSCs is consistent with the fact that DS individuals show an increased number of astrocytes. Our results demonstrate an increased number of differentiated neurons and glial cells, and dysregulation in cell lineage specification early in development of the DS brain, which may lead to altered synapse formation, function, and elimination, decreasing the efficiency of neuronal

transmission. Due to the lack of previous research on this topic, this experimental data provides evidence that trisomy 21 affects EB formation and NPC cell fate specification during neurogenesis.

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F-4041

A MUTATION IN SCN1A SELECTIVELY IMPAIRS IPSCS-DERIVED INHIBITORY NEURONS DERIVED FROM A PATIENT WITH DRAVET SYNDROME

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Dravet syndrome (DS), a devastating type of infantile-onset epilepsy that presents with cognitive deficits and autistic traits, is caused by a mutation in SCN1A, which encodes the α-subunit of the voltage-gated sodium channel, Nav1.1. Several types of mutations, including nonsense, frame-shift, and missense mutations, located at different sites in SCN1A have been identified in patients with DS. Excitatory/inhibitory (E/I) imbalance in the cerebral cortex can cause central nervous system disorders, such as DS (or epilepsy). However, the underlying cellular disturbance remains ill-defined owing to the reliance of available knowledge on animal models that are not readily transferable to the syndrome in humans. Recently, we generated induced pluripotent stem cells (iPSCs) derived from a DS patient (D1) with a c.4933C>T substitution in SCN1A predicted to cause truncation in the fourth homologous domain of the protein (p.R1645*). Moreover, to elucidate the mechanism of neurodegeneration in DS caused by c.4933C>T mutation, we performed gene correction in D1 iPSCs using TALEN (transcription-activator-like effector nuclease)-mediated genome editing, generating D1 TALEN iPSCs. In this study, we generated excitatory or inhibitory neurons by employing direct in vitro conversion of iPSCs through the overexpression of specific transcription factor cocktails as a novel approach for neuronal differentiation. These cells were seeded on multi-electrode array (MEA) systems, which is a measuring device with multiple electrodes integrated in a cell culture dish, and the spontaneous neuronal activity was recorded. We present data comparing excitatory or inhibitory neurons derived from healthy (WT), DS (D1), and isogenic control (D1 TALEN) iPSCs that were measured using MEA systems. We found differences in physiological activity between WT and D1 inhibitory neurons. The spontaneous firing of D1 inhibitory neurons was significantly impaired compared to that in WT neurons. Inhibitory neurons derived from iPSCs with the c.4933C>T mutation in SCN1A (D1) showed fewer spontaneous spikes in the burst. This phenotype was rescued in the isogenic control inhibitory neurons (D1 TALEN) to a level equal to that in the WT neurons.

F-4043

PHYSIOLOGICAL ANALYSIS OF PATIENT SPECIFIC IPSC DERIVED DOPAMINERGIC NEURONS; PARKINSON'S DISEASE MODELING

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Neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and other age related dementias are incurable diseases. Parkinson's disease (PD) is the second most common neurodegenerative disease which is a slowly progressive disease and is the result of degeneration of neuromelanin(NM)-containing dopamine neurons(DA) in the substantia nigra par compacta. There are many PD disease models, but in vitro models are limited which have difficulties in mimicking PD. Here, we observed clinical potentials of iPSC derived dopaminergic neurons from PD patients. First data showed distinct differentiation markers were expressed each steps of differentiation, such as progenitor, development and maturation. Dopamine secretion was confirmed by LC-MS method. Next step, Drug test was performed using Patch-Clamp detection. Action-potential is detected at –57mV. The frequency decreased at 10 uM of L-Dopa but two action potentials appeared simultaneously, 30 μM L-Dopa increased the frequency compared to the action potential of the control in the current clamp mode. In the voltage clamp mode, the frequency of dopaminergic neurons were increased and the amplitude of L-Dopa increased at 30uM. However, the 60uM L-dopa increased the frequency compare to 10uM L-dopa but decreased level than 30uM L-dopa. Dose dependent dopamine releasing result using high sensitive ELISA showed similar releasing patterns as patch clamp data. As a result, dopaminergic neurons differentiated from PD patient iPSCs were showed recovery results as L-dopa treatment, so patient specific iPSCs can be useful tools for modeling Parkinson's disease.

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F-4045

3D MODEL OF PARKINSONS DISEASE SPECIFIC-DOPAMINERGIC NEURONS FOR HIGH-THROUGHPUT PHENOTYPING AND DRUG SCREENING

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Parkinson's disease (PD) is a neurodegenerative disease which is characterized by the progressive loss of dopaminergic neurons in the substantia nigra which leads to motor dysfunction. The high heterogeneity of the disease and the lack of preclinical models that recapitulate the features of PD prohibiting successful development of neuroprotective therapies. By using induced pluripotent stem cell (iPSC) technology we have a powerful tool in hand to develop in vitro PD disease models applicable in the field of regenerative medicine. Here, we describe the development of a human 3D Parkinson's disease model of iPSC-derived dopaminergic neurons in Mimetas' OrganoPlate® to discover new drug candidates. The culture platform contains 96 tissue chips with 3D microfluidic channels that support the development of neuronal cultures of patient-derived iPSCs towards an in vitro phenotype resembling that of dopaminergic neurons in vivo. Here, intermediate human neuroepithelial stem cells were seeded pre-mixed in Matrigel into the OrganoPlate® and differentiated for 4 weeks towards functional dopaminergic neurons. Immunostaining confirmed the presence of dopaminergic neurons in a 3D environment. Imaging of calcium fluctuations showed the spontaneously electrophysiological activity of the neurons, which is a characteristic feature of mature neurons in vivo. Mito-functionality assay has been developed to screen potential mitochondrial dysfunction. We developed an industrial quality microfluidic PD model containing, mature, differentiated, midbrain-like dopaminergic neurons. This PD model will be applied in high-throughput screening to identify mitochondrial dysfunction in PD patients and finally used to develop new candidate neuroprotective agents for PD patients.

F-4047

IN VIVO SIMULTANEOUS TRANSCRIPTIONAL ACTIVATION OF MLTIPLE GENES IN THE BRAIN USING CRISPR-DCAS9-ACTIVATOR TRANSGENIC MICE

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Despite rapid progresses in the genome-editing field, in vivo simultaneous overexpression of multiple genes remains challenging. We generated a transgenic mouse using an improved dCas9 system that enables simultaneous and precise in vivo transcriptional activation of multiple genes and long noncoding RNAs in the nervous system. As proof of concept, we were able to use targeted activation of endogenous neurogenic genes in these transgenic mice to directly and efficiently convert astrocytes into functional neurons in vivo. This system provides a flexible and rapid screening platform for studying complex gene networks and gain-of-function phenotypes in the mammalian brain.

F-4049

REPROGRAMMING ENRICHES FOR SOMATIC CELL CLONES WITH SMALL SCALE MUTATIONS IN CANCER-RELATED GENES

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Recent studies observed high mutational load in iPSCs, which is largely derived from their parental cells, but as yet it is unknown whether reprogramming may enrich for individual mutations that pre-exist in the parental cell population. We have derived 30 human iPSC clones from neonatal and aged individuals under comparable conditions. High accuracy exome and amplicon sequencing showed that all analyzed small scale variants pre-existed in their parental cell population. We demonstrate that individual mutations present in small subpopulations of parental cells become enriched among iPSC clones during reprogramming. Evaluation of the variant impact and gene function in cellular processes and in cancer development imply a potential role of some of those mutations as putatively actionable driver mutations. Especially as some of the enriched, putatively actionable mutations affect genes of cell death / survival, cell cycle control, and pluripotency, somatic cells carrying such a mutation might experience a selective advantage during reprogramming. In view of the various common characteristics of (pluripotent) stem cells and cancer stem cells, the same mutations are likely to account for an increased tumor risk. Notable, on average, iPSCs of aged donors carry a higher number of these putatively actionable mutations. The reprogramming-associated selection for individual potentially pathogenic or carcinogenic mutations that have been acquired during lifetime may impact the clinical value of patient-derived iPSCs.

F-4051

SAFER AND MORE STABLE IPSC GENERATED USING DOGGYBONE DNA VECTORS

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The application of induced pluripotent stem cell (iPSC)-derived cells in clinical trials is in its infancy but the potential is vast. A key asset of iPSCs is the ability to apply autologous cell therapies, but to date most current or approved clinical trials are using fully characterised allogeneic or non-allogeneic cell banks alongside immunosuppressive drugs. Until now, all current or approved clinical trials worldwide utilise iPSC generated using EBNA1 expressing plasmids containing the OriP sequence to maintain a self-replicating episome. These vectors are amplified in bacterial hosts and contain bacterial DNA motifs recognised by the transfected cell's innate and intrinsic interferon host defense responses. Moreover, the continued forced expression of the Epstein-Barr virus EBNA1 protein is known to cause widespread alterations in gene expression as well as elevated oxidative stress and DNA damage. All of these have potentially significant implications for the safe clinical use of iPSC generated using OriP/EBNA1 plasmid episomes. We describe efficient iPSC reprogramming by applying equivalent gene sequences transiently expressed from Doggybone DNA (dbDNA) vectors free of OriP/EBNA1 sequences, bacterial motifs and produced in a chemically defined, low endotoxin, cGMP compliant manufacture. In direct comparator experiments with the current state-of-the-art OriP/EBNA1 episomes, dbDNA vectors produced iPSC colonies with the same efficiency but dbDNA-iPSC displayed evidence of greater stability in terms of maintenance of pluripotency. Mechanistic evaluations showed that the persistence of OriP/EBNA1 episomes resulted in elevated STAT1/IFN signalling in iPSC when compared to dbDNA generated iPSC. This in turn resulted in increased spontaneous differentiation and slowing of the cell cycle in OriP/EBNA1-iPSC. We propose a potential that utilising dbDNA vectors presents a safer and more stable approach to iPSC production and development.

F-4053

INDUCING ANTI-TUMOR IMMUNITY WITH DENDRITIC CELL REPROGRAMMING

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Cell fate reprogramming towards pluripotency or unrelated somatic cell-types have highlighted the plasticity of adult somatic cells, providing new technologies to generate any desired cell type for regenerative medicine. Dendritic cells (DCs) are professional antigen presenting cells specialized in the recognition, processing and presentation of antigens to T-cells. In particular, the mouse conventional DC type 1 (cDC1) subset excels on the ability to perform antigen cross-presentation on MHC-I, a critical step for inducing cytotoxic responses. We hypothesized that the unique properties of cDC1s could be induced in unrelated cell-types, allowing the direct control

of immune responses with cell reprogramming. Here, the requirements to induce cDC1s were investigated using a combinatorial overexpression of transcription factors (TFs) in Clec9a-tdTomato mouse fibroblasts. In the hematopoietic system, this reporter system specifically marks the DC lineage, including all cDC1 cells. We have identified Pu.1, Irf8 and Batf3 as sufficient and necessary to induce Clec9a reporter activation, establish DC morphology and activate a cDC1 transcriptional program in mouse fibroblasts. The overexpression of the three factors ignites the expression of cDC1 markers, major histocompatibility complex (MHC) class I and II molecules and co-stimulatory receptors. Functionally, induced DCs (iDCs) secrete inflammatory cytokines and engulf, process and present antigens to CD4+ T cells. Remarkably, iDCs have established the competence for cross-presentation, a hallmark of cDC1s, eliciting antigen-specific CD8+ T-cell responses. We have shown that intra-tumoral vaccination of B16-OVA and EG7-OVA syngeneic tumor mouse models with iDCs conferred protection against tumor growth. Moreover, injection of iDCs increased infiltration of antigen-specific CD8+ T cells in the tumor, as well as promoted cytotoxic profile of T-cells in the draining lymph nodes. Hence, we have shown for the first time that antigen presentation can be programmed by a small combination of TFs providing evidence that mouse immune cells and triggered immune responses can be modulated through cell reprogramming. Collectively our work represents a platform for future development of cancer immunotherapies based on DC reprogramming.

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F-4055

DISTINCT ROUTES CONVERGE ON A UNIFYING TRANSITION LOGIC TO ESTABLISH NAÏVE PLURIPOTENCY

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To decipher how cellular identity is instructed by interplay between transcription factors and signals, we employ defined reprogramming systems in which genetic and signalling parameters can be independently varied and successfully transitioning cells isolated. We show that naïve pluripotency can be induced from EpiSCs along transcriptionally and mechanistically distinct routes. Relative to development, one route moves forward, with productive cells acquiring mesodermal signature prior to naïve pluripotency induction. In contrast, another route goes backwards, transcriptionally resembling the earlier embryo and gaining its greater developmental potency. Nevertheless, these distinct trajectories reach the same endpoint, demonstrating surprising flexibility for the establishment of a single identity from a single origin. We reconcile route differences, revealing precise Oct4 expression as a unifying, essential and sufficient feature. We propose that fine-tuned regulation of this “transition” factor underpins multidimensional access to the naïve identity. This offers a conceptual framework for the understanding of cell identity transitions.

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F-4057

RHINO INDUCED PLURIPOTENT STEMCELLS TO RESCUE THE NORTHERN WHITE RHINO

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The northern white rhinoceros (NWR, Ceratotherium simum cottoni) is the most endangered mammal at present. Only two females have survived and even before the death of the last male, both were incapable of natural reproduction. The only way to create a self-sustaining population of the NWR is the use of assisted reproduction technologies combined with emerging stem cell technologies. A crucial step in this process is gaining knowledge of the regulation of pluripotency and differentiation. Here, we show that pluripotency states of northern white rhino (NWR) and the human are comparable in terms of growth conditions and certain marker gene expression. We derived intrinsically renewing NWR iPSCs and show that these cells can differentiate to progeny of the germ layers when applying protocols optimized for human iPSCs. Finally, we ectopically express Bcl2 and show that ground state cells can integrate in mouse epiblast-stage chimeras. Moreover, we were able to establish ESC from southern white rhinos which also can be differentiated into three germ layers. We hope by using these “gold standard“ pluripotent stem cells to establish a germ cell differentiation protocol. Taken together, we have generated NWR iPSCs and ESCs, as well as validated both pluripotent maintenance as well as germ layer differentiation in this critically endangered mammal, with a view to optimizing germ lineage differentiation. This could one day provide a tool to help restore the NWR using cryopreserved somatic cells.

F-4059

DCM-TIME-MACHINE: A NOVEL METHOD TO DETECT TRANSCRIPTION RETROSPECTIVELY

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A doxycyclin inducible DCM-RpolIIb fusion gene is able to methylate actively transcribed genes. Bacterial DCM methylation introduced in the murine epigenome is detected using MeD-seq together with the endogenous CpG methylation and does not affect gene expression. DCM methylation is propagated to daughter cells enabling retrospective analysis of gene expression. Using the murine small intestine as a validation model we were able to trace stem cell expression. DCM methylation changes during pulse labeling generated distinct DCM methylation profiles corresponding to specific intestinal cell types.

F-4061

A SCALABLE AND PHYSIOLOGICALLY RELEVANT SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELL EXPANSION AND DIFFERENTIATION

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Human induced pluripotent stem cells (iPSCs) and their derivatives are needed in large. However, scalable and cost-effective manufacturing of high quality iPSCs and their derivatives remains a challenge. In vivo, cells reside in a 3D microenvironment that has cell-cell and cell-extracellular matrix interactions, sufficient nutrients and oxygen, and minimal hydrodynamic stress. The current iPSC culturing methods provide highly-stressed microenvironments, leading to low efficiency. For instance, iPSCs typically expanded 4-fold/4 days to yield ~2.0x10^6 cells/mL with current 3D suspension culturing. These cells occupy ~0.4% of the bioreactor volume. To our best knowledge, the largest culture volume for iPSCs is less than 10 liters. There is a critical need to develop new culture technologies to achieve the iPSCs' potential.We report a novel technology that can overcome all limitations of current methods and provide a physiologically-relevant culture microenvironment. iPSCs are processed into and cultured in microscale alginate hydrogel tubes termed stress-free intratubular cell culture (SFIT) that are suspended in the cell culture medium (Figs. 1A and B). The hydrogel tubes create free microspaces that allow cells to interact with each other and expand. Meanwhile, they protect cells from hydrodynamic stresses in the culture vessel and confine the cell mass 10 passages) of iPSCs without uncontrolled differentiation and chromosomal abnormalities. Cultures between batches and cell lines were very consistent. iPSCs in SFIT had high viability, growth rate (1000-fold/10 days/ passage in general) and yield (~5x10^8 cells/mL microspace). The expansion per passage (e.g. up to 4200-fold/passage) and volumetric yield are much higher than current methods, which significantly reduce the culture volume and time, and the production cost, making large-scale cell production technically and commercially feasible. iPSCs could be efficiently differentiated into various cells in SFIT. Additionally, other human cells, such as T cells, could also be efficiently cultured in this technology. Two SFIT-based automated bioreactors for producing autologous and allogenic iPSCs and their derivatives are under developing. This technology has high potential to address the cell manufacturing challenge.

F-4063

CHARACTERIZATION OF HUMAN ADIPOSE STEM CELLS GROWING IN OPTIMIZED SERUM-FREE XENO-FREE MEDIA

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Adipose stem/stromal cells (ASCs) are a type of adult mesenchymal stem cells (MSCs) that can be easily isolated from adipose tissue. ASCs can be cultured in vitro over several passages, but conventional culture conditions involve the use of human or animal serum to enhance cell attachment and promote cell survival and proliferation. Since these cells or their conditioned media, which contains many beneficial secretory factors, are increasingly being used in regenerative medicine, it is crucial to eliminate serum supplements to ensure scalability and prevent adverse immune reactions. We aim to formulate a unique serum-free media (SFM) that supports the growth and expansion of human ASCs in vitro, and, to characterize their cell viability, proliferation, differentiation potential and secretory factor profiles. ASCs were isolated from adipose tissue obtained from human donors through liposuction. These cells were then cultured in vitro using either media supplemented with 2% human serum or SFM developed in our laboratory. Morphology, viability, proliferation rates, differentiation potentials, and cytokine profiles were evaluated in both groups of cells. Our SFM supported the growth and proliferation of ASCs up to passage 14 in vitro while maintaining their mesenchymal stem cell characteristics and stemness. These cells were successfully cultured in monolayers and three-dimensional scaffolds, and, were able to differentiate into adipocytes, chondrocytes and osteocytes. In addition, they secreted less pro-inflammatory chemokines, unlike ASCs cultured in conventional media supplemented with 2% human serum. These ASCs or their

conditioned media, which exhibits biological activity, may thus be safely used for downstream cosmetological or regenerative applications without the risk of viral transmission or adverse immunological reactions.

F-4065

HIGH THROUGHPUT BANKING AND CARDIAC DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN A SUSPENSION BIOREACTOR

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Human induced pluripotent stem cells (hiPSCs) are one of the most promising options for regenerative medicine, disease modeling, toxicity testing and drug discovery. However, the current reliance on 'traditional' culture systems for propagation of hiPSCs and differentiated progenitors are intensive in time, labour and reagent costs, restricting investments into actual research using these tools. To provide, at low cost, the needed quantity of high-quality hiPSCs and hiPSC derived differentiated cells, technologies and methodologies for cell culture have to fundamentally evolve. To address this problem, in conjunction with the European Bank for induced Pluripotent Stem Cells (EBiSC), Fraunhofer-IBMT have established a novel system for culturing and differentiating hiPSCs in suspension. Here, the bioreactor system BioLevitatorTM (now CERO from OLS OMNI Life Science) was used in combination with innovative impeller-free vessels to avoid high shear forces and culture multiple hiPSC lines in parallel, simultaneously. Disease-unaffected EBiSC hiPSC lines were thawed and directly inoculated with alginate microcarriers and after only one passage between thaw and harvest, at least 54 vials with 1x106 cells per vial were banked for each line. Media volume, matrix volume, staff time and overall culture time were significantly reduced in comparison to traditional culture methods. All banked lines showed viability, recovery, adhesion rate, expression of pluripotency markers and karyology comparable to banking using traditional two-dimensional culture systems. Additionally, using this bioreactor system, bulk production of spheroids and subsequent differentiation of two hiPSC lines into autonomously contracting cardiac clusters was achieved. This generated 7500 cardiac clusters in just 10 ml of differentiation medium for each hiPSC line. Cardiac clusters successfully expressed lineage-specific markers Cx43 and α-Actinin and at least 93% were spontaneously contracting. In conclusion, we present a multifunctional system for expansion and banking, as well as cardiac differentiation of hiPSCs in suspension. EBiSC2 aims to integrate such valuable tools to overcome current limitations in generating large numbers of high-quality cells from many donors in parallel.

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F-4067

EFFICIENT OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS BY DIRECT DELIVERY OF LINEAGE-SPECIFIC TRANSCRIPTION FACTORS

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Mesenchymal stem cells (MSCs) have a great potential for regenerative medicine due to their multi-lineage differentiation potency. Especially, many studies have revealed that the osteogenic differentiation of MSCs can contribute to the treatment of bone diseases such as osteoporosis. However, the precise control of their lineage commitments has been regarded as a key issue in the therapeutic applications because MSCs have multipotency and many factors are involved in MSC differentiation to each lineage. Although some groups have reported the methods to control MSC differentiation by delivering lineage-specific genes, these approaches have limitations such as clinical incompatibility or low efficiency. We hypothesized that direct delivery of osteoblast-specific transcription factors (TFs) can induce osteogenesis of MSCs without any genetic manipulations. In this study, we produced Osterix, a typical osteogenic TF, as recombinant protein using E.coli expression system. To intracellularly deliver the recombinant protein, a silkworm-derived cell-penetrating protein, 30Kc19 was fused to the Osterix. The fusion protein was efficiently delivered into the cells without any significant cytotoxicity and some of the protein was located at nucleic space. By alizarin red S and alkaline phosphatase staining, we determined that osteoblast differentiation of MSCs was significantly augmented when the cells cultured in osteogenic medium were repeatedly treated with Osterix-30Kc19. Quantitative real-time PCR analysis also demonstrated the expression of osteoblast-specific markers such as Runx2 and Osteocalcin was increased by Osterix-

30Kc19. Consequently, our strategy to directly deliver Osterix using cell-permeable 30Kc19 protein is anticipated to hold great potential for treating bone disorders in stem cell-based therapies.

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F-4069

CAR-T MEDIA DEVELOPMENT: NOVEL FORMULATIONS FOCUSED ON IMPROVED TRANSDUCTION EFFICIENCY AND PRESERVATION OF RELEVANT T CELL SUBPOPULATIONS.

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Adoptive immunotherapy involves patient T cell expansion in optimal conditions for the purpose of re-infusing their progeny as therapy. This calls for the generation and characterization of novel media supplements and formulations to boost the progress of the cell therapy market by providing physiologically relevant protein sources in concentrations suitable for biological activity. PhysiologixTM XF Human Growth Factor Concentrate (hGFC) is a cGMP, xeno-free serum replacement that replaces supplements such as fetal bovine serum (FBS) or human serum (HS). In this study, T cell media such as OpTmizer and X-VIVO 15 were supplemented with 5% human serum or 2% PhysiologixTM XF. In addition, RPMI 1640 with 10% FBS was compared due to its prevalent use in non-clinical settings. T cells from three healthy donors were activated with CD3/CD28 Dynabeads and then cultured for 14 days in varying media formulations. Proliferative capacity was assessed with flow cytometry. PhysiologixTM XF showed increased potency relative to human serum when used along X-VIVO 15 or OpTmizer media. Differentiation status was assessed using CCR7 and CD45RO to distinguish T cell subpopulations. We detected an increase in naÃ¯ve and central memory populations when cells were cultured in PhysiologixTM XF compared to human serum. These phenotypes are highly relevant for the outcome of patients undergoing CAR-T cell therapy. To further define the advantage of our supplement in generating therapy ready products, we compared the transduction efficiency of a lentiviral-GFP in OpTmizer, X-VIVO 15 or RPMI supplemented with 2% PhysiologixTM XF or 5% HS at different multiplicities of infection (MOIs). A higher transduction efficiency was consistently observed when using PhysiologixTM XF at a high MOI of 4 down to a MOI of < 1. These results were also observed for anti-GD-2 virus. This increased transduction efficiency and preservation of T cell subpopulations can translate into a higher number of transfusable CAR+ cells with relevant phenotypes which allow for reduced overall costs and better clinical outcomes. The ability of anti-GD-2 CAR-T cells expanded in medium supplemented with PhysiologixTM XF to directly kill target cells and control tumor burden in an aggressive leukemia xenograft model is discussed.

POSTER III - EVEN
19:00 – 20:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

F-2002

A QUEST FOR OPTIMIZING IN VITRO HMSCS EXPANSION PRECONDITIONS: A COMPARATIVE PROTEOMIC ANALYSIS EMPHASIZES 3D-SPECIFICATIONS WITH 1% OXYGEN

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Human Mesenchymal Stem/Stromal Cells (hMSCs) has the potential for numerous regenerative clinical applications. But so far there is no defined culture condition, as a standard protocol for the in vitro expansion of hMSCs. We hypothesized that the common norm of two-dimensional (2D) culture expansion with atmospheric oxygen levels (21% O2) could alter the properties of the de novo hMSCs. Thus, we attempted to provide natural homely 3D-microenvironmental space with 1% physiological oxygen. In this study, we have done the comparative proteomic analysis of human Wharton's Jelly-derived MSCs (hWJMSCs) cultured in four different culture conditions (2D Normoxia (21% O2), 2D Hypoxia (1% O2), 3D Normoxia (21% O2) and 3D Hypoxia (1% O2) by two-dimensional gel electrophoresis and mass spectrophotometry. The comparative proteomic data revealed that among the culture groups of 2D Normoxia Vs. 2D Hypoxia 12 protein spots; 2D Normoxia Vs. 3D Normoxia 20 protein spots; 2D Normoxia Vs. 3D Hypoxia 23 protein spots; 2D Hypoxia Vs. 3D Hypoxia 16 protein spots and between 3D Normoxia Vs. 3D Hypoxia 5 protein spots were differentially expressed. Further identification of 20 spots by LC-MS/MS revealed that several filaments network proteins including plectin, vimentin, keratin, tropomyosin, and myosin isoforms differentially expressed between 2D and 3D culture conditions; insisting on the role of the microenvironment in cytoskeleton reorganization, cell shape, cell binding, focal adhesion and migration of hMSCs. Interestingly, 3D Hypoxia (1% O2) cultured hWJMSCs showed enhanced expression of galectin-1 than

other culture conditions. Galectin-1 involved in the inhibition of immune effector cells in an inflammatory setting. These findings along with relatively superior multilineage-differentiation capability, better in vivo performing exosomes packed with key therapeutic molecules, elevated pluripotency-associated gene expression, greater levels of immunomodulatory cytokine expression, higher expression of genes related to cellular migration is implicating that 3D Hypoxia (1% O2) culture condition maintains better intrinsic properties of hMSCs and also primes them with enhanced immunomodulatory potential, in turn providing better culture condition for expansion of hWJMSCs for clinical applications.

Funding Source: We would like to thank UGC for SRF to Balasubramanian. DBT, Govt of India for Ramalingaswami Fellowship to Sanjay and Research grants. Also appreciate Core support grants from CSCR. Technical help from CSCR core is acknowledged.

F-2004

TISSUE-SPECIFIC INTERACTIONS OF HUMAN MESENCHYMAL STROMAL CELLS (HMSCS) IN MODULATING PERIPHERAL B LYMPHOCYTES MATURATION

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B lymphocytes, also called B cells, are a type of white blood cell within the immune system. Circulating in the blood and activated by pathogens/antigens, peripheral B cells play an important role in both physiological and pathological conditions, being responsible for the humoral immunity of the adaptive immune system as well as acting as professional antigen-presenting cells (APCs) to critically orchestrate T cell responses. Despite the importance of B cells, surprisingly little is known about their interactions with mesenchymal stromal cells (MSCs), a type of multilineage somatic progenitor cell with strong immunomodulatory properties. First isolated from the bone marrow (BM), MSCs have since been found to exist in numerous adult as well as fetal-derived tissues/organs. Given that the BM is the site for B lymphocyte development, interactions between B cells and MSCs from this source are likely to be different from MSCs derived from other sources. We therefore were interested in the interactions of B cells to BM-MSCs and term placenta-derived MSCs (P-MSCs). We found that P-MSCs but not BM-MSCs affect the maturation of activated B cell at several stages. Moreover, similar findings were seen in an in vivo mouse model of B cell activation. Our preliminary findings demonstrate that B cell interactions with MSCs can differ significantly depending on the source of MSCs. Studies are ongoing to elucidate the mechanisms mediating tissue-specific MSCs interactions with this important population of the adaptive immune system.

ADIPOSE AND CONNECTIVE TISSUE

F-2006

HUMAN ADIPOSE-DERIVED STROMAL CELL PROTEOME

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Human adipose-derived stromal cells are often marketed as stem cell treatments despite the fact that there is little to no scientific evidence of their safety let alone efficacy. The number of predatory clinics offering these unproven stem cell treatments is rising, with clinics across the globe in countries such as Australia, Japan, Canada and the United States. Despite their wide use, surprisingly little is known about these cells. This study is the first to characterise the proteome of human adipose-derived stromal cells and compares both fresh and cryogenically preserved samples. 10 patients underwent liposuction and adipose-derived stromal cells were extracted and expanded. Whole cell lysates, membrane bound fractions and extracellular vesicles were analysed on Q Exactive Plus Orbitrap mass spectrometer, resulting in the detection of more than 5000 proteins per patient sample. Whole cell lysates provided vital insight into cellular function, while analysis of membrane bound proteins provided an extensive catalogue of cell surface markers that are useful for antibody-based assay development. Extracellular vesicles were investigated as adipose-derived stromal cells secrete them in substantial quantities and they are known to play a significant role in cancer, injury healing and immune suppression. As expected, there was variation in the detectable proteome across patients, however almost half of the detected proteins were conserved across all 10 patient samples, providing unique insight into the phenotype and functionality of these cells. 27 secreted cytokines were also investigated through the utilisation of a Bioplex multiplex immunoassay as cytokines facilitate cellular communication of immune signals. With adipose-derived stromal cell use rising in unproven stem cell treatments it is crucial that more research is conducted to identify exactly how these cells function. This novel study provided fundamental insight by identifying and comparing the proteome of fresh and cryogenically preserved human adipose-derived stromal cells.

F-2008

CHARACTERIZATION OF HUMAN BONE MARROW- AND ADIPOSE TISSUE DERIVED- MESENCHYMAL STROMAL CELLS IN AN IMPROVED ANIMAL COMPONENT-FREE CULTURE MEDIUM

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We characterized Mesenchymal Stromal Cells (MSCs) derived from human bone marrow (BM) and adipose (AD) tissues in an improved animal component-free (ACF) culture medium (MesenCult™-ACF Plus) and in medium containing fetal bovine serum (FBS). Clonogenic growth was evaluated by plating BM mononuclear cells (MNCs) or AD-derived stromal cells (SCs) at low density in the Colony-Forming Unit-Fibroblast (CFU-F) assay. The proliferative potential of BM and AD-derived MSCs was measured by determining cell number at each passage (P) up to P6 (BM-MSCs) or P9 (AD-MSCs). MSCs from both tissue sources were plated at 1.5–3x10^3 cells/cm^2 in each medium for long-term cell expansion. Immunosuppression of CD4+ T cells by BM-MSCs cultured in MesenCult™-ACF Plus (MACF-P) was evaluated by co-culture of BM-MSCs with Peripheral Blood Mononuclear Cells (PBMCs). The PBMCs were labelled with eFluor450, activated with human CD3/CD28 T Cell Activator and then analyzed by flow cytometry for T cell proliferation after 5 days. Total CFU-F per 10^6 AD-SCs and per 10^6 BM-MNCs was comparable in MACF-P and FBS containing medium (AD-SCs: 236 ± 127 vs. 268 ± 120 [mean ± SEM; n=3]; BM-MNCs: 37 ± 7 vs. 32 ± 5 [mean ± SEM; n=6] in MACF-P and FBS-containing media, respectively). However, the average fold-expansion of AD-MSCs at each subculture over 9 passages was significantly higher in MACF-P medium (12.2 ± 0.9; mean ± SEM; n=5) than in FBS-containing medium (3.8 ± 0.3; mean ± SEM; n=5; $p<0.05$). Similarly, the average fold-expansion of BM-MSCs at each subculture over 6 passages was also significantly higher in MACF-P (11.6 ± 1.2; mean ± SEM; n=5) than in FBS-containing medium (3.9 ± 0.4; mean ± SEM; n=5; $p<0.05$). Both AD-MSCs and BM-MSCs cultured in MACF-P differentiated more robustly in vitro under the appropriate conditions into adipogenic, osteogenic and chondrogenic cells compared to the same cells cultured in FBS-containing medium. Co-culture of BM-MSCs with PBMCs in MACF-P indicated that proliferation of CD4+ T cells is suppressed in a cell concentration-dependent manner when BM-MSCs were added in vitro. These data demonstrate that both BM-MSCs and AD-MSCs can be efficiently derived and expanded in MACF-P medium under complete ACF conditions and that BM-MSCs cultured in MACF-P medium exhibit immunosuppressive activity in vitro

F-2010

SUV39H1 AND CITED2 ARE NEGATIVE REGULATORS OF HUMAN ADIPOGENESIS

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Human adipogenesis is the process through which uncommitted human mesenchymal stem cells (hMSCs) differentiate into adipocytes. Understanding the molecular and cellular regulation of human adipogenesis may provide a way to prevent and/or treat obesity and obesity related diseases. Based on a siRNA high throughput screen, a list of genes whose expression knock-down by its siRNAs led to enhanced adipogenic differentiation of hMSCs were uncovered, including SUV39H1 and CITED2. SUV39H1 encodes a histone methyltrasferase that catalyze H3K9Me3, while CITED2 encodes a CBP/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2. The role of SUV39H1 and CITED2 during human stem cell fate commitment has never been studied. Here we report that both SUV39H1 and CITED2 are down regulated during adipogenic differentiation of hMSCs. Single knockdown of either SUV39H1 or CITED2 by siRNA significantly promoted adipogenic differentiation of hMSCs by both accelerating fat accumulation in individual adipocytes and increasing the number of adipocytes, despite slight reduction in total cell numbers as compared to control treatment. In addition, both siSUV39H1 and siCITED2 were able to promote hMSCs to commit to adipogenic lineage even in the presence of osteogenic inducing media, which normally only induces osteogenic differentiation of hMSCs in the absence of siSUV39H1 or siCITED2. Furthermore, siSUV39H1 significantly enhanced adipogenic differentiation of human dermal fibroblasts, which was further improved by siCITED2. Concomitantly, expression of CEBPα and PPARγ, two master regulators of adipogenesis, were both markedly increased in siSUV39H1 and siCITED2 individually transfected cells as compared to siControl cells. Finally, simultaneous knockdown of both SUV39H1 and CITED2 demonstrated an accumulative effect on promoting both adipogenesis and the expression of CEBPα and PPARγ. Taken together, this study demonstrates that SUV39H1 and CITED2 are both negative regulators of human adipogenesis, whose expression knockdown exerts

accumulative effect in promoting adipogenic differentiation efficiency through both accelerated adipogenic maturation and enhanced adipogenic fate commitment, and such effect was mediated by promoting the expression of CEBPα and PPARγ.

Funding Source: NIH grant (1SC3GM116720) 2016 - 2020, PI Yuanxiang Zhao

MUSCULOSKELETAL TISSUE

F-2012

IN VITRO REGULATION OF MOUSE MUSCLE STEM CELL DIFFERENTIATION BY ESTROGEN (E2) AND EICOSAPENTAENOIC ACID (EPA)

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Myogenesis involves interactions between signal transduction pathways and transcription factors resulting in the expression of myogenic regulatory factors (Mrfs). In-vivo, skeletal muscle mass is maintained by E2 and polyunsaturated fatty acids (ie: EPA) due to their anti-inflammatory characteristics and ability to modify the expression of Mrfs suggesting that they should be incorporated into in-vitro models of skeletal muscle differentiation. However, inconsistent reported effects of E2 and EPA on in-vitro myogenesis discourage their use in skeletal muscle regenerative studies. Using C2C12 mouse myoblasts to reiterate myogenesis in-vitro, the present study provides (a) time-dependent (0-120hr) immunofluorescence imaging and targeted gene expression (qPCR) analyses during differentiation in 10nM E2, 50μM EPA and 10nM E2/50μM EPA treatments, (b) a bench mark global transcriptome (Illumina HiSeq 2500 RNA-seq) at 48hr differentiation in E2 or EPA. We show that E2 augments myotube formation and fusion index at 48 and 120hr, while EPA and E2/EPA treatments significantly inhibit tube formation and reduce fusion index ($P<0.001$). E2 increases the expression of ERα and the signal transduction pathway genes MAPK and Akt ($P<0.01$) within 1hr from treatment leading to increased expression of Mrfs genes (MyoD, myogenine, Myh1) and the membrane fusion gene Tmeme8c ($P<0.01$) at 48hr or 120hr. EPA and E2/EPA treatments have no effect on E2 receptor expression and significantly reduce the expression of genes associated with the myogenic pathway and membrane fusion ($P<0.01$). Cell number and viability are not affected by treatment. We also show that a whole transcriptome profile following E2 treatment is similar to control profile but significantly different to EPA treatment with 4,000/15,000 transcripts expressed differently ($P<0.05$-0.001). The latter are associated primarily with the myogenic, muscle contraction, E2 metabolism, fat metabolism and signal transduction pathways. Results from the present study suggest that in-vitro regulation of myogenesis is significantly different between E2 and EPA. What directs the cellular response to augment or attenuate in-vitro myogenesis by E2 and EPA or which cellular mechanism(s) they use to regulate myogenesis remains equivocal and requires further research.

Funding Source: ACURF project grant 2016 to Orly Lacham-Kaplan

F-2014

THE IDENTIFICATION OF SMALL MOLECULES REGULATING PAX7 EXPRESSION IN SKELETAL MUSCLE PROGENITOR CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Embryonic and fetal skeletal muscle progenitor cells (SMPCs) and adult skeletal muscle stem cells (satellite cells, SCs), identifiable by their expression of transcription factor PAX7, participate in developmental myogenesis and postnatal muscle regeneration, respectively. The lack of ability to maintain pure SMPC and SC populations in vitro poses great challenges for analyzing these populations in detail, both for better understanding of the basic biology of human skeletal muscle development as well as for potential translational utilization in cell replacement therapies including for muscle disorders such as Duchenne Muscular Dystrophy. Human pluripotent stem cells (hPSCs) present a source for modeling human skeletal myogenesis and generating SMPCs and SCs for regenerative medicine. However, current hPSC directed myogenic differentiation protocols always generate heterogenous cultures comprised of both myogenic and other cell types. Moreover, different myogenic commitment states coexist within the myogenic subpopulations themselves, and PAX7 expression gets lost over continuous passaging in culture. To address these concerns, we developed a high-throughput small molecule screen and identified small molecule candidates that maintain PAX7 expression in hPSC-derived SMPCs. We hypothesize that these novel small molecules modulate survival or self-renewal pathways in SMPCs and enable expansion while maintaining stemness. In conjunction with developing strategies to expand SMPC populations, we have utilized single-cell RNA-sequencing to identify cell surface markers that not only enable isolation of myogenic cells from other cell types, but also enrich for PAX7-expressing SMPCs while excluding those of more differentiated MYOD- or MYOG-positive myogenic states. This is crucial considering that previous studies in mice and human clinical trials have shown that only cells of high stemness with the ability to self-renew are capable of conferring long-term regeneration in vivo. In summary, our combined approaches to purify and sustain

hPSC-derived SMPCs will enable detailed mechanistic studies of SMPCs and potentially SCs derived from hPSCs and human development and facilitate the evaluation of potential myogenic populations for cell-based therapies to treat muscle diseases.

Funding Source: Support from the UCLA Center for Duchenne Muscular Dystrophy Ruth L. Kirschstein National Research Service Award T32AR065972, CIRM Grant DISC2-10695, and NIH/NIAMS Grant R01 AR064327.

F-2016

G-RESIST: A DEEPLY QUIESCENT STATE ADOPTED BY STEM CELLS IN RESPONSE TO DISTANT LIVER INJURY

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Skeletal muscle stem cells (MuSCs), also known as satellite cells, are the central factors responsible for muscle repair and regeneration. In normal conditions, these cells reside in host muscle in a quiescent state. An injury to their host muscle induces these cells to enter the cell cycle and divide, a process known as activation. Previous work has shown that following muscle injury, or isolation-induced activation, MuSCs from adult mice require ~50 hours to complete cell division, MuSCs from humans require ~70 hours. In our previous work, we found that speed of MuSC activation was plastic and could be regulated. We identified that the HGFA-HGF-cMet-mTORC1 pathway could induce MuSCs into a primed state of quiescence in which, following muscle injury, they are able to activate much more quickly than normal, in ~35 hours. We termed this primed quiescent state “GAlert”. Because the systemic factor responsible for stimulating MuSCs into GAlert, Hepatocyte Growth Factor Activator (HGFA), is primarily produced and regulated by the liver, we decided to test how MuSCs could response to changes in liver function. We performed liver injury by partial hepatectomy (PHX) and, to our surprise, found that 2 days after PHX, MuSCs required significantly longer time, ~70 hours, to activate compared to the MuSC from animals that received a sham surgery. Furthermore, PHX had a significant impact on muscle regeneration. Animals that were subject to muscle injuries several days after PHX displayed profound impairments and delays in muscle regeneration. Interestingly, these PHX-induce defects in MuSC function and muscle regeneration were transient, 30 days after PHX MuSC function and muscle regeneration returned to normal. Using mouse genetic models, we have identified that these PHX-induce MuSCs functional changes are independent of HGFA levels or activity. We propose that we have identified a novel functional response by MuSCs in which they enter a, reversible, deeply quiescent state and have low muscle regenerative potential. We putatively term this state “GResist”. It is well known that patients with liver disease and impaired liver function have a potently impaired ability to heal wounds and injuries, anywhere on their body, we suspect that this systemic, stem cell, response to liver injury is a mechanism that underlies this connection.

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F-2018

PLURIPOTENT STEM CELL DERIVED MUSCLE FUNCTION ASSAY FOR DUCHENNE AND BECKERS MUSCULAR DYSTROPHY

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Duchenne’s Muscular Dystrophy (DMD) and Becker’s muscular dystrophy (BMD) are X-linked diseases that are caused by a mutation in the DMD gene that will eventually progress to fatality. Recent evidence suggests that SkM cells die in DMD due increased mechanical stress, which activates NADPH-oxidase (NOX2) and reactive oxygen species (ROS) production. This in turn activates calcium influx via TRPC channels, causing calcium overload and inhibition of mitochondrial function ultimately leading to SkM lost, reducing ambulatory and respiratory function, and a reduced life expectancy to 35 years. Currently, there is no effective cure for either DMD or BMD and current therapies at best, only delay progression of symptoms. Our lab has recently developed a platform, kinetic image cytometer (KIC), that will accelerate drug discovery for skeletal muscles disorders by creating a more precise/reproducible translational assay utilizing hPSC- disease-derived cells to quantify physiological functions of DMD. For this study, we devised aims to develop normal, DMD, and BMD hPSC-SkMs, develop methods to quantify physiological function of hPSC-SkM, and develop methods to stress the hPSC-SkMs cultures. Both healthy and DMD-derived hPSCs will be differentiated into mature skeletal muscle myotubes and examined for DMD-specific defects. Furthermore, we have identified a variety of clinically relevant DMD phenotypes that are amenable to high-throughput utilizing automated digital microscopy, including elevated levels of creatine kinase, calcium transient, contractile motion and ROS. While our data supports that calcium reuptake is disrupted more in BMD and DMD lines further investigation is needed to determine the correlation of electrode stimulation strength with overall cell fatigue, shock and degradation. Additionally, data suggest that the organization of the myotubes, regardless of cultivation densities, could be a key contributing factor to the rapid degradation in DMD. This platform is a proof-of concept for DMD but could be applicable to many other myopathic disorders.

F-2020

CELLULAR REPROGRAMMING REJUVENATES HUMAN SYNOVIAL FLUID-DERIVED STEM CELLS FOR ENHANCING PROPERTIES AND FUNCTIONS

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Cellular senescence is one of the major concerns associated with the use of mesenchymal stem cells (MSCs) for regenerative applications. Among different methods that have been developed to address the issue, rejuvenation of MSCs through cellular reprogramming has been shown particularly promising. In this study, we reprogrammed human synovial fluid-derived MSCs (SF-MSCs) into induced pluripotent stem cells (iPSCs), which were then induced to differentiate into MSCs to establish iPSC-MSC lines. Activities of iPSC-MSCs and their parental SF-MSCs were compared to determine if reprogramming is able to alter their age-associated properties and functions. Our results showed that compared to SF-MSCs, iPSC-MSCs exhibited similarity in the morphology and immunophenotype but an increase in cell proliferation, osteogenesis, and chondrogenesis. Functional enrichment analysis indicated that senescence-related hallmark gene sets, including those associated with reactive oxygen species, the p53 pathway, and senescence-associated secretory phenotype-related inflammatory cytokines and chemokines were down-regulated in reprogrammed MSCs compared to non-reprogrammed ones. We also demonstrated that senescence-associated β-galactosidase activity and the expression of p53 and p21CIP1 were attenuated in iPSC-MSCs compared to those in SF-MSCs. Cellular reprogramming resulted in an increase in the expression of telomerase reverse transcriptase and telomerase activity, in turn lengthening telomere and a decrease in the expression of p53 and p21CIP1 through regulation of β-catenin in iPSCs and their derived-MSCs compared to non-reprogrammed cells. Taken together, this study demonstrates that cellular reprogramming is able to rejuvenate MSCs through β-catenin-mediated telomere lengthening and p53/p21CIP1 attenuation, providing a potential solution to the concern of MSC senescence.

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F-2022

HUMAN SKELETAL STEM CELLS FROM ACUTE FRACTURES MAINTAIN SELF-RENEWAL AND DIFFERENTIATION INDEPENDENT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID)

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Whether NSAIDS hinder human fracture healing by direct action on osteochondrogenic differentiation remains unknown. While animal studies suggest NSAIDs are deleterious to osteoblast differentiation in vitro and in vivo, human clinical trials have yet to demonstrate an effect of NSAIDs on fracture healing. We have recently identified a purified bona fide skeletal stem cells (SSCs) as the source for osteogenic and chondrogenic cell populations, which can be isolated from intact bones and fracture sites and sought to investigate functional changes by NSAID administration. Human SSCs (Podoplanin+, CD146- CD73+ CD164+) and osteoprogenitors (hOPs (MSCs); Podoplanin-CD146+) were FACS-isolated from human fractures. Purified SSCs were cultured in the presence or absence of three common NSAIDs and analyzed subsequently for colony-forming units (CFU-F). After osteogenic and chondrogenic differentiation, cell avidity for Alizarin Red and Alcian Blue was quantified by spectrophotometry. Experiments were performed in triplicate on n =4 healthy adults (age. 44-85 years). Physiologic and supra-physiologic concentrations, as well as initial pulsed and continuous NSAID administration, failed to inihibit hSSC (and hOPs) differentiation into osteoblasts and chondrocytes. NSAID administration did not affect clonogenicity of hSSCs. In contrast and as expected from results of previous studies, mouse SSCs showed impaired osteochondrogenic differentiation potential when treated with physiological concentrations of NSAIDs. Microarray gene expression data of mouse and human SSCs suggests this could be due to the lack of Cox-2 expression in hSSCs. We demonstrate that hSSCs prospectively isolated

from human fracture sites maintain functionality independent of NSAID application. Our results add evidence to emerging clinical data suggesting NSAID administration for post-operative analgesia is safe for fracture healing.

CARDIAC TISSUE AND DISEASE

F-2024

THE ROLE OF TNNI3K IN ADULT MAMMALIAN HEART REGENERATION

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Adult mammalian heart regeneration capacity is thought to be extremely limited. All fetal and newborn mouse cardiomyocytes are mononuclear and diploid, and most become polyploid during the first postnatal week. Our lab showed that mononucleated diploid cardiomyocytes (MNDCMs) retain regenerative capacity, and that the percentage of this subpopulation in the adult mouse heart is variable and can be surprisingly high. We reported that cardiac troponin I-interacting kinase (TNNI3K) is a key regulator of MNDCM frequency. Nonetheless, a detailed mechanism of how Tnni3k regulates MNDCM frequency is still lacking. Studies from another group showed that Tnni3k mediates adult cardiomyocyte oxidative stress response. We therefore hypothesize that similar pathways might explain how Tnni3k affects cardiomyocyte ploidy. We found that reducing postnatal oxidative stress by transgenic expression of mitochondrial catalase increased MNDCM frequency. MNDCM frequency was further increased with Tnni3k deletion. We also found that several common human TNNI3K polymorphisms compromise kinase activity, which may influence variation in MNDCM frequency and heart regenerative capacity in the human population.

F-2026

CARDIOMYOCYTES FROM A SET OF ISOGENIC HUMAN IPSC LINES HARBORING MUTATIONS IN KCNH2 DISPLAY DIFFERING RESPONSES TO A TORSADOGENIC COMPOUND

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Congenital long QT syndrome type 2 (LQT2) is one of the most common genetic cardiac channelopathies leading to life-threatening arrhythmias and sudden cardiac death. A broad range of phenotypes are associated with this cardiac channelopathy and nearly 500 variants identified in the gene KCNH2, encoding the ion channel hERG, have been associated with this disease. Studies have shown that the location of the mutation within KCNH2 is an important determinant of arrhythmic risk in LQT2 patients, with patients harboring mutations in the pore-loop region at higher risk of cardiac events than those with mutations located in other regions of the channel. An understanding of how the mutation type and location contributes to phenotypic variability could lead to improved intragenotype risk stratification of patients. Patient-derived human induced pluripotent stem cells (hiPSCs) can be used to investigate the pathogenicity of mutations identified in LQT2 patients. However, as these lines are from different individuals, each harbours additional genetic variants that may functionally influence the disease phenotype observed and complicate their use for broad intragenotype risk stratification. To overcome this issue, we have generated a set of isogenic hiPSC lines by introducing KCNH2 mutations into a wild-type hiPSC line using Crispr/Cas9 technology. Analysis of cardiomyocytes derived from these isogenic lines (hiPSC-CMs) show a prolongation of both action potential and field potential duration when compared to wild type, consistent with the cardiomyocyte phenotype observed in the LQT2 patient iPSC lines. Treating the cardiomyocytes from this isogenic set of KCNH2-variant hiPSC lines to a hERG channel-blocking compound revealed different sensitivities between the lines, with the hiPSC-CMs containing a mutation in the pore-loop region more susceptible to the proarrhythmic effects of the drug. These findings demonstrate the potential of hiPSC-CMs to correlate the functional effects of KCNH2 mutations with patient risk when using genetically-matched lines, and offers new opportunities for predicting risk in patients as well as in the development of patient-specific pharmacotherapy.

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F-2028

INTRA-MYOCARDIAL TRANSPLANTATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIAC LINEAGE CELLS INTO NOD SCID GAMMA (NSG) MICE: PRECLINICAL ENGRAFTMENT AND SAFETY EVALUATION

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Heart disease is a major cause of death and morbidity in the western world. Transplantation remains the only viable long-term treatment for patients with progressed cardiac failure. For this reason, stem cell-derived cardiomyocyte-based therapies have emerged as a potential therapeutic approach to initiate cardiac remodeling and delay transplantation. Cardiac stem cell therapy challenges include the ability of cells to engraft into myocardium, mitigation of stem cell-induced tumor formation, and life threatening arrhythmias. A population of Day 20 human iPSC-derived cardiac lineage cells (iPSC-CL) was produced though 3D culture and injected into the myocardium of NSG mice. Mice were divided into 4 groups that received either the placebo (cardiomyocyte media) or increasing doses of Day 20 iPSC-CL; placebo group (n=10), and treatment groups receiving iPSC-CL at 0.3x106/animal (n=25), 1.0x106/animal (n=25), and 3.0x106/animal (n=25). A telemetry device was implanted into 6 mice from each group to detect ventricular ectopic activity. Each subject was assessed for iPSC-CL engraftment, tumor formation, and cardiac side effects. No test article-related effect was observed on the heart rate or PR, QRS, or QT intervals at any dose level. Ventricular premature complexes were observed in mid and high dose groups early in the study; however, these events were not detected at week 14. All surviving mice were sacrificed 14 weeks post-treatment. Hematology, biochemistry, and troponin data collected at that time indicated no significant abnormalities or variability between groups. Histologically, no evidence of tumor in the myocardium or other ectopic sites was observed and immunohistochemistry indicated mature grafts of viable transplanted iPSC-CL present within the myocardium. Results demonstrate 3D culture production of Day 20 iPSC-CL achieved engraftment within the myocardium, the cells maintained a high survival rate in a dose-dependent manner, induced no teratomas, and incurred no significant risk factors. Results from our study suggest that grafting high-purity iPSC-CL at a dose of 3.0x106 does not form tumors and persistent ventricular arrhythmias are not observed.

Funding Source: Todd and Karen, Wanek Family Program for Hypoplastic Left Heart Syndrome

F-2030

EFFECT OF CONNEXIN 43 AND SGSM3 THROUGH HIF-1A-MEDIATED MODULATION IN RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Connexin 43 (Cx43) contributes gap junction-mediated communication as a gap junction protein but has shown channel independent functions. Many reports have suggested that Cx43 regulates other cellular mechanisms, including cell cycles, differentiation, and proliferation. Recent evidence suggests that connexins, and in particular Cx43, may have additional effects that may be important in cell death and survival by mechanisms independent of cell to cell communication. In the previous study, we found that Cx43-interaction protein, small G protein signaling modulator 3 (SGSM3) plays a critical role in stress cells. Moreover, their interaction plays a key role in Cx43 internalization for connexin turnover in cardiomyocytes of infarcted hearts. Here, we investigated for SGSM3, a potential partner of Cx43, in an attempt to identify for enhancing survival markers in bone marrow-derived mesenchymal stem cells (MSCs). Cx43 co-immunoprecipitated analysis identifying two proteins, and gap junction proteins were predicted that SGSM3 was highly

correlated with Cx43 in GeneMANIA network analysis. Results of Hif1a and Sgsm3 siRNA knockdown experiments suggest that SGSM3 possibly plays a role in the cellular response to stress or ischemia with Cx43 dependently on Hif1α. In conclusion, these data demonstrate a role for SGSM3 in Cx43 endocytic trafficking and further substantiate its role in Cx43 turnover. This knowledge of SGSM3-mediated regulation of Cx43 may help to identify a novel therapeutic target to counteract the loss of Cx43 or impairment of Cx43-GJIC that disrupt normal cell functions and are associated with many human diseases.

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F-2032

MIR148A FAMILY REGULATES CARDIAC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS BY INHIBITING THE DLL1-MEDIATED NOTCH SIGNALING PATHWAY

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MicroRNAs (miRNAs), as a type of naturally occurring RNAs, play important roles in cardiac physiology and pathology. There are abundant miRNAs showing multifarious expression patterns during the cardiomyocyte genesis. Here, we focused on the MIR148A family, which is composed of MIR148A, MIR148B and MIR152, three highly conserved miRNAs sharing same seed sequences. The expression levels of all MIR148A family members were progressively increased during the differentiation of human embryonic stem cells (hESCs) into cardiomyocytes. The deletion of MIR148A family (MIR148A-TKO) resulted in decreased cardiomyocyte proportion after cardiac induction, which could be restored by the ectopic expression of MIR148A family members. The transcriptome analysis indicated that MIR148A family was the potential repressor of paraxial mesoderm after primitive streak formation. These miRNAs, in turn, promoted the differentiation of lateral mesoderm and cardiomyocytes. Furthermore, the NOTCH ligand Delta-like1 (DLL1) was validated as the target gene of MIR148A family, and the knockdown of DLL1 could promote cardiac differentiation of MIR148A-TKO hESCs. Thus, our results demonstrate MIR148A family promotes cardiomyocyte differentiation as a novel paraxial mesoderm repressor, which provides a new insight into heart development and cardiac differentiation.

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F-2034

MESENCHYMAL STEM CELLS MIGHT BE MOBILIZED FROM BONE MARROW TO THE PERI-INFARCTION AREA BY SYSTEMIC HMGB1 ADMINISTRATION VIA SDF1-CXCR4 SIGNALING COMPLEX IN RAT INFARCTION MODEL

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High-mobility group box 1 (HMGB1) is reportedly a regenerative factor to mobilize CXCR4 positive bone marrow mesenchymal stem cells (BM-MSC) to the damaged tissue to promote tissue regeneration. However, actual mechanism of BM-MSCs recruitment remains uncertain. Whereas, SDF-1, which is a ligand of CXCR4, plays an important role in various stem cells migration, including BM-MSCs. So, we hypothesized that, in rat myocardial infarction (MI) model, systemic administration of HMGB1 could mobilize BM-MSCs to the damaged myocardium via SDF-1/CXCR4 signaling, leading to inhibition of left ventricular (LV) adverse remodeling. HMGB1 (3 mg/kg) or PBS (3 ml/kg) was administered intravenously for 4 days to 26 MI model rats, and cardiac function was evaluated by ultrasonography; antifibrotic action, by immunostaining. Using GFP-bone marrow transplantation (GFP-BMT) rat, BM-MSC recruitment was evaluated. Furthermore, in MI model rat, SDF-1 expression was evaluated by QT-PCR and histology. Four weeks after each injection, the LVEF was significantly improved in the HMGB1 group than in the control (HMGB1 vs. control; 48.6% ± 5.5% vs. 33.6% ± 5.4%; $p < 0.01$). LV remodeling exhibiting interstitial fibrosis, cardiomyocyte hypertrophy and

decrease of capillary density were significantly attenuated in the HMGB1 group compared with the control. On QT-PCR analysis, VEGF mRNA level was significantly higher in the HMGB1 group than in the control (border zone; 1.6 ± 0.6 vs. 1.1 ± 0.2; p = 0.02). In GFP-BMT rat MI model, confocal microscopy image showed that there were more GFP+/PDGFRα+ cells in the HMGB1 group compared with the control. Some of those cells were present at vessel constituent cells in the peri-infarction area. On PCR analysis, SDF-1 expression significantly increased in MI model rat than in normal rat, with SDF-1 level of peri-infarction area was the highest in all area (MI rat vs. normal; peri-infarction area; 2.1 ± 0.4 vs 0.9 ± 0.1; p < 0.01). Histological analysis also showed SDF-1 expression along the border zone. Based on these findings, systemic administration of HMGB1 could inhibit LV adverse remodeling by enhancing BM-MSCs recruitment to the damaged myocardium, leading to LV functional recovery. This study also suggests that those BM-MSCs may be mobilized to the peri-infarction area via SDF-1/CXCR4 signaling complex.

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F-2036

THE ROLE OF GLUCOSE AS A PROMOTER FOR CARDIAC REGENERATION

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Heart failure is the leading cause of death worldwide. Our focus is on non-genetic mechanisms by which cardiac regeneration can be lengthened or enhanced. Specifically, we are interested in the cyto-protective effects of glucose in cardiomyocyte growth, differentiation and proliferation and how this knowledge can be applied to regeneration therapies. Our preliminary data showed that glucose induces cardiomyocyte proliferation and inhibits cardiomyocyte maturation in human embryonic stem cells derived cardiomyocytes (hESC-CM) via the Pentose Phosphate Pathway in a dose dependent manner. Whether this pathway can be a therapeutic target for heart regeneration is unknown. Our hypothesis is that glucose promotes neonatal heart regeneration in a murine model. Non-Transmural cryoinjury was performed to the apex of the left ventricle in wild-type pups and cardiac specific overexpression of Glucose Transporter 1 Transgenic pups. In the acute phase (P1-P7), the level of cardiomyocyte cell proliferation was measured via flow cytometry analysis and immunostaining with PH3 and cTnnt. Glucose uptake by cardiomyocytes was measured by 18F-FDG assay and Glut1 immunostaining. In the chronic phase (P14, P21, P40), we quantified the level of fibrosis by histology (H&E and Picrosirius Red) and neovascularization by immunostaining with PECAM. Increased cardiomyocyte proliferation was observed in the Transgenic Glut1 pups. Myocardial glucose uptake declines from the muscular layer towards the trabecular layer, corresponding with maturation of the heart. We observed that Glut1 cardiomyocyte-specific overexpression resulted in improved cardiac repair compared to wild type (WT) mice at 21 days postnatally. Compared to wild-type, Glut1 hearts showed increased angiogenesis around the site of injury. We believe that increased in blood vessel formation is secondary to an increase in cardiomyocyte proliferation. This study would be the first to demonstrate the potential role of glucose as a promoter for cardiac regeneration and reveal a potential mechanism for congenital cardiomyopathy associated with diabetic pregnancy.

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F-2038

INVESTIGATION PATHOGENIC MECHANISMS OF PEDIATRIC DILATED CARDIOMYOPATHY BY USING PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELL MODEL

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Disease models are essential for understanding cardiovascular disease pathogenesis and developing new therapeutics. The human induced pluripotent stem cell (iPSC) technology has generated significant enthusiasm for its potential application in basic and translational cardiac research. Patient-specific iPSC-derived cardiomyocytes (iPSC-CMs) offer an attractive experimental platform to model cardiovascular diseases, study the earliest stages of human development, accelerate predictive drug toxicology tests, and advance potential regenerative therapies. Harnessing the power of iPSC-CMs could eliminate confounding species-specific and inter-personal variations, and ultimately pave the way for the development of personalized medicine for cardiovascular diseases. Unlike in adult dilated cardiomyopathy (DCM) patients, demonstrated benefits of RAAS inhibition and -adrenergic receptor blockade are not observed in DCM children. Independent of classic G-protein coupled receptor signaling pathway, the pediatric DCM pathogenic progression maybe related to other causes such as microRNAs. Compared to current animal models, we applied DCM iPSCs which are reprogramed from pediatric patient blood as our DCM patient-specific model. Compared to healthy control iPSC-CM, the DCM iPSC-CM are intensively characterized. The potential microRNAs have been identified by using patient specific iPSC-CM model. Overexpression of the specific microRNA

aggregated the DCM progression. On the other hand, the inhibition of the microRNA is reverse the disease progression. Our results enhanced current understanding of DCM pathogenic progression and provide novel therapeutic targets.

F-2040

ELEVATED EXPRESSION OF MITF IN FAR-INFRARED-PRECONDITIONED RAT BONE MARROW-DERIVED STEM CELLS PROTECTS CELL SURVIVAL AGAINST METABOLIC STRESS

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Bone marrow-derived stem cells (BMSCs) have been broadly investigated for treatment of ischemic heart diseases. However, there are many uncertainties that are prevailing against bench-to-bedside research related to BMSC-based therapy: like the optimal rout of cell transplantation, appropriate dosage, duration, safety, and efficacy of applications. Herein, we focus on a strategy for improving the low survival rate of BMSC after transplantation. To achieve this, we extend our previous study about the effects of preconditioning with far-infrared irradiation (FIR) on survival of BMSCs under condition of metabolic stress, such as oxidative stress, low temperature, and ischemic hypoxia condition. BMSCs were isolated and harvested from femur bone marrow of 6-week-old male Sprague-Dawley rat. To determine the effects of a FIR generator with an energy flux of 0.13 mW/cm2 on viability of rat BMSCs following metabolic stress, survival of BMSCs was measured by crystal violet staining and PI staining. FIR preconditioning was observed to significantly increase BMSC survival against H2O2, low temperature, and ischemic hypoxia condition. Of note, qRT-PCR and Western blot analysis demonstrated that FIR induced microphthalmia-associated transcription factor (MITF), BCL2, HIF-1a, mTOR and CD63 at mRNA and protein levels. It is well known that the mTOR or MITF is a master regulator of various important cellular responses, including protein synthesis, cellular growth, proliferation, autophagy, lysosomal function, and cell metabolism. In agreement with these observations, MITF-depleted BMSCs or rapamycin-treated BMSCs is decreased proliferation and survival of preconditioned BMSC by FIR-associated with up-regulation of MITF and mTOR. Overall, our results demonstrated for the first time that preconditioning with FIR can open new insights to help therapeutic efficacy of BMSCs, and the expression of MITF and mTOR-mediated cellular response is a key to understanding its role in survival or death of BMSCs after transplantation.

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ENDOTHELIAL CELLS AND HEMANGIOBLASTS

F-2042

ENDOTHELIAL CELLS DERIVED FROM HEMOPHILIA PATIENT-SPECIFIC IPSCS FOR SUSTAINED FVIII DELIVERY AND THE TREATMENT OF HEMOPHILIA A

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Hemophilia A is a bleeding disorder characterized by spontaneous and prolonged hemorrhage. The disease is caused by mutations in the coagulation factor 8 gene (F8) leading to factor VIII (FVIII) deficiency. Since FVIII is primarily produced in endothelial cells in a non-diseased human being, endothelial cells hold great potential for development as a cell therapy for hemophilia A. We showed that hemophilia A patient-specific induced pluripotent stem cells (HA-iPSCs) could provide a renewable supply of endothelial cells. The HA-iPSC-derived endothelial cells were transduced with lentiviral vectors to stably express the functional B domain deleted F8 gene, the luciferase gene and the enhanced green fluorescent protein gene (GFP). When transplanted intramuscularly into neonatal and adult immune deficient mice, the HA-iPSC-derived endothelial cells were retained in the animals for at least 10-16 weeks and maintained their expression of FVIII, GFP and the endothelial marker CD31, as demonstrated by bioluminescence imaging and immunostaining, respectively. When transplanted into neonatal hemophila A mice, these transduced HA-iPSC-derived endothelial cells significantly reduced blood loss in a tail-clip bleeding test. Thus, our studies provide proof-of-concept that HA-iPSC-derived endothelial cells can serve as a relatively long-term cell factory to deliver FVIII for the treatment of hemophilia A in not only adults but also newborns.

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F-2044

ROBUST DIFFERENTIATION PROCEDURE INTO HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS AND THEIR PROPERTIES

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Primary human endothelial cells (ECs) such as HUVECs are widely used for vascularization study, various organoids formation or safety/toxicological test. However, primary ECs sometimes show uncontrollable lot-to-lot variances in these researches due to genetic diversity of derived donors, and thereby may lead to the difficulty of obtaining the reproducibility of the experiment. Human induced pluripotent stem cell-derived ECs (iPS-ECs) are expected to use for these research field as cell source having stable properties among manufacturing batches from single donor. Nevertheless, iPS-ECs have not been widely utilized in those experiments, since current iPS-ECs have some problems such as unstable differentiation from iPS cells or poor growth capacity. To overcome this, we have developed robust differentiation procedure into iPS-ECs in which continuous proliferation has been observed. We tested their ability of the procedure to differentiate into ECs from various iPS cell clones. All iPS-ECs from tested iPS cell clones showed more than 90% of both CD31 and CD144 positive population, which are typical endothelial cell surface markers. Then, these iPS-ECs were evaluated for their growth capacity for one month. Although all iPS-ECs exhibited almost similar endothelial cell-related genes and markers expression, there were big differences in the proliferation rate. The most proliferated iPS-ECs showed about one thousand fold expansion, even though one out of 6 iPS-ECs could little proliferate after differentiation (about a few fold expansion). In spite of the differences in growth capacity, all iPS-ECs retained both CD31 and CD144 positive population during observation period. We also confirmed whether iPS-ECs could be used for angiogenesis inhibition assay by observing the tube formation by measuring the fluorescence of calcein-labeled ECs. As a result, the inhibition of tube formation was observed in the addition of Wortmannin in a dose-dependent manner. Overall we have successfully developed iPS-ECs differentiation system. It can be overcome the lot variation problems of primary endothelial cells, making them suitable for various kinds of vascularization researches.

F-2046

LONG-TERM PRIMING BY THREE SMALL MOLECULES IS A PROMISING STRATEGY FOR ENHANCING LATE ENDOTHELIAL PROGENITOR CELL BIOACTIVITIES

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Endothelial progenitor cells (EPCs) and outgrowth endothelial cells (OECs) play a pivotal role in vascular regeneration in ischemic tissues; however, their therapeutic application in clinical settings is limited due to the low quality and quantity of patient-derived circulating EPCs. To solve this problem, we evaluated whether three priming small molecules (tauroursodeoxycholic acid, fucoidan, oleuropein) could enhance the angiogenic potential of EPCs. Such enhancement would promote the cellular bioactivities and help to develop functionally improved EPC therapeutics for ischemic diseases by accelerating the priming effect of the defined physiological molecules. We found that preconditioning of each of the three small molecules significantly induced the differentiation potential of CD34+ stem cells into EPC lineage cells. Notably, long-term priming of OECs with the three chemical cocktail (OEC-3C) increased the proliferation potential of EPCs via ERK activation. The migration, invasion, and tube-forming capacities were also significantly enhanced in OEC-3Cs compared with unprimed OECs. Further, the cell survival ratio was dramatically increased in OEC-3Cs against H2O2-induced oxidative stress via the augmented expression of Bcl-2, a prosurvival protein. In conclusion, we identified three small molecules for enhancing the bioactivities of ex vivo-expanded OECs for vascular repair. Long-term 3C priming might be a promising methodology for EPC-based therapy against ischemic diseases.

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HEMATOPOIESIS/IMMUNOLOGY

F-2050

EFFICIENT MODIFICATION OF CCR5 LOCUS IN NONHUMAN PRIMATE INDUCED PLURIPOTENT STEM CELLS AND THEIR USE FOR PRODUCTION OF LYMPHOID AND MYELOID CELLS

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The discovery that CCR5 serves as an R5-HIV-1 receptor, coupled with findings of protection from HIV infection in individuals lacking CCR5, led to the exploration of novel therapeutic strategies for HIV infection based on targeting of CCR5. Among them are technologies based on iPSCs with edited CCR5 locus which have emerged as promising option for providing unlimited source of HIV-resistant blood cells for cellular therapies. To advance preclinical models for assessing iPSC-based therapies for AIDS, we established method for efficient CCR5 gene disruption in nonhuman primate (NHP) iPSCs using the CRISPR/Cas9 system. NHP T-iPSC lines were generated from peripheral blood T cells from Mauritian cynomolgus macaque (MCM) using Sendai Reprogramming kit. To successfully disrupt CCR5, we designed two CCR5 gRNAs to target sequences within exon 2, including 24-bp deletion region which was found to prevent functional CCR5 expression in NHPs. Following CCR5 editing in T-iPSCs using these gRNAs, we were able to achieve CCR5 disruption in 50% of colonies, 33% of which showed biallelic editing. NHP T-iPSCs with biallelic CCR5-disruption (CCR5mut-T-iPSC) showed normal traits of pluripotent stem cells and efficiently differentiated into CD34+CD45+ multipotent hematopoietic progenitors (MHPs). MHP from CCR5mut-T-iPSCs were differentiated into CD45+CD14+MHCclassII+ macrophages in presence M-CSF and IL-1b for a week to yield about 6x106 macrophages from 1x10^6 MHPs. For T cell differentiation MHP were cultured with OP9-DLL4 in presence of IL7 and SCF. Resistance of immune cells from wild and CCR5mut NHP T-iPSCs to SIV infection was evaluated in vitro following challenge with SIV virus. Overall these studies provide a platform for further exploration of AIDS therapies based on gene-edited iPSCs in NHP preclinical model.

F-2052

PRODUCTION OF ASSAY-READY HUMAN IPSC-DERIVED CD34+ CELLS, MONOCYTES, AND MESENCHYMAL STEM CELLS

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There is an unmet need for highly characterized and reproducible cell models for a wide range of applications including mechanisms of action, drug development and toxicity testing. Human induced pluripotent stem cells (iPSCs) have the capability to differentiate into all somatic cell types and therefore hold great promise for development into cell models for a wide range of applications. We have developed processes for the scalable generation of functional CD34+ cells, monocytes, and mesenchymal stem cells (MSCs) derived from iPSC lines. It is well documented that starting cell types and donor background play an important role in the efficiency of iPSCs to terminally differentiate. We screened four iPSC lines, three from bone marrow CD34+ cells and one foreskin fibroblast-derived iPSC line, for differentiation into CD34+, monocytes, or MSCs. All CD34+ cells-derived iPSC lines exhibited high efficiency for differentiation into CD34+ cells and MSCs, while the fibroblast-derived iPSC line favored differentiation into monocytes. To assess the differentiation potential of the iPSCs-derived cells, we demonstrated that CD34+ cells could be differentiated into erythroid cells, myeloid cells, and megakaryocytes while monocytes were capable of differentiating into dendritic cells and functionally active macrophages. Compared to primary MSCs, iPSCs-derived MSCs exhibited similar immunophenotypes and T-cell suppression activity as well as the ability to differentiate into adipocytes, osteocytes, and chondrocytes. In addition, these iPSC-derived cells exhibited very stable phenotypes across multiple lots. The ability to obtain sufficiently large quantities of certain cell types, including CD34+ cells, for high throughput assays for drug and toxicity screening can be an issue. To address this issue, in this study we show that we have developed processes for the scalable and reproducible generation of high purity bio functionally active assay-ready iPSCs-derived CD34+ cells, monocytes, and MSCs. In addition, these stable phenotype cells remove the inherent variability of donor-derived material. The availability of these highly characterized cells for screening applications will serve as a catalyst for developmental studies, drug screening, and toxicity testing.

F-2054

A CORRELATIVE LIGHTSHEET AND ELECTRON MICROSCOPY APPROACH TO CHARACTERISE THE ADULT HEMATOPOIETIC STEM CELL NICHE IN ZEBRAFISH

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Hematopoietic stem and progenitor cells (HSPCs) originate from the hemogenic endothelium in the dorsal aorta, then migrate to colonize the fetal liver, before finally homing and engrafting within the fetal bone marrow (BM). Within the BM, HSPCs are retained in a quiescent state in a complex microenvironment and divide occasionally to self-renew and to repopulate the blood lineages. While it is known that HSPCs are maintained by surrounding niche cells such as megakaryocytes, peripheral nerves, endothelial and mesenchymal stromal cells, the ultrastructure of these HSPCs within the niche is not well defined, as current imaging technology does not allow direct visualization of the fetal BM niche. Zebrafish have a similar hematopoietic ontogeny to mammals, and because the embryos are transparent, intrinsic HSPC interactions with the niche can be directly visualized. To track HSPCs during niche colonization, we used our previously validated HSPC-specific transgenic reporter lines (Runx:GFP and Runx:mCherry). We could visualize the presumptive adult niche, kidney marrow (KM) in fixed larvae at 5 days post fertilization using a tissue clearing technique and detected ~100 HSPCs/larva. To precisely locate these rare HSPCs within the larger dense KM, we genetically tagged endogenous HSPCs to track them live using lightsheet microscopy, followed by high resolution serial block-face scanning electron microscopy (SBEM) (XY=10.8 nm/pixel, Z=70 nm/pixel). Using this technique, we could visually track single mCherry+ HSPCs, then confirm their exact location in a SBEM dataset with high contrast APEX2 peroxidase label. We found HSPC clusters within vessel lumens, as well as a novel perivascular HSPC niche with a defined cellular assembly. In this perivascular site, a single HSPC was seen to simultaneously contact one mesenchymal stromal cell, multiple endothelial cells, a glial-like cell, and other hematopoietic cells. This shows that within a distinct anatomical niche, multiple cell types can directly contact and regulate HSPCs. Our technique can be used as a general approach to identify the ultrastructure of single rare cells within dense tissues by using multiple imaging techniques. Further, we can now identify novel intercellular structures that form between an unperturbed HSPC in its endogenous perivascular niche.

F-2056

BIOACTIVE PEPTIDE SL-13R EXPANDS HUMAN UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cell (HSC) transplantation is a curative treatment of hematological disorders that has been utilized in clinical setting. Although umbilical cord blood (UCB) is a promising source of HSCs, insufficient number of HSCs in UCB limits their use, prompting need for ex vivo HSC amplification method. HSCs emerge from hemogenic endothelium in the aorta-gonads-mesonephros (AGM) region, and then expand in liver. We previously showed that Delta-like 1 (Dlk1) positive hepatoblasts are the niche-like cells of HSCs in fetal liver. Dlk1 is a transmembrane protein and also secreted as soluble protein known as fetal antigen 1 (FA1). We hypothesized that hepatoblasts regulate HSC self-renewal via DLK1/FA1. Several bioactive peptides were generated from extracellular domain of human DLK1 and a bioactive peptide, SL-13R, which has ability to expand HSCs was identified. UCB CD34+ cells were cultured with the SL-13R in xeno- and serum-free medium containing a cytokine cocktail for 9 and 14 days. The number of CD34+CD38- cells were increased by SL-13R compared to control (9 days: 1.5 fold, 14 days: 2.5 fold). Transplantation of CD34+ cells cultured with SL-13R into immunodeficient NOG mice confirmed that the cultured cells possess long-term reconstitution ability. Moreover, we performed second transplantation and showed the self-renewal ability of the cultured cells, but not untreated control cells. These results suggest that SL-13R has ability to expand HSCs and maintain its self-renewal capacity. To understand the mechanisms of HSC expansion by SL-13R, we investigated peptide binding proteins using biotin-conjugated SL-13R and identified PLEC and ERLIN2 proteins as interactors of SL-13R by LC-MS/MS and MASCOT analysis. PLEC knockdown UCB CD34+ cells cultured with bioactive peptide showed a decreased number of hematopoietic colonies relative to peptide-treated, non-knockdown controls. By contrast, ERLIN2 knockdown had little effect in the presence of SL-13R. These results suggest that PLEC functions in HSC expansion promoted by SL-13R. In summary, we have identified a novel bioactive peptide promoting expansion of UCB CD34+ cells with long-term reconstitution ability. Its use may facilitate clinical use of UCB HSCs.

F-2058

PENTRAXIN 3 PLAYS A KEY ROLE IN HUMAN MYELOID ANGIOGENIC CELLS PHAGOCYTIC FUNCTION

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Myeloid angiogenic cells (MACs) were originally described as endothelial progenitors, but we and others have demonstrated that their revascularisation properties are explained by release of soluble factors. Furthermore, MACs do not differentiate into endothelial cells, but exhibit a defined myeloid phenotype CD14+CD163+CD204+CD54- related to alternative activated macrophages. Their therapeutic potential has been tested in preclinical models for ischaemic disease in heart, retina, and lower limbs. Besides their paracrine pro-angiogenic effect, MACs may also clear cellular debris from ischaemic tissues to reduce inflammation. Pentraxin 3 (PTX3) is a pattern recognition receptor expressed in myeloid cells with reported functions in inflammation, angiogenesis, and phagocytosis. Therefore, here we studied PTX3 role in MACs function. First, MACs were exposed to 25 mM D-glucose to model diabetes or to 1% O2 to mimic tissue hypoxia. After 7 days exposure to high glucose, PTX3 mRNA expression was significantly ($P < 0.01$) downregulated, while IL1-β mRNA was significantly ($P < 0.05$) increased when compared to untreated controls. Similarly, there was a significant reduction in PTX3 mRNA expression after 2, 4, and 6 hours exposure to hypoxia. This was associated with a significant decrease in MACs phagocytic index using pHrodo™ Bioparticles™ and GFP-positive apoptotic bodies, when exposed to a high glucose milieu for 7 days ($P<0.05$). Second, we tested whether treatment with recombinant (r) PTX3 rescue MACs from high glucose-induced decrease in phagocytosis. Pre-treatment with 100 ng/ml rPTX3 for 24 hours restored the phagocytosis of pHrodo bioparticles and GFP-apoptotic bodies ($P<0.05$) in high glucose-treated MACs. Finally, we isolated MACs from PTX3 knockout (KO) and wild type (WT) mice. MACs derived from PTX3 KO mice showed less uptake of pHrodo bioparticles as compared to MACs from WT mice ($P<0.05$). This evidence demonstrates that PTX3 promotes phagocytosis in MACs. Furthermore, treatment of MACs with rPTX3 significantly reduced the expression of IL1-β mRNA ($P < 0.05$) with an increase in CD163 expression ($P < 0.05$), suggesting an anti-inflammatory role for PTX3. These findings support our hypothesis that MACs act as phagocytic cells and their dysfunction under diabetic conditions can be corrected by PTX3.

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F-2060

IDENTIFICATION OF THE SPATIO-TEMPROAL VULNERABILITIES TO GENOTOXIC STRESS IN FETAL HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Childhood leukemia demonstrate being prenatal in origin and associated with chromatin alterations. However, the developmental vulnerabilities to genotoxic stress and underlying mechanism in fetal hematopoietic stem and progenitor cells (HSPCs) remain much unknown. Here we report genotoxic insults of Etoposide or UV, both in vivo and ex vivo, induced more DNA breaks in mouse HSPCs in 12.5~14.5 dpf (days post fertilization) fetal livers (FLs) than that in 12.5dpf placentas and in 15.5~18.5 dpf FLs. Consistently, lineage-tracing observation showed that relatively retarded hepatocyte development in early FLs exposed HSPCs in an unprotected environment to genotoxic stress. ATAC-seq and RNA-seq showed that the chromatin in 12.5dpf FLs HSPCs was more accessible, more enriched in CTCF occupancy, but less in YY1 binding, which might determine the chromatin fragility in early FLs HSPCs. These findings will be important in further studies of the evolutionary “trade-off” of development and etiology of childhood malignancies.

F-2062

DIAPHANOUS-RELATED FORMINS MODULATE BETA 2 INTEGRINS THROUGH SERUM RESPONSE FACTOR TO REGULATE THE ENGRAFTMENT OF MURINE HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Mature blood cells are derived from hematopoietic stem and progenitor cells (HSPC) that possess self-renewal and engraftment ability. The diaphanous-related formins are effectors of Rho GTPases and involved in membrane and cytoskeleton physiology. How they are involved in the regulation of HSPC remains unknown. Here we show that loss of formins mDia1 or mDia2 impaired the competitive repopulation capacity of HSPC, in which mDia2 deficiency exhibited a more prominent phenotype. mDia1 partially compensates the defects in mDia2 deficient HSPC since mice with double knockout of mDia1 and mDia2 exhibited rapid lethality after birth with severely reduced HSPC populations. The major role of mDia2 in HSPC was further implicated in that mice transplanted with mDia2 deficient bone marrow mononuclear cells showed decreased HSPC with loss of stem cell quiescence and rapid lethality under serial transplantation. Unexpectedly, loss of mDia2 did not affect HSPC localization to the bone marrow vasculatures during competitive transplantation. However, these HSPC failed to undergo trans-endothelial migration to the bone marrow niche. Mechanistically, loss of mDia2 reduced the linear actin filaments in HSPC, which led to a repression of the transcriptional activity of serum response factor (SRF). We identified that the expression levels of beta 2 integrins were significantly attenuated in mDia2 deficient HSPC, which could explain the trans-endothelial migration defects in these cells. We further revealed both Itgam and Itgb2 as new downstream targets of SRF with intronic binding in their genomic loci. Ectopic expression of SRF or a naturally occurred SRF variant rescued dysregulated gene expression profile as well as engraftment defects of mDia2 deficient HSPC. Consistently, mice with hematopoietic specific ItgaM and Itgb2 knock out through CRISPR-Cas9 exhibited serve defects in HSPC engraftment and long-term self-renewal. In addition, exogenously expressing human ItgaM is sufficient to rescue the engraftment defects in mDia2 deficient HSPC. Collectively, our data revealed a novel mDia-SRF-beat 2 integrin axis that regulates HSPC engraftment in mice.

F-2064

OPTIMIZED POST-CRYOPRESERVATION RECOVERY OF HUMAN STEM-CELL DERIVED BETA-CELLS FOR TREATMENT OF TYPE 1 DIABETES

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Semma Therapeutics is developing a novel therapy for Type 1 diabetes using human pluripotent stem cells (hPSCs) to derive functional insulin-producing islets (SC-Islets). These SC-Islets can be cryopreserved and thawed, and are glucose-responsive in vitro and in vivo with the capacity to ameliorate hyperglycemia in diabetic mice. To optimize the post-cryopreservation SC-Islet yield and function, we have characterized the contribution of signaling pathways that improve CHGA+ endocrine cell re-aggregation. We identify novel signaling requirements that enhance re-aggregation efficiency and cluster size. Further, these conditions improve SC-islet composition and SC-beta function in-vitro. These findings will contribute to the development of a scalable process suitable for Phase 1 clinical trials and future commercial manufacturing.

PANCREAS, LIVER, KIDNEY

F-2066

REDUCTION OF HEPATIC DAMAGE INDUCED BY RADIATION IN MICE TREATED WITH G-CSF

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Although liver cancer cells are sensitive to radiation, this treatment cannot be used at very high doses because normal liver tissue is also easily damaged by radiation. To overcome this problem, we investigated the role of Granulocyte Colony Stimulating Factor (G-CSF) in a model of alcohol and ionizing

radiation induced liver injury. C57BL/6 mice (n=45) were equally divided into 3 groups: irradiated (IR) receiving vehicle; irradiated and G-CSF (IR+G-CSF) and non-irradiated control (CT). The IR and IR+G-CSF groups were subjected to local irradiation with a single dose of 18Gy. Three days prior irradiation, animals from the IR+G-CSF group received 50 µg/kg of G-CSF subcutaneously every 24 hours. To confirm the mobilization of bone marrow hematopoietic precursors, a set of CD45+low, CD117+, CD90.2 and CD34+ markers were analyzed by flow cytometry. The animals were submitted to euthanasia 7, 30 and 60 days after the irradiation date, serum was collected for biochemical analysis of albumin (ALB) and alanine amino transferase (ALT) and liver fragments processed for H&E analysis. Flow cytometry showed that animals receiving G-CSF presented 0.3 ± 0.1% of hematopoietic precursors, whereas those receiving vehicle showed 0.1 ± 0.02%. The animals submitted to IR showed ALB reduction on days 7 (1.4 ± 0.15g/ dl), 30 (1.5 ± 0.09g/dl) and 60 (1.2 ± 0) compared to CT group: 7 (2.2 ± 0.1g/dl), 30 (2.5 ± 0.3g/ dl) and 60 (2.5 ± 0.5g/dl), however, the IR+G-CSF group showed that albumin level were similar to control: 7 (2.1 ± 0.14g/dl), 30 (2.2 ± 0.11g/dl) and 60 (2.5 ± 0.19g/dl). IR and IR+G-CSF groups showed a significant increase in ALT-IR: 7 (50 ± 7U/L), 30 (100 ± 10U/L) and 60 (50 ± 8 U/L) and IR + G-CSF: 7 (45 ± 5U/L), 30 (55 ± 3U/L) and 60 (62 ± 1 U/L) - when compared to CT: 7 (37 ± 5U/L), 30 (40 ± 7U/L) and 60 (41 ± 5U/L). H&E staining showed the presence of inflammatory infiltrate in the IR group; however, this change was not observed in both IR+ G-CSF and control groups. The data suggest that irradiation caused hepatic dysfunction (ALB reduction, ALT increase and presence of inflammatory infiltrate). In the G-CSF group, there was no inflammatory infiltrate and albumin levels were similar to those in the control group. Therefore, mobilization of hematopoietic progenitors, by administration of G-CSF prior to irradiation showed hepatoprotective effects.

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F-2068

AUTOLOGOUS CO-CULTURE HUMAN IPSC MODEL FOR PANCREATIC ENDOCRINE AND VASCULAR ENDOTHELIAL CELLS

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Diabetes is a clinical condition that affects millions of people, and its major causes are the death or dysfunction of insulin-producing beta-cells within the pancreatic islets, resulting in improper insulin secretion and failure to maintain normal glycemia. Most of the patients are reliant on multiple exogenous insulin injections as treatment, and some of them are recipients of cadaveric islet transplantations; however, the source of cadaveric islets is scarce, and these cells are not long-term functional. Thus, new strategies to create scalable and compatible pancreatic islets containing insulin-producing beta cells are necessary. Differentiation of human induced pluripotent stem cells (iPSCs) into pancreatic beta cells appears to be a promising alternative cell source for diabetic patients, as well as for other applications such as disease-modeling and to study pancreas development. Several protocols to generate iPSC-derived beta cells have been developed over the past years; however, improvements are still necessary since many immature polyhormonal cells remain and cannot attain a monohormonal and functional state. During human development, pancreas co-develops with endothelium and shares signals, allowing for better maturation of beta cells, and these are not currently included in the current differentiation protocols. Thus, here we describe a novel autologous co-culture model using iPSC-derived pancreatic endocrine and endothelial cells from the same patient to obtain more functional and monohormonal pancreatic beta-cells from iPSCs. We have established an efficient "in-house" protocol to differentiate iPSCs into pancreatic endocrine cells, with high expression of beta-cell markers such as C-PEPTIDE, NKX6.1 and NGN3. However, these cells have limited glucose stimulated insulin secretion response. To improve functionality of the iPSC-derived pancreatic endocrine cells, we have co-cultured them with iPSC-derived endothelial cells, as well as with primary HUVECs, in trans-wells and Organ-Chip devices. Preliminary data indicate that iPSC-derived pancreatic endocrine and endothelial cells can be adapted to trans-wells and Organ-Chip devices, and co-culture improves endothelial cell markers.

F-2070

CHARACTERISTICS AND DIFFERENCES OF HEPATIC PROGENITOR CELLS ACCORDING TO DONOR AGE IN MICE AND HUMANS

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Due to a shortage of organ donors, liver transplantation being the only choice of treatment for end-stage liver disease is problematic. Therefore, researchers are now leaning towards stem cells as a solution. We recently reported human hepatocytes can convert into human chemically derived hepatic progenitors

(hCdHs) through a cocktail of three small molecules (A83-01, CHIR99021, HGF). Interestingly, clinical feature analysis revealed that age is to be the most important factor in CdH generation. To analyze features of intergenerational mCdHs, we used E16.5 hepatoblast, young mice (6-8 weeks old) and old mice (72-96 weeks old) hepatocytes. Mouse hepatocytes of different ages were stably reprogrammed. In particular, the generation efficiency of mCdHs was different in hepatocytes of different ages. The best of them were liver precursor cells (EmCdHs) made from E16.5 hepatoblasts. These various age-matched mCdHs expressed precursor cell markers, and they also had the ability to differentiate into functional hepatocytes. Afterwards, mCdHs generated from the hepatocytes of various age groups were transplanted into FRG - / - mice and the cell viability was analyzed. Furthermore, significant survival results were seen in the mCdH transplantation group compared to non-transplanted mice. Hepatocytes of various ages were reprogrammed as hepatic progenitor cells. The generation, differentiation, and hepatic function efficiency of the youngest E16.5 hepatoblast-derived mCdHs (EmCdHs) showed a significant difference when compared to others. Also, we obtained the same results from human samples in the process of studying mice. Specifically, human hepatoblasts (14.2 weeks old) showed the same generation efficiency as old hepatocytes (75 years old) similarly to the mice model. The efficiency of hepatic differentiation and function showed adequate results which are better correlated with age. In conclusion, our study has showed that age is an important factor in the generation of CdH which can open the door and provide opportunities for future specific liver disease treatments.

F-2072

REPRESSING OF A-CELL FUNCTIONS IN INDUCE PORCINE MONOHORMONAL PANCREATIC BETA-CELLS GENERATED BY INSERTION OF TRANSCRIPT FACTORS, PDX1/MAFA/PAX4

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Transcription factors, such as Pdx1 and the intrinsic signal pathways involved in pancreatic organogenesis developmental pathways have been revealed recently. As an alternative source for pancreas or islet transplant in the treatment of type 1 diabetes, studies have been carried out on stem cells or somatic cells to generate functional insulin-producing β-cells via a combination of small molecules and/or transcription factors. Therefore, we tested whether pig fibroblasts treated with specific transcription factors (Pdx1/MafA/Pax4) and a small molecule (A83-01) could be differentiated into direct monohormonal cells (β-cells). The generation of insulin-producing β-cells was carried out through the transfection (MO1=1~2) of a lentivirus vector inserting 3 transcription factors (Pdx1/MafA/Pax4) into pig ear fibroblasts for 1 day. The cells were cultured in N2B27 medium supplemented with A83-01/bFGF (basic culture medium, BCM) + activin-A for the first 6 days, BCM + retinoic acid for 2 days, and BCM + ITS for 3 weeks. In induced β-cell-like cells (iβ-cells), we tested the expression of specific islet proteins (Pdx1/c-peptide/glucagon/somastatin/pancreatic polypeptide) and performed whole-transcriptome analysis and DTZ. Morphologically, iβ-cells were observed 1 week after transfection. They formed many clusters of > 100 mm diameter after 1 month and maintained their cluster formation ability until 15 passages. They even formed spherical structures like isolated pancreatic islets and showed strong Pdx1/c-peptide but no Pdx1/glucagon co-expression, and a DTZ-positive reaction. Whole-transcriptome analysis confirmed the activation of endocrine cell differentiation and ²-cell maturation pathway-related genes, such as FOXA3, NKX2-3, NKX6-1, PAX6, ISL1, NEUROD1, in the i²-cells. Overall, i²-cells shared characteristics with real pancreatic ²-cells. Repressing ±-cells thus induces characteristic monohormonal cell (²-cell) functions and could be used to develop cellular therapy for T1D.

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F-2074

DISCOVERY OF SMALL-MOLECULE CELL GROWTH ACTIVATORS THAT PROMOTE LIVER REGENERATION

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The liver is the only internal organ that has a remarkable capacity for repair following injury. The ability for the liver to regenerate is critical for patients of liver diseases when the partial removal of the liver is an indispensable treatment process. Recently, regenerative medicine of the liver is expected as a new method to cope with a shortage of living donors for liver transplantation. However, the mechanism of liver regeneration has not been fully elucidated, and further improved therapeutic approaches are needed to accomplish the realization of liver regenerative medicine. In this study, we have performed a phenotypic high

throughput screening (HTS) using FCeM, a three-dimensional (3D) cell culture medium with gellan gum, for human ovarian cancer cell line SKOV3 to identify small-molecule compounds that promote cell proliferation under 3D cell culture conditions. As the result of screening, we obtained active compounds named GA series. They promoted the proliferation of various cells including cancer cells, fibroblasts, endothelial cells, mesenchymal stem cells under 3D cell culture conditions. DNA microarray-mediated transcriptional profiling of SKOV3 cells cultured with GA series revealed that GA series led to up-regulation of yes-associated protein (YAP) downstream target genes including CTGF and Cyr61 at higher level than vehicle treated control did. Consistently, cells treated with GA series showed decreased levels of phosphorylated YAP and elevated levels of nuclear YAP. Next, we investigated the effect of GA series in mice with a predamaged liver after a one-third partial hepatectomy. Administration of GA series significantly promoted the recovery of liver weight as compared to that of vehicle. In conclusion, we identified GA series as a consequence of HTS under 3D cell culture conditions. GA series augmented the proliferation of various cells, which were probably caused by activated YAP. In addition, GA series promoted liver regeneration in vivo. GA series will contribute to development of novel and efficient treatment for liver diseases.

F-2076

GENERATION OF AN INSULIN REPORTER GENE KNOCK-IN HESC LINE USING CRISPR-CAS9 GENE EDITING

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Loss of dysfunction of pancreatic insulin-producing beta cells is the hallmark of diabetes mellitus. Human pluripotent stem cells can generate functional pancreatic beta cells, which in turn, can effectively be used in the therapeutics for treating diabetes. However, current protocols differentiate pancreatic beta cells from human embryonic stem cells (hESCs) are unable to generate glucose-responsive, functionally mature beta cells. This is partially due to the heterogenous population of cells that is generated with differentiation. It is suggested that the presence of undifferentiated cells in hESC-derived cultures inhibit glucose stimulated insulin secretion (GSIS). Therefore, a strategy to enrich for insulin-producing mature beta cells would improve beta cell function in these cultures. To accomplish this, we aim to develop a fluorescent-based insulin reporter in hESCs that will enable us to enrich for mature beta cells in our differentiation cultures using fluorescence-activated cell sorting (FACS). Here, we will use CRISPR-Cas9 mediated homologous recombination to knock-in the fluorescent reporter, insulin-T2A-GFP, at the insulin locus of the hESC H1 line. This insulin reporter one would be a powerful tool for diabetes disease modeling as well as for beta cell-specific studies at the molecular level.

Funding Source: EDUC2-08381

F-2078

GENERATION OF HUMAN IPSC-DERIVED QUIESCENT HEPATIC STELLATE CELLS FOR DRUG DISCOVERY AND LIVER DISEASE MODELING

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Hepatic stellate cells (HSCs) are liver-specific mesenchymal cells present in the perisinusoidal space of the liver. HSCs in a healthy liver are quiescent and store vitamin A, whereas they are converted into activated HSCs by virus infection or hepatic toxins. As activated HSCs play a central role for the progression of liver fibrosis by producing collagen, it is necessary to recapitulate the HSC activation process in vitro to develop effective drugs for fibrosis and a liver disease model. However, freshly isolated HSCs from normal liver rapidly undergo activation in culture and the supply of human quiescent HSCs is also limited. Therefore, it is strongly desired to develop a method to prepare quiescent HSCs for drug screening and disease modeling. In this study, we aimed at developing a culture system to generate quiescent HSCs from human induced pluripotent stem cells (hiPSCs). By mimicking the HSC development process, we have established a culture system to generate HSCs highly expressing HSC marker genes, such as NGFR, DES, NES, and LRAT, but not ACTA2, a key activation marker. Moreover, they exhibited a stellate morphology and stored vitamin A. These results indicated that hiPSC-derived HSCs are quiescent state. Upon activation by TGFb, those HSCs started to express activation marker genes, ACTA2, and COL1A1, indicating that they were converted into activated HSCs. The conversion of quiescent to activated HSCs was suppressed by inhibition of TGFb or WNT signaling. Finally, to establish a method for quantitative assessment of HSC activation in vitro, we generated the ACTA2-RFP reporter hiPSC line by inserting the RFP gene at the ACTA2 locus. The quiescent HSCs derived from the reporter hiPSC expressed RFP fluorescence after the conversion into the activated state, indicating that the reporter cell line is a useful tool for monitoring the activation process of HSCs and for drug discovery and liver disease modeling.

Funding Source: Japan Society for the Promotion of Sciences (JSPS) and Japan Agency for Medical Research and Development (AMED)

EPITHELIAL TISSUES

F-2080

MODELING AND ENHANCING MUCOCILIARY CLEARANCE IN PULMONARY DISEASE USING HUMAN AIRWAY BASAL STEM CELLS

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Airway basal stem cells (ABSCs) of the proximal airway can self-renew and differentiate into mucus and ciliated cells for host defense. Effective mucociliary clearance (MCC) is essential for lung health. In conditions with impaired MCC, ABSCs become metaplastic causing the bronchioles to grow too far distally in the lung parenchyma, a finding known as bronchiolization. While bronchiolization is widely acknowledged as a pathological finding, it is unclear why these ABSCs proliferate towards the alveolar region. The pathophysiology of several lung diseases such as Idiopathic Pulmonary Fibrosis (IPF), bronchiectasis, Cystic Fibrosis (CF) and Chronic Obstructive Pulmonary Disease (COPD), involve impaired MCC. To date, no study has directly targeted bronchiolization and MCC for therapeutic intervention. The development of rationally designed treatments for pathologic MCC has been hindered by a lack of understanding of the mechanisms of mucus dysfunction and its clearance. We believe that the bronchiolization observed in the lungs is a futile attempt of the lung to heal itself. It attempts this by the self-renewal and differentiation of ABSCs, but instead becomes hyperplastic and develops a stratified bronchiolar epithelium. In the case of IPF, which is a progressive scarring lung disease of unknown etiology, a gain-of-function promoter variant of the mucus producing MUC5B gene, is a genetic risk factor for the disease. In these patients, we observed desquamation of the ciliated cells of the bronchiolar epithelium, linked to the aberrant activity of the serine proteases KLK10 and KLK7. We observed that the cilia in these desquamated cells continue to beat unidirectionally, causing them to spin and injure the bronchiolar epithelium. We hypothesize that this persistent injury is responsible for the bronchiolization and progression of the disease. To further test our hypothesis, we are currently modeling bronchiolization, using ABSCs from IPF patients with the MUC5B variant, differentiating them into a mucociliary epithelium, and selectively detaching the ciliated cells using recombinant KLK7. Using this model, we aim to characterize the injury response and changes in the rheological properties of the mucus to determine its role in the progression of the disease and provide a target for therapeutic intervention.

F-2082

HISTONE PHOSPHORYLATION PATTERN IN CORNEAL EPITHELIUM AND IN CAOMECS

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Oral mucosa epithelial stem cells (OMECS) are used to engineer cultured autologous oral mucosa epithelium cell sheets (CAOMECS), to repair epithelial damaged tissues. A lot of effort is put in the proteomic characterization of the epithelium and much less is done on the epigenetic modification such as the DNA and the histones (Histone 3: H3). The purpose of the study is to study the pattern of expression of phosphorylated H3S10 and phosphorylated H3S28 in rabbits' corneal epithelium, oral mucosal epithelium and CAOMECS. Immunostaining were performed on rabbit fixed tissues and CAOMECS. H3S10-p is lightly expressed in the microvilli of the oral mucosa (OM), on the limbal region, on the upper layers of the corneal epithelium and mainly on the basal side of the CAOMECS. H3S28-p is not present on the cornea, but it is present on few cells of the limbal region, on the OM (with a decreasing gradient of expression from the microvilli to the apical side of the OM) and highly present on CAOMECS. The H3S10-p and H3S28-p are new markers that could be used to characterize epithelial progenitor stem cells and confirm the proliferative/apoptotic activity of the cells, on epithelium. H3S10-p is involved in active transcription, chromatin condensation (cell division or apoptosis) and UVB response. The cornea blocks a significant part of the UVB, through the activation of H3S10-p, which could damage the cornea until hazy cornea appears. H3S28-p is involved in cell division and active transcription, and can work in synergy with H3S10-p. In addition, the activation of pathways (Aurora Kinase, A/B, Msk1, JNk1, RSK2, erk1/2) controlling H3S10-p and H3S28-p could ameliorate CAOMECS engineering by shortening the time of growth. The presence of these markers and downstream genes could also be used to show the healthiness of the CAOMECS grafting on cornea, and to show that they are capable to protect the eye from UVB damage, and they play a role in the renewal of the epithelium.

Funding Source: Emmaus Medical, Inc.

F-2084

USING SINGLE CELL RNA-SEQ DISSECT DRUG-RESISTANT CANCER STEM CELLS AND THEIR NICHES

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Treatment or Drug-resistance is a fundamental biological question and clinical challenge. To dissect the cell population that are drug-resistant and also responsible for regrowth of tumor (cancer stem cell, CSC) post chemotherapy will shed light on clinical treatment of tumor or cancer. Here we report our recent work using single cell RNA-seq (scRNA-seq) to dissect CSCs and their microenvironment (TME), particularly myeloid derived cells (MDCs) as well as T cells. We first used the APCMin/+ adenoma model to study the dynamic interaction between MDCs and CSCs in vivo following chemoradiotherapy. Using 3D scanning electronic microscopy, we observed that while proliferating tumor cells were dying following chemoradiotherapy, MDCs or its derivative tumor associated macrophages (TAMs) were recruited to the site where CSCs paired with necroptotic cells. TAMs functioned to promote activation of slow-cycling CSCs. Dissected with single cell RNA-seq, we observed that while proliferating tumor cells were declining following chemotherapy, an increase in MDCs was accompanied with a reduction of T cells as well as an activation and short-term expansion of initially quiescent CSCs. Depletion of TAMs using a drugs or genetic means resulting in reduced CSCs and attenuated tumorigenesis. On the other hands, adding MDCs to the adenoma derived organoid culture increase but with EP (Pge2 receptor) inhibitor reduced organoid growth. Thus, we show that MSCs with secreted PGE2 activated Akt and Wnt signaling to promote activation of CSCs. Hence, targeting TAMs will benefit clinical treatment of colorectal tumor and cancer by reducing drug-resistant CSCs.

F-2086

ENTEROCOLITIS MOUSE MODEL: PHENOTYPIC AND MECHANISTIC ANALYSIS OF FZD7 DELETION IN MICE

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Frizzled7 (Fzd7) receptor is the important signaling receptor in the Wnt signaling pathway. The purpose of Fzd7 receptor is to receive Wnt signaling molecular and to activate beta-catenin for the downstream pathway processing the transcription of the target genes. Currently, the research had indicated that these target genes involved in regulating the proliferation and differentiation of intestinal stem cells (ISCs). Intestinal epithelial cells are considered to be rapid-renewing cells, and intestinal stem cells keep differentiating to maintain the homeostasis of intestinal epithelial cells. Therefore, it is predicted that the function of intestinal stem cells will be affected when Fzd7 gene undergoes deletion. In this study, we hypothesize the Fzd7 deficiency causes the defects on intestinal epithelium, and the specific aims as follow, (1) to establish and characterize the Fzd7 gene knockout mouse model, especially focus on intestine tract; (2) to analyze the effect of Fzd7 deletion on regeneration/ repair in intestine; (3) to verify the mechanisms of Fzd7 in regulating the proliferation and differentiation of intestinal stem cells and enterocolitis formation. It can be seen from the results that the deletion of the gene of Fzd7 affects the expression of goblet cells and causes functional defects, resulting in reduced secretion of mucus and destroyed the intestinal mucosal barrier, finally triggering an inflammatory response. We expect this animal model can facilitate to understand the functions of Fzd7 on intestine development and regeneration, and furthermore explore therapeutic for human patients with enterocolitis.

F-2088

CDC42 CONTROLLED EPITHELIAL POLARITY REGULATES INTESTINAL STEM CELL TO TRANSIENT AMPLIFYING CELL FATE TRANSITION AND CRYPT EXPANSION VIA HIPPO-MTOR SIGNALING

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Epithelial polarity is a fundamental cell function controlled by delicate polarity machinery including the Rho family GTPase Cdc42 and the SCRIBBLE/PAR complex. In Drosophila, polarity regulated hippo signaling is known for epithelial cell proliferation regulation. Previously we have shown that intestinal epithelial deletion of CDC42 by Villin-Cre in CDC42 lox/lox mice causes intestinal hyperplasia and crypt expansion associated with a Microvillus Inclusion Disease-like phenotype. Here, by using intestinal stem cell (ISC)-specific deletion of CDC42 in Olfm4-IRES-eGFPCreERT2;CDC42 lox/lox mice, we found that ISC-

initiated CDC42 loss causes similar intestinal hyperplasia with drastic hyper-proliferation of transient amplifying (TA) cells and disrupted epithelial polarity as found in Villin-Cre;CDC42 lox/lox mice, indicating that the epithelium defects are due to ISC-intrinsic effects. Intestinal TA cell population expands in the CDC42-null crypts at the expense of ISCs, accompanied with significantly elevated canonical hippo signaling through YAP/TAZ - Ereg and mTOR activation, without detectable changes in Wnt or Notch signaling. Conditional knockout of three alleles of YAP/TAZ genes is able to restore the balance of ISC/TA cell populations and crypt proliferation but not the altered intestinal morphology in the CDC42 KO small intestine. Administration of mTOR inhibitor rapamycin into CDC42 KO mice also exhibits similar rescuing effects without affecting YAP/TAZ signaling activity, indicating that mTOR acts downstream of YAP/TAZ. Inducible ablation of SCRIBBLE gene in intestinal epithelial cells mimics that of CDC42 KO defects including crypt hyperplasia and TA cell expansion. Our results demonstrate that mammalian epithelial cell polarity is critical for ISC maintenance and progenitor proliferation via a hippo-mTOR signaling cascade.

EYE AND RETINA

F-2090

TRANSCRIPTION FACTOR-MEDIATED REPROGRAMMING OF HUMAN MULLER GLIA INTO PHOTORECEPTOR-LIKE CELLS USING CRISPR ACTIVATION

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The loss of photoreceptors is a key hallmark of many incurable blinding diseases and regenerative medicine has great potentials of alleviating blindness in patients. Previous studies have highlighted the feasibility of using transcription factors to reprogram glial cells into different types of neurons both in vitro, and in vivo in the brain, spinal cord and retina. This study aims to use CRISPR activation to develop reprogramming technology to convert human Müller glia into photoreceptors (induced photoreceptors, iPH) as an innovative approach for photoreceptor generation. We have adapted the CRISPR activation (CRISPRa) system to activate expression of endogenous genes, which allow us to activate up to 9 transcription factors simultaneously. Using this CRISPRa platform, we have screened and identified cocktails of transcription factors that allow reprogramming of human Müller glia into iPH in vitro. qPCR and immunocytochemical analysis demonstrated that the reprogrammed iPH expressed a panel of rod markers including RHO, PDE6B and GNAT. To comprehensively analyse the iPH, we performed single cell transcriptome profiling of ~10000 iPH cells using 10X Chromium. Transcriptome analysis demonstrated the transition of glial to neuron through reprogramming, the activation of photoreceptor markers in iPH and the presence of different stages of reprogrammed cells. We also generated a single-cell transcriptome atlas for human retina using ~20000 adult retinal cells as a benchmark. Using this atlas, we showed that iPH reprogramming promoted transcriptome changes and transitions from Müller glia to photoreceptors. Our study demonstrated the feasibility of using CRISPRa to induce cell reprogramming of Müller glia into photoreceptors, providing a potential cell source for tissue engineering and regenerative medicine. Future application for in vivo reprogramming provides an exciting regenerative approach to replace photoreceptor losses in retinal diseases.

F-2092

IDENTIFICATION OF DIFFERENTIALLY METHYLATED WNT5A AS A DIABETIC MARKER IN HUMAN CORNEOLIMBAL CELLS AND LIMBAL-DERIVED IPSC

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Diabetes mellitus appears to be an epigenetic disease with DNA methylation and histone acetylation changes. Diabetic corneal epithelial alterations include persistent defects and impaired wound healing, which may occur due to the dysfunction of limbal epithelial stem cells (LESC). In this study, DNA methylation analysis was performed using Illumina Infinium Methylation 450k Beadchips to compare the methylation

patterns between 3 diabetic and 4 non-diabetic primary cultured human limbal epithelial cells (LEC) enriched in LESC as well as induced pluripotent stem cells (iPSC) derived from these primary cells. A 98-gene cluster showed common significant methylation differences between non-diabetic and diabetic LEC. Conversely, another 90-gene cluster showed similarity between diabetic iPSC and non-diabetic LEC but not with diabetic LEC. In the differentially methylated gene cluster, WNT5A gene was hypermethylated in diabetic LEC. This finding was validated by the decreased WNT5A expression in the limbus and LEC from 5 diabetic vs. 5 non-diabetic donors by Western blot and immunostaining. Scratch wound healing assay in diabetic LEC treated with recombinant WNT5A (200 ng/mL) showed accelerated wound healing as compared to the untreated controls. In contrast, siRNA knockdown of WNT5A expression in non-diabetic LEC showed decreased wound healing as compared to the controls. In summary, this study reported for the first time the similarity in the methylation patterns of certain genes in diabetic iPSC and non-diabetic primary LEC, indicating a possible way of normalizing diabetic LEC through iPSC generation. There is also reproducible differential methylation of diabetic and non-diabetic primary LEC, confirming epigenetic changes in diabetic limbal epithelium. WNT5A was identified as a new diabetic marker in the cornea with increased gene methylation and decreased protein expression, which can be modulated to accelerate diabetic corneal epithelial wound healing.

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F-2094

JAGGED 1-MEDIATED NOTCH SIGNALING CONTROLS THE DIFFERENTIATION AND STRATIFICATION OF HUMAN LIMBAL EPITHELIAL STEM/PROGENITOR CELLS IN VITRO

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Limbal epithelial stem/progenitor cells (LSCs) maintain the homeostasis and regeneration of the corneal epithelium. Understanding the regulation of the limbal stem cell niche is crucial to efficiently expand the LSC population in vitro and treat patients suffering from limbal stem cell deficiency characterized by the loss or deficiency of these stem cells. Notch signaling plays a pivotal role in regulating stem cell maintenance, differentiation and homeostasis. We investigated the role of Jagged 1-mediated Notch signaling activation in the regulation of human limbal stem/progenitor cells (LSCs) in vitro. To better understand the role of Notch signaling in the LSCs, we analyzed the expression of several Notch family members on the human sclerocorneal tissue at the mRNA and protein level. Primary human LSCs were cultured on Jagged 1-coated (Jag1-Fc) plates to activate Notch signaling. Air-lifting induction was performed in the LSC cultures to study the stratification and differentiation capacity of the limbal epithelium. The expression of Notch receptors (Notch 1, Notch 2), ligands (Jagged 1, Jagged 2, Delta-like 1) and target genes (Hes1, Hey1) was detected in the human corneal epithelium at the mRNA and protein level. Jag1-Fc activated Notch signaling in the LSCs. Upon Notch activation in the cultivated LSCs, cell morphology was less compact, and cell growth and the amount of small cells (≤12 µm) were significantly reduced. We also observed a significant lower expression of the stem cell marker p63αbright. After induction of air-lifting, the number of epithelial layers was reduced (lower stratification), and differentiation, given by the amount of cytokeratin 12+ cells, was increased in the Jagged 1-activated cultures. Our data suggest that Jagged 1-mediated Notch signaling activation in the LSC cultures induces differentiation.

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F-2096

DIRECT PRODUCTION OF CORNEAL ENDOTHELIAL (SUBSTITUTE) CELLS FROM HUMAN IPS CELLS BY OMITTING NEURAL CREST DIFFERENTIATION

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In order to provide regenerative medicine for the unmet millions of patients waiting for corneal transplants globally, we have derived corneal endothelial substitute cells from iPSCs (CECSi cells) for the treatment of corneal endothelial dysfunction (bullous keratopathy). At first we tried to develop CECSi cells step by step via neural crest cells (NCC) differentiated from iPS cells, but the stepwise protocol was a disadvantage for mass production. DNA microarray analysis between NCC from iPS cells and human corneal endothelial cells from donor corneas revealed three important intracellular pathways for corneal endothelial development. We therefore improved the protocol to produce CECSi from iPS cells directly by activating three pathways, omitting neural crest development. Hexagonal

confluent monolayer cells with Na,K-ATPase alpha 1 subunit (ATP1A1), ZO-1, N-cadherin, and PITX2 expressions could be produced from two of three different iPS cell clones (Ff-I01s04, Ff-MH09s01, Ff-WJs513). Real time PCR analysis revealed that pump function marker ATP1A1 expression levels of CECSi cells from two different iPS cell clones are equal to human corneal endothelial cells from donor corneas. Since this protocol is simple, it may be more suitable for mass production of corneal endothelial cells compared to previous methods.

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F-2098

TRANSPLANTATION OF CONE PHOTORECEPTORS PURIFIED FROM RETINAL ORGANOIDS GENERATED WITH A CONE-SPECIFIC HUMAN REPORTER IPSC LINE

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Human vision is particularly reliant on cone photoreceptor dependent high acuity and colour vision. A strategy to restore vision in retinal degenerative diseases is the replacement of lost cells with stem cell-derived photoreceptors, however, efficient human cone-enrichment strategies are lacking. Here, we describe the enrichment and transplantation of purified cones through the use of a human iPSC cone reporter line. Applying piggyBAC technology, hiPSCs were transduced to express GFP under the control of the mouse cone arrestin promoter and subsequently used to generate retinal organoids. Organoids were dissociated at 140 or 200 days of age and FACS sorted for GFP+ cells. Sorted cells were transplanted into the subretinal space of wild-type mice and three mouse lines exhibiting aberrant retinas: CPFL1 (cone degeneration), CPFL1; Rho-/- (cone and rod degeneration) and the Nrl-/- line which displays a cone-like cell enriched retina. Mice were immune-suppressed with triamcinolone and eyes were analysed 3 or 9 weeks later. GFP+ cells comprised 8% of all organoid cells at day 140 and 27% by day 200. Enrichment of cones as well as the cone identity of GFP+ cells were confirmed by immunostaining. This showed 90% of sorted cells co-express GFP, the pan-photoreceptor marker recoverin and the cone marker ARR3 (GFP- fraction: < 4% ARR3+ cells). Grafted cells formed clusters in the subretinal space and expressed human cone markers. While GFP+ cells were almost absent in the photoreceptor layer of wt, CPFL1, and CPFL1; Rho-/- hosts, in Nrl-/- mice some transplanted cones appeared to incorporate into the host retina. The transfer of cytoplasmic material between donor and host photoreceptors, as was recently observed in mouse-to-mouse transplantation, was not detected. In summary, FACS of dissociated retinal organoids derived from a hiPSC cone-GFP line yielded a greatly enriched cone population suitable for transplantation. Human cones survived in the murine subretinal space but did not appear to integrate into the recipient tissue or engage in material transfer, with the exception of Nrl-/- hosts, where a subset of donor cones incorporated into the retina. The viability of organoid-derived cones and the specificity of the reporter line suggests the outlined strategy to be a promising approach for further translational studies.

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F-2100

FORWARD PROGRAMMING OF HUMAN PHOTORECEPTORS

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The replacement of photoreceptors represents a promising option to counteract retinal degenerative diseases. However, for a viable cell therapeutic intervention, one requires human photoreceptors in high quantity and quality. While it is possible to obtain photoreceptors in low quantities by direct reprogramming from fibroblasts or from human stem-cell-derived 3D retinal organoids, an efficient 2D forward programming protocol to generate photoreceptors in vitro from human induced pluripotent stem cells (hiPSCs) needs to be established. Forward programming relies on transcription factors' (TF) abilities to activate distinct differentiation pathways in stem cells. Aiming to find TF combinations that drive efficient differentiation of stem cells into photoreceptors, we performed a TF-library-on-library screen. A conditional fluorescent photoreceptor hiPSC reporter line was transduced with lentiviral particles each carrying one of 16 TFs known from in vivo photoreceptor development and with a comprehensive library consisting of 1748 human TFs. We sorted 87 out of 8.4 million cells that

were qPCR-tested for photoreceptor markers and sequenced to identify the overexpressed TFs at single cell resolution. 90% of the sorted cells were qPCR-positive for at least one of the tested photoreceptor-specific genes indicating the cell-type-precision of our screen. One validated TF combination (two known TFs and one unbiasedly-screened TF) led to a significant loss of the pluripotency marker TRA-1-60 and upregulation of the neuronal marker NCAM within 5 days of overexpression, indicating that cells are differentiating towards the neuronal lineage. Furthermore, fluorescence microscopy and flow cytometry detected high numbers GFP-positive cells suggesting the presence of photoreceptor-like cells. We are currently characterizing these cells in-depths. Our data suggest that the known TFs were insufficient to drive photoreceptor differentiation, indicating that photoreceptor genesis from hiPSCs requires additional TFs. In-vitro-engineered photoreceptors might serve as donor material for cell transplantation to treat blindness or as biomedical testbeds as sufficient quantities can be generated within few days compared to hundreds of days if dissociated from 3D human retinal organoids.

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F-2102

PEDF PEPTIDE FACILITATES SIMPLE LIMBAL EPITHELIAL TRANSPLANTATION FOR OCULAR SURFACE RECONSTRUCTION IN THE RABBIT MODEL OF TOTAL LIMBAL DEFICIENCY

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To demonstrate the capacity of a pigment epithelial-derived factor (PEDF) peptide 44-mer to facilitate ocular surface epithelialization by enhancing simple limbal epithelial transplantation (SLET) in a rabbit model of total limbal stem deficiency (LSCD). Total limbal stem cell deficiency (LSCD) was created surgically by total circumferential limbal excision and total corneal epithelial removal. Two months later, ocular surface reconstruction was achieved by removal of the fibrovascular pannus over the cornea and limbus, followed by SLET and PEDF peptide treatment. A 2×2 mm strip of donor limbal tissue was obtained from the contralateral healthy eye and divided into eight to ten small pieces. The structure of the restored ocular surface was analyzed at one- and three-months follow-up. Immunohistochemical analysis was performed with antibodies to DNp63a and BrdU to identify proliferating and slow cycling cells. Cells harvested from the regenerated limbal and corneal epithelium were analyzed for colony forming capacity and expressions of limbal and mesenchymal putative stem cell markers were analyzed by immunostaining assay and quantitative real-time PCR (qPCR). The ocular surface epithelialization occurred in vehicle control eyes with conjunctivalization and vascularization of the cornea and limbus. In PEDF 44-mer treated eyes, a completely epithelialized and stable corneal surface was seen in all recipient's eyes by three months follow-up. The reconstructed ocular surface showed anatomic and functional recovery of the corneal and limbal epithelia. Immunohistochemical staining demonstrated a wide distribution of BrdU labelled slow-cycling cells throughout the regenerated corneal and limbal epithelia and superficial stromal cells. The PEDF peptide enhances ocular surface reconstruction by SLET and sustains ocular surface integrity. The addition of PEDF peptide facilitates SLET survival and reconstruction of the ocular surface with better recovery of the ocular surface epithelium than SLET only in severe LSCD eyes.

STEM CELL NICHES

F-2104

COMMITMENT OF MOUSE NEPHRON PROGENITOR CELLS TO NEPHROGENESIS IS DRIVEN BY A β-CATENIN-DRIVEN SWITCH IN TCF/LEF TRANSCRIPTION FACTOR BINDING TO ENHANCERS OF DIFFERENTIATION PROMOTING TARGET GENES

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Wnt signaling and the Wnt pathway transcriptional co-activator β-catenin are required for both the self-renewal and differentiation of mammalian nephron progenitor cells (NPCs). To investigate the molecular mechanism underlying Wnt/β-catenin-driven NPC self-renewal and differentiation, we modeled these processes in nephron progenitor expansion medium (NPEM) culture (Brown et al., 2015) supplemented with low (maintenance/expansion of NPCs) or high (differentiation of NPCs) levels of CHIR99021 (CHIR), a small molecule GSK3 inhibitor. Gene expression profiling detected a downregulation of transcriptional repressor Tcf7l1 and Tcf7l2, and a dramatic

up-regulation of transcriptional activators Tcf7 and Lef1 in differentiated NPCs. Fluorescent in situ analysis and single-cell RNA-Seq data in the developing kidney suggest Tcf7l1 and Lef1 localized to self-renewing and differentiated NPCs, respectively. As a result of changing β-catenin levels, Tcf7/Lef1 factor binding replaced Tcf7l1/Tcf7l2 interactions at enhancers regulating expression of key differentiation promoting genes, correlating with the activation of these genes. Notably, Hi-C analysis showed Tcf7/Lef1/β-catenin utilizes both pre-established and CHIR-induced enhancer-promoter loops to activate gene expression. Gene-editing approaches are being used to mechanistically dissect key interactions in this regulatory framework. Together these data suggest NPCs are in a primed state ready to undergo a rapid commitment to the nephrogenic program in which repressive interactions mediated by Tcf7l1/Tcf7l2 silence differentiation promoting enhancers while a β-catenin driven replacement by activating Lef1/Tcf7/β-catenin activation initiates NPC differentiation. We expect the findings here will have broader implications for Wnt pathway regulation of stem/progenitor systems in invertebrate and vertebrate developmental systems and Wnt-driven cancers.

Funding Source: NIDDK

F-2106

MACROPHAGE-HEMATOPOIETIC STEM CELL INTERACTIONS IN THE EMBRYONIC NICHE DETERMINE ADULT HSC CLONE NUMBER IN THE MARROW

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In development, hematopoietic stem cells (HSCs) emerge and ultimately comprise the fetal and adult hematopoietic system. Using a brainbow color barcoding system, we have previously demonstrated that zebrafish produce 20-30 HSCs in the developing aorta. Following emergence, HSCs traffic to a sinusoidal vascular niche, exit circulation, and divide. The number of HSC clones that support hematopoiesis is thought to be regulated by the niche. Using spinning disk confocal microscopy, we imaged HSPCs in the niche and found surprising HSC 'grooming' behavior by primitive macrophages, marked with the specific mpeg1:GFP transgene: macrophages contacted newly lodged HSCs and scanned their cell surface for 30-45 minutes. To evaluate clone number, we made use of a brainbow labeling system in fish in which macrophages were depleted. Unique color barcodes were induced in individual HSCs at 24 hours post fertilization (hpf), just prior to HSC emergence, and clodronate loaded liposomes were injected into circulation at 28 hpf. The resulting embryos showed rapid loss of primitive macrophages and were raised to 3 months for color analysis of the adult marrow. On average, animals injected with clodronate liposomes had only 14 unique HSC color clones, compared to an average of 24.6 HSC color clones in sibling controls ($p = 0.0002$), with a larger average clone size of 7.8% of total marrow compared to 5% in controls. Similar results were produced when macrophages were reduced through a macrophage differentiation-blocking irf8 morpholino, suggesting that this result is due to a specific loss of macrophages. Based on these data, we undertook further imaging studies. In many cell-cell interactions fluorescent protein can be seen moving from the stem cell (runx1+23:mCherry) into the macrophage. Between 48 – 72 hpf, around 20% of HSPCs interact with a macrophage in this manner. Macrophages harvested from runx1+23:mCherry;mpeg1:GFP embryos exhibit punctate mCherry signal, and in some cases appear to have engulfed entire mCherry+ cells. Our barcoding studies taken together with our imaging establish a role for macrophages in determining the number of HSC clones during development and impacts our understanding of clonal diseases in the adult hematopoietic system.

F-2108

MICRO-SCALED TOPOGRAPHIES DIRECT DIFFERENTIATION OF HUMAN EPIDERMAL STEM CELLS

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Human epidermal stem cells initiate terminal differentiation when spreading is restricted on ECM-coated micropatterned islands, soft hydrogels or hydrogel-nanoparticle composites with high nanoparticle spacing. The effect of substrate topography, however, is incompletely understood. To explore this, primary human keratinocytes enriched for stem cells were seeded on a topographical library with over 2000 different topographies in the micrometre range. 24h later the proportion of cells expressing the differentiation marker Transglutaminase-1 was determined by high content imaging. As predicted, topographies that prevented spreading promoted differentiation. However, we also identified topographies that supported differentiation of highly spread cells. The topographical features that promoted differentiation of rounded and spread cells were different.

Topographies supporting differentiation of spread cells were more irregular than those supporting differentiation of round cells. Topography coverage was also important: low topography coverage promoted differentiation of spread cells, whereas high coverage promoted differentiation of round cells. Based on these observations we fabricated a topography in 6-well plate format that supported differentiation of spread cells, enabling us to examine cell responses at higher resolution. We found that differentiated spread cells did not assemble significant amounts of hemidesmosomes, focal adhesions, adherens junctions, desmosomes or tight junctions; they did, however, organise the actin cytoskeleton in response to the topographies, suggesting a potential role for actin polymerization and SRF signalling in the topography-induced differentiation of spread cells.

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F-2110

ENDOGENOUS MOUSE NEURAL STEM CELLS ARE IRREPLACEABLE

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Quiescent tumor-initiating with neural stem cell-like properties are resistant to therapy and enable tumor recurrence. As such, quiescent tumor stem cells are increasingly targeted by novel anti-tumor therapies. Quiescent neural stem cells (NSCs) are extremely similar to tumor stem cells and may be targeted by these therapies. The capacity of the quiescent NSC niche to recover following ablation remains unknown. Nestin-creERT2::diphtheria toxin reporter (DTR) mice were induced to express DTR on NSCs with 3 days via 150mg/kg i.p. tamoxifen. Ablation and control mice were treated with 100ug/kg diphtheria toxin DT or vehicle, respectively. Proliferative cells were labeled with BrdU prior to sacrifice. For cell replacement experiments, embryonic stem cell (ESC)-derived NSCs were stereotactically implanted into the dentate gyrus and lateral ventricle. Results: Ablation caused 50-80% reduction of quiescent NSCs and BrdU+ cells in the subventricular zone and dentate gyrus within 10 days. NSC loss persisted for at least 2 months, and was accompanied by 48% loss of neurogenesis at 2 months ($p<0.01$), suggesting inability of the partially vacated neurogenic niche to be repopulated over time by surviving NSCs. ESC-derived NSCs increased proliferation of endogenous NSCs, though only modestly impacted the quiescent NSC pool size. Implanted NSCs also differentiated into neurons with appropriate morphology and projections but failed to engraft long term into the partially vacated quiescent NSC niche. We demonstrate for the first time that adult NSCs are unable to repopulate the quiescent NSC niche after partial ablation of quiescent NSCs, even though surviving NSCs remain functional and responsive to proliferative stimuli. Primitive ESC-derived NSC also appear unable to engraft into the partially vacated quiescent NSC niche. These findings suggest that loss of quiescent NSCs irreversibly decreases niche size, and suggest endogenous NSC could comprise an irreplaceable component of the niche itself. Given the importance of neurogenesis for cognition and memory, the potentially irreversible impacts of novel stem cell-targeted antitumor therapies on quiescent NSC pool size should be carefully evaluated.

Funding Source: California Institute of Regenerative Medicine Regenerative Medicine Minnesota

F-2112

MYOKINE SIGNALING BY MYF6/MRF4 IS REQUIRED TO SUSTAIN ADULT SKELETAL MUSCLE STEM CELL POOL

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In metazoans, skeletal muscle evolved to contract and produce force. Recent experimental evidence, however, suggests that skeletal muscle has also acquired endocrine functions and produces a vast array of myokines. The mechanisms that regulate myokine production and their effect on the resident stem cell population in skeletal muscle remain unknown. Here, we report that in adult skeletal muscle, Myf6/MRF4 is a major regulator of myokine expression. Genetic deletion of Myf6 in skeletal muscle leads to reduction of the muscle stem cell (MuSCs) pool in adult mice in a myokine-dependent manner but, surprisingly, does not disrupt muscle differentiation. Using ChIP-Seq and gene expression analyses of myogenic factors, we show that Myf6/MRF4 is a direct regulator of many myokines and muscle-secreted proteins, including ligands for canonical signaling pathways such as EGFR and VEGFR. Consequently, in Myf6-deficient animals MuSCs increasingly break quiescence, but can nevertheless undergo differentiation. Lastly, we show

that Myf6 and its gene network rapidly respond to aerobic and anaerobic exercise. Thus, Myf6 may play a unique role in regulation of muscle-derived cytokines and growth factor collectively called myokines during exercise.

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F-2114

IDENTIFYING THE NICHE FOR SPERMATOGENESIS

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Mammalian spermatogenesis is a complex developmental process based on a robust stem cell system, which is controlled by a special microenvironment in the testis. Although multiple cell types have been proposed as potential niche(s) for spermatogenesis, direct in vivo evidence is lacking among these studies. SCF/c-kit pathway have been implied in the regulation of spermatogenesis and is essential for maintaining normal sperm production. For this purpose, we employed different cell type-specific Scf knockout mice to investigate the physiological source and function of SCF-expressing cells, aiming to identify the niche for spermatogenesis. Using ScfGFP mice, we found that Sertoli cells were the major source of SCF in mouse testis. Conditional deletion of Scf from Sertoli cells by Amh-Cre did not alter the number of spermatogonial stem cells, but depleted most of the differentiating spermatogonial cells, differentiated spermatocytes and spermatids in the testis of six-week-old mice, suggesting that SCF-expressing cells regulate the differentiation but not the self-renewal of spermatogonial stem cells, which was further supported by the result that transplantation of SSC from Scf conditional knockout mice restored spermatogenesis in busulfan-treated wild type recipient mice. Interestingly, overexpression of SCF in DDX4-positive germ cells led to an increase of differentiated germ cells with a tamoxifen-inducible system, suggesting a dose-dependent regulation of spermatogenesis by SCF. In summary, using in vivo systems, our study provided key evidence that SCF-expressing sertoli cells create a niche for spermatogenesis, which regulate the differentiation of spermatogonial stem cells and ensure constant production of sperms.

CANCERS

F-2116

MAYO CLINIC HIGH-RISK MAMMARY GLAND AND FALLOPIAN TUBE LIVING ORGANOID BIOBANKS FOR THE DISCOVERY OF MOLECULARLY GUIDED CANCER PREVENTION APPROACHES

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A woman's life-time risk for breast and ovarian cancers are more than 10% and 1. 5% respectively and this risk is further elevated in patients with family history of associated cancers and/or deleterious mutations in genes such as BRCA1/2. A significant proportion of high-risk patients succumb to their disease. The prevention strategy currently available to these patients involve highly invasive and least desirable surgical debulking of breast and/or ovaries/fallopian tubes. Understanding of developmental and mechanistic origins of cancer cells in high-risk patients is urgently needed to develop molecularly guided cancer prevention approaches. The cell-of-origin for breast cancer and high-grade serous ovarian cancer are believed to be from epithelial cells lining the mammary gland and fallopian tubes. Organoids from dissociated tissues or single primitive epithelial cells obtained from high-risk patients are vital resource to study mechanisms regulating tissue homeostasis and track disease origin. Such viable tissue resources are currently unavailable. With a vision to boost women's cancer prevention research at Mayo, we have established the first and largest clinically and genetically-annotated patient-derived organoid biobank for mammary gland and fallopian tube tissues from average and high-risk patients undergoing surgeries at different sites of Mayo Clinic. We have viably frozen >1000 vials of mammary tissue-organoids from 100 patients including carriers of BRCA1/2 and other breast cancer-associated mutations who underwent prophylactic mastectomy, reduction mammoplasty or autopsy, and >17,000 individual fallopian tube stem cell derived organoids from 27 patients who underwent salpingectomy. Our large-scale and successful effort to create the first, well-annotated 'living' organoid biobanks for women's cancer prevention program, attendant challenges in its establishment will be discussed, and our preliminary characterization of primitive epithelial cells with the intention to identify molecular targets for prevention will be presented.

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F-2118

ACTIVE TARGETING EFFECTS OF THERAPEUTIC NANOCOMPLEX USING NOVEL BIOMARKER OF BREAST CANCER STEM CELL IDENTIFIED BY PROTEOGENOMIC ANALYSIS

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Breast cancer in women is one of the most determined life-threatening malignancy and the leading cause of cancer death. Although many conventional therapies have been applied for its treatment, it still has many handicaps to overcome. Among them, cancer stem cells (CSC) are well known to be responsible for tumor formation, development, and differentiation that offers to cellular heterogeneity as well as the recurrence cause of cancer. Therefore, to fully cure breast cancer, the treatment of both cancer and CSC is required. For CSC targeting, we developed a new surface biomarker (Factor X) of breast-derived CSC (BCSC) via proteogenomic analysis and evaluated the possibility as the specific marker and its biological effects. Next, we generated liposome-based on smart nanocomplex for selective targeting against BCSC. Selective and increased transduction efficiency was observed in BCSC treated with rhodamine included nanocomplex conjugated with anti-Factor X monoclonal antibody depending on the expression level of factor X. Doxorubicin-encapsulated nanocomplex selectively showed increased cell killing effects in BCSC with high Factor X expression level and improved anti-tumor effects in mice. Targeting of our excellent therapeutic agent using novel surface biomarker of cancer stem cell may be a more effective strategy to overcome cancer resistance.

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F-2120

QUANTITATIVE EVALUATION AND BIODISTRIBUTION ANALYSIS OF INTRAVENTRICULAR DELIVERY OF THERAPEUTIC NEURAL STEM CELLS TO ORTHOTOPIC GLIOMA IN MOUSE BRAIN

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Neural stem cells (NSCs) are inherently tumor-tropic, which allows them to migrate through normal tissue and selectively localize to invasive tumor sites in the brain. We have engineered a clonal, immortalized allogeneic NSC line (HB1.F3.CD21; CD-NSCs) that maintains its stem-like properties, a normal karyotype and is HLA Class II negative. It is genetically and functionally stable over time and multiple passages, and has demonstrated safety in phase I glioma trials. These properties enable the production of an 'off-the-shelf' therapy that can be readily available for patient treatment. The route of NSC delivery and the distribution of NSCs at tumor sites are key determinants in the efficacy of cell-based therapies. Stem cells can be engineered to deliver and/or produce many different therapeutic agents, including prodrug activating enzymes which convert prodrugs to active chemotherapeutic agents; tumor-targeted antibodies; therapeutic nanoparticles; oncolytic viruses; and extracellular vesicles that contain therapeutic oligonucleotides. By targeting therapeutics selectively to tumor foci, we aim to minimize toxicity to normal tissues and maximize therapeutic benefits. Here we quantitatively analyze biodistribution of HB1.F3.CD21 NSCs administered via ventricular injection (IVEN). IVEN delivery enables repeat administrations for patients through an Ommaya reservoir, potentially resulting in improved therapeutic outcomes. In preclinical studies using multiple glioma cell lines, we quantify NSC migration and biodistribution in mouse brain. We find robust migration of our clinically relevant HB1.F3.CD21 NSC line toward invasive tumor foci, irrespective of their location of origin. These results establish proof-of-concept and demonstrate the potential of IVEN delivery of therapeutic options using modified NSCs.

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F-2122

AN ASSAY FOR THE DETECTION AND ISOLATION OF SINGLE LIVE CTCS USING ACCUCYTE/ RARECYTE PLATFORM

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Circulating tumor cells (CTCs) contain critical metastatic precursors with cancer stem cell properties and are at the interface between the primary tumor and the target secondary organs. Identification and analyzing CTCs at single cell level will help understand the properties associated with metastatic precursors. To develop methods for detecting and isolating single CTCs from whole blood with the goal for transcriptomic studies via single cell RNA sequencing and biological characterization via ex-vivo culture of single CTCs. Healthy donor blood samples were spiked with cells from breast cancer patient-derived CTC lines and processed using the AccuCyte system. CTCs are enriched by AccuCyte platform in a layer termed “Buffy Coat”. Buffy coat containing spiked CTCs was stained live with a cocktail of PE-594 antibodies conjugated to immune cell markers including CD14, CD16 and CD45. Buffy coats were simultaneously stained with either a live cell dye or with a cocktail of Alexa Fluor 488 antibodies conjugated to cancer cell surface markers (EpCAM, Her2, and EGFR). Stained cells were then plated on chamber slides in the presence of serum free RPMI media and investigated using RareCyte fluorescence platform for the presence of CTCs. For cells stained with live cell dye and immune markers, CTCs were identified based on the lack of immune cell marker expression and the presence of the live cell dye and hence termed “negative selection”. For cells stained with cancer cell surface markers and immune markers, CTCs were identified based on their surface expression of cancer markers and lack of immune cell markers and hence termed “positive selection”. Single CTCs detected by both approaches were retrieved using an optimized interactive picking protocol. Cells detected by “negative selection” were tested for their ability to proliferate ex-vivo, whereas those isolated by “positive selection” were processed for single cell RNA sequencing analyses. Detection rates of the optimized protocol reached up to 100% (24 out of 24 CTCs detected by negative selection). Furthermore, single CTCs were spiked in whole blood can be detected by positive and negative selection. Moreover, cells picked by “positive selection” are currently validated for RNA sequencing analyses.

F-2124

AN IN-VITRO ANALYSIS OF THE CROSS TALK BETWEEN DENTAL PULP MESENCHYMAL CELLS(DPSCS) AND CANCER CELLS

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Mesenchymal stem cells (MSCs) have shown to inhibit or proliferate cancer cells depending on the lineage of the cancer cells and the tissue of origin of the MSCs. Compared to other MSCs (bone marrow-derived, placenta-derived, adipose tissue-derived etc) the effect of dental pulp-derived MSCs (DPMSCs) on cancer cells have been relatively less explored. Thus, the present study analysed the effects of DPMSCs on 4 different cancer cell lines including melanoma, oral, breast, and prostate cancer cells. The DPMSCs were isolated from human teeth. The isolated DPMSCs were characterized to confirm their lineage. The cancer cell lines were bought from national center for cell sciences (NCCS), Pune. The DPMSCs were co-cultured with each of the cancer cell lines using both direct and indirect methods (trans-well and conditioned media). Two common phenomena were observed in the direct co-cultures: Homo-fusion between the cancer cells and hetero-fusion (hybrid cells) between the cancer cells and the DPMSCs. In homo-fusion, the fused cancer cells formed polyploid giant cancer cells which were more aggressive than the parent cell line. In the hetero-fusion, the fused hybrid cells exhibited properties different from the parent cell. The properties of the fused cells (from both homo and hetero fusion) were compared with the parent cancer cells using the MTT assay (to estimate metabolic potential), the wound healing assay (to estimate migratory potential), and the colony forming assay (to estimate clonogenic potential). Flow cytometric analysis for cancer stem cell markers like CD44, CD133 and SOX 2 were performed using the hybrid cells and the results were compared to the parent cancer cell line expression. As tumour microenvironment (hypoxic, acidic pH) is favourable for cell fusion, there could be an association between the frequency of cell fusion and cancer progression. An increased proportion of fused cell population in cancer could potentially influence the overall tumour behaviour. Thus, inhibiting cell fusion through manipulation of the tumour microenvironment (oxygen saturation, increasing the pH) could aid in modifying the cancer cells behaviour including its sensitivity to therapeutic modalities.

F-2126

TOWARDS A MECHANISTIC UNDERSTANDING OF THE TUMOR SUPPRESSOR FUNCTION OF WISKOTT-ALDRICH SYNDROME PROTEIN

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Wiskott-Aldrich syndrome (WAS) is a rare pediatric disorder caused by mutations in the WAS gene. The biological features of this disease include thrombocytopenia, eczema, complex immunodeficiency, and malignancy. WAS protein (WASP), encoded by the WAS gene, is a classical actin nucleation-promoting factor. Yet, the well-known functions of WASP fail to fully explain the high rate (13%~ 22%) of cancer in children with WAS. Recently, WASP was identified as a tumor suppressor by Chiarle's group; however, the mechanism of its tumor suppressor function is not clear. Mounting evidence has already demonstrated that the ribosomal DNA (rDNA) gene inside the nucleolus is critical for genome stability, chromatin structure, and cancer pathogenesis. In addition, the perinucleolar heterochromatin shows structural alterations in cancer cells. Here, we use induced pluripotent stem cells (iPSCs) from patients with WAS (WAS-iPSC), isogenic gene-corrected cells (cWAS-iPSC), wild type B lymphocytes, and immortalized WASP-deficient cell lines to study the mechanisms of WAS pathogenesis. Our results showed that WASP deficiency results in the abnormal cell proliferation, over-expression of oncogenic genes, and perinucleolar heterochromatin lost. Besides, WASP physically interacts with partners inside the nucleolus. WASP binds to the ribosomal DNA (rDNA). We are examining gene expression, genome stability, and copy number variations. Taken together, our data suggests that WASP is a tumor suppressor in blood cells, and reveals important mechanisms associated with this function.

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F-2128

ACTIVATION OF GSK3 BETA SUPPRESSES CANCER STEM CELLS AND OVERCOMES THE CETUXIMAB RESISTANCE IN HUMAN COLORECTAL CANCER

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The limited efficacy of anti-EGFR therapies such as Cetuximab and Erlotinib in advanced cancer patients remains to be resolved to enhance the prognosis of cancer patients. Clonal evolution, which is derived from survived cancer stem cells, is the barrier to effective anti-epidermal growth-factor receptor (EGFR) therapies. In this study, we observed that the protein levels of EGFR and RAS, especially mutant KRAS, are increased in Cetuximab-resistant CRC patient tissues. We also identified that these increases are attributed to the mutations of adenomatous polyposis coli (APC) which occur in 90% of human CRC. In addition, the increment of these proteins by APC loss synergistically promotes activation of cancer stemness of CRC. Therefore, we tested the effects of reducing the levels of EGFR and Ras in overcoming the cetuximab resistance by KYA1797K, a recently identified small molecule that degrades Ras and suppresses transcriptional level of EGFR via GSK3β activation. KYA1797K effectively suppresses the anti-EGFR therapy resistances with decreased cancer stem cell markers in the CRC animal models as well as patients derived tumor organoids (PDTOs) having KRAS mutations. Therefore, therapeutic strategy lowering levels of both RAS and EGFR would be an effective strategy that overcomes current limits of the anti-EGFR therapies in CRC and other types of cancer with mutations of EGFR and RAS.

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F-2130

HIGHER ACCURACY DETERMINATION OF IMMUNE CELL IDENTITY AND PURITY USING THE PUREQUANT REAL-TIME PCR ASSAY

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In the past several years significant improvement has been made in treating certain kinds of cancers by harnessing the power of immune system, in particular, T cells. One of the key challenges in developing immune cells as therapeutic agents is the accurate estimation of their identity and purity. Current methods used for characterization of immune cell types rely on flow cytometry. Flow cytometry can accurately estimate CD8+ T lymphocytes and other surface markers. However, this method is challenging to implement in a GMP manufacturing environment posing logistical challenges such as requirement for live cells, variability leading to difficult in standardizing and high throughput. In addition, cytometric methods are not accurate for specific intracellular targets that positively identify Regulatory T (Treg) cells and T Helper 17 (Th17) cells. Therefore, there is an emerging need for alternative assay methods. Epigenetic DNA methylation is known to be unique for specific cell types and can thus be used as an identifier in heterogeneous population of cells. Exploiting differences in cell type-specific methylation signatures, we developed assay kits that quantify the percentage of Treg and Th17 by detecting methylation status of FoxP3 and IL17A via qPCR of bisulfite converted genomic DNA. In contrast to flow analysis, sample requirement is minimal and the assay works well with fresh/frozen cells or genomic DNA. This assay has been implemented to accurately identify and estimate different T cell population in Chimeric Antigen Receptor (CAR)-modified T cells. The combination of accuracy, low sample requirement and flexibility provides an ideal measurement system for confirmation of identify and purity of T cell types specifically critical for therapeutic applications.

NEURAL DEVELOPMENT AND REGENERATION

F-3002

DISTINCTIVE RESPONSE OF NEUROEPITHELIAL STEM CELLS WITH CORTICAL AND SPINAL CORD IDENTITY TO THE NOTCH SIGNALING IN INJURED SPINAL CORD MICROENVIRONMENT

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Transplantation of tripotent neural stem/progenitor cells is a promising therapeutic strategy for traumatic spinal cord injury (SCI), however, the optimal temporal and spatial developmental stage for these cells remains to be determined. In this study, we compared the fate determination of neuroepithelial stem/progenitor cells (NECs) with an anterior cortical identity to NECs patterned to acquire a ventral spinal cord identity in the injured spinal cord microenvironment. Human-induced pluripotent stem cell (hiPSC) derived cortical NECs (cNECs) and spinal NECs (spNECs) were generated and transplanted into the injured spinal cord. cNECs mainly differentiated into neurons, while spNECs mainly differentiated to myelinating oligodendrocytes. The unique differentiation profiles were mainly due to differential Pax6 expression between the two lines and were affected by activation of Notch signaling in the injured spinal cord microenvironment. cNECs excreted their effect in functional recovery in part through differentiation to neurons, migration towards cavity and making a cellular bridge, while spNECs implemented their effect partially through remyelination. Both lines provided trophic support for tissue preservation and regeneration.

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F-3004

MESENCHYMAL STOROMAL CELL DELIVERY THROUGH CARDIOPULMONARY BYPASS FOR NEUROPROTECTION IN A JUVENILE PORCINE MODEL

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Neurodevelopmental impairment is emerging as one the most important current challenges for survivors after pediatric cardiac surgery. Cardiopulmonary bypass (CPB) can cause substantial systemic inflammation and trigger prolonged microglial activation. Mesenchymal stromal cells (MSCs) have significant immunomodulatory properties and regulate microglia activation. We hypothesize that intra-arterial MSC delivery through CPB is neuroprotective by modulating systemic and neuro-inflammatory responses. Two-week old piglets were randomly assigned to one of 3 groups: (1) Control, (2) CPB, (3) CPB followed by MSC administration. 18F-FDG or superparamagnetic iron oxide (SPIO)-labeled MSCs (10x106 per kg) were delivered through CPB. PET was performed at 1hr after MSC delivery. Animals were sacrificed 3hrs after CPB. It has been well demonstrated that intra-venous injection of MSCs resulted in high accumulation of cells primarily into lungs. In contrast our PET study showed that intra-arterial delivery through CPB uniformly distributed MSCs to all organs analyzed such as the brain, heart, and kidney except that lungs and intestine showed lower uptake. Brain MRI showed diffuse distribution of hypointense voxels (SPIO particles) throughout the entire brain. We have previously demonstrated an increase in permeability of the blood-brain barrier after DHCA. Consistently we identified MSCs located in the extra-vascular space. MSC delivery through CPB modulated plasma cytokine/chemokine expression following surgery. In the brain MSC treatment reduced microglia expansion/activation and inhibited caspase activation resulting from CPB. Analysis of the RNA sequencing data identified 262 differentially expressed genes. Of these, 53 upregulated genes were significantly enriched for WNT signaling, indicating a potential mechanism through which MSCs mediate their neuroprotective effects. Various biomarkers after MSC delivery did not differ compared with CPB group. No evidence of either embolic events or microstrokes were observed by MRI and histology. MSC delivery during CPB is highly effective and shows translational potential to minimize CPB-induced systemic inflammation and reduce microglial expansion and caspase activation in children with CHD.

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F-3006

TFII-I ISOFORMS REGULATE TYROSINE HYDROXYLASE EXPRESSION IN COOPERATION WITH NURR1 AT BOTH THE TRANSCRIPTIONAL AND EPIGENETIC LEVEL

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Our previous study showed that Nurr1 actively represses human tyrosine hydroxylase (hTH) transcription in precursor cells, while it activates hTH expression in dopaminergic (DA) neuronal cells. Using proteomic analysis, we identified TFII-I as an interacting partner of Nurr1. Here we report that two alternative splicing forms of TFII-I acts in switching from repression to activation of hTH expression. TFII-IΔ preferentially interacts with Nurr1 in neural stem cell F3, while TFII-Iγ in SH-SY5Y cells. In addition, each TFII-I isoform majorly occupies hTH promoter in each cells. The transcriptional outcome of each isoform is totally different; repression for TFII-IΔ and activation for TFII-Iγ. Next, to investigate if SUMOylation of TFII-IΔ represses Nurr1 transcriptional activity via synergy control motif, we mutated SUMO sites of TFII-I isoforms as K221R and K240R. While de-SUMOylation of TFII-IΔ did not change nuclear localization, it loses transcriptional repression activities in F3 cells. Oligoprecipitation and ChIP experiments showed that deSUMOylation result in higher DNA-binding activity on hTH promoter. Last, TFII-IΔ preferentially co-localizes with bivalent chromatin marks H3K4/K27me3 in F3 cells compared to SH-SY5Y cells. All our results demonstrated that two alternative splicing forms of TFII-I gene may play an important role in fine tuning of hTH gene expression during DA neurogenesis.

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F-3008

UNDERSTANDING THE DEVELOPMENT OF SPINAL SENSORY INTERNEURONS THROUGH IN VITRO DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Spinal cord injury (SCI) patients can lose somatosensation, the ability to sense the environment if sensory interneurons (dI1-dI6) are damaged. These six classes of interneurons arise in the developing dorsal spinal cord through the combined actions of the Bone Morphogenetic Protein (BMP) and retinoic acid (RA) signaling pathways. However, it remains unresolved how RA and BMP signaling direct a variety of dI fates in the spinal cord. Using developmental signals, we have identified the conditions to derive four classes of dI neurons (dI1-dI4) from mouse embryonic stem cells (mESCs). We have found that RA alone is sufficient to direct ESCs towards dI2 (unknown function) and dI4s (pain-sensing) neurons while RA with BMP4 suppresses dI2/dI4 fates and concomitantly induces dI1 (proprioception) and dI3 (mechanosensing) neurons. We hypothesize that RA and BMP4 activate different transcriptional networks to drive ESCs towards bipotential progenitors, which then differentiate into either dI2/dI4 or dI1/dI3 fates. We are evaluating this model using RNA-Seq analysis of differentiating dI neurons to identify the RA and BMP4 specific transcriptional networks. This information will enable us to design clinically relevant ESC protocols to generate specific dI neurons that can be used as cell replacement therapies to treat SCI.

F-3010

RECONSTRUCTION OF MOTOR FUNCTION IN PERIPHERAL NERVES BY TRANSPLANTATION OF HUMAN IPS CELLS-DERIVED MOTOR NEURON PROGENITORS

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Skeletal muscles denervated by nerve injury or motor neuron disease often fall into irreversible motor dysfunction. To overcome this issue, regeneration of peripheral nerves has been attempted to reconstruct damaged motor functions. Previously, we reported that transplantation of motor neurons derived from spinal cord of rat embryos into peripheral nerves facilitated muscle contraction by electrical stimulation. In this study, human iPSC-derived motor neuron progenitors (hiPSC-MNPs) were transplanted into injured peripheral nerves and were evaluated for the effectiveness of functional neuromuscular reconstruction. Peripheral nerve injury model was generated by the transection of peroneal nerves of 8-week-old Nude rat (F344/NJcl-rnu/rnu). One week after the transection, one million hiPSC-MNPs stably expressing ffLuc (fusion protein of Venus and luciferase) as a fluorescent and luminescent marker (201B7-ffLuc), were transplanted into the distal portion of the transected nerves. Transplanted cells were evaluated over time by in vivo bioluminescent imaging (BLI), followed by the fixation at 12 weeks for histological evaluation. In BLI, luminescence intensity gradually increased until 12 weeks. Compared to the surgical control group, transplanted rats showed suppression of the atrophy of the tibialis anterior muscles, and functional contracture of tibialis anterior by electrical stimulation. Action potentials were also detected in electromyogram. Maximum muscle contraction force by tetanus stimulation was about 1/5 of the healthy side. Histological analysis has shown the cells stained for Venus (GFP) or human specific antigens in the grafted peroneal nerves, with axonal elongation to form a-BTX+ neuromuscular junctions (NMJs) in tibialis anterior muscle. In this study, we confirmed in vivo NMJ formation by hiPSC-derived motor neurons, as well as functional muscle contraction by electrical stimulation. This system could be applicable not only to the reconstruction therapy of motor function, but also to the in vivo analysis of neuromuscular pathology using disease specific iPSCs as a humanized model.

F-3012

EFFICIENT INDUCTION OF ASTROGLIOGENESIS ENABLES AUTOMATED, HIGH-TROUGHPUT GENERATION OF ASTROCYTES FROM HUMAN PLURIPOTENT STEM CELLS

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Astrocytes play important roles in normal brain development, synaptic function, neurodegenerative diseases, and various pathological conditions (e.g. opioid addiction). Derivation of human astrocytes from a scalable source such as induced pluripotent stem cells (iPSCs) is an attractive approach for disease modeling and drug discovery; however, currently

available protocols are variable, inefficient, and lengthy (lasting up to several months). Here, we developed a highly efficient and controlled astrocyte differentiation protocol that overcomes the limitations of previously published methods. By identifying and simultaneously manipulating several critical pathways, we directly induced astrogliogenesis from iPSCs with over 90% efficiency in less than 30 days. These cells displayed astrocyte morphologies and expressed typical markers such as GFAP, NF-IA and S100-B. Unlike previous protocols, our approach enabled the direct transition of pluripotent cells into PAX6+ neuroepithelia and then into BLBP+ radial glial cells in only 7 days. By day 14, radial glial cells differentiated into S100B+ astroglia, thereby largely bypassing neurogenesis, followed by NF-IA+ expression at day 21 as demonstrated by immunocytochemistry and time-course RNA-Seq experiments. Single-cell analysis and comparison of iPSC-derived neuroepithelia to astrocytes confirmed strong enrichment of astroglial genes and absence of genes indicative of other cell types (e.g. neurons, oligodendrocytes, microglia, endothelial cells, pluripotent cells). Importantly, iPSC-derived astrocytes were functional and capable of taking up the neurotransmitter glutamate, storing glycogen intracellularly, and promoting neuronal survival and synaptic activity when co-cultured with neurons. Finally, the differentiation protocol was automated using a robotic cell culture system, which now enables standardized production of large quantities of astrocytes for high-throughput screening and other translational applications.

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F-3014

CHARACTERIZATION OF FETAL BRAIN TISSUE AND VENTRAL MIDBRAIN-PATTERNED HUMAN EMBRYONIC STEM CELLS AT SINGLE CELL RESOLUTION

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Parkinson disease (PD) is the most common neurodegenerative disorder, characterized by progressive loss of dopamine neurons in midbrain. The relatively focal degeneration makes it a good candidate for cell-based therapies. Clinical trials using cells derived from human fetal brain have shown dopamine release restored to normal levels and in some PD patients produced substantial long-term clinical improvement. To move to large-scale clinical applications, the current challenge is to recreate authentic and functional dopamine neurons from human embryonic stem cells (hESCs) in vitro, thereby opening up unprecedented opportunities to gain access to a renewable source of cells potentially suitable for PD therapeutic applications. In this study we used unbiased single cell RNA sequencing to transcriptionally compare fetal VM tissue and VM-patterned hESCs after in vitro differentiation. Our analysis confirmed the diversity of cells types during midbrain development and revealed both developmental similarities and differences between the fetal VM cells and the ones differentiated from pluripotent stem cells.

F-3016

HUMAN STEM CELL-DERIVED NEURONS REPAIR CIRCUITS AND RESTORE NEURAL FUNCTION

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The adult mammalian brain has a limited capacity to regenerate after injury or diseases. Transplantation of neural cells to repair the damaged circuitry is a potential treatment, but whether and to what extent by which the function of the repaired circuit is restored is not known. By transplanting midbrain dopamine (mDA) neurons and forebrain glutamate neurons, derived from hESCs, into the substantia nigra (SN) of adult Parkinson's disease model mice, we found that both neuronal types extended long axons but to different brain regions with mDA neurons predominantly projecting to the dorsal striatum via the nigra-striatal pathway. The grafted mDA neurons received area-specific synaptic inputs and these inputs became functional 3-6 months after transplantation. Transplantation with mDA neurons, but not forebrain glutamate neurons, resulted in motor functional recovery in the PD mice, which was abrogated or enhanced by regulating the activity of the grafted mDA neurons.

These results highlight the capacity of hESC-derived neurons for specific circuit integration both pre- and post-synaptically in the adult brain and the dependence on reconstruction of functional circuitry for therapeutic outcomes.

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NEURAL DISEASE AND DEGENERATION

F-3018

DEVELOPMENT OF A NEUROLOGICAL DISEASE MODELING PLATFORM USING DIRECTLY INDUCED ASTROCYTIC CELLS FROM HUMAN NEURAL STEM CELLS

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It has becoming apparent that glial cells play pivotal roles in the pathogenesis of neurodegenerative disease. To achieve pathologic elucidation and innovative drug development against the neurological disorders, it is important to establish disease-in-a-dish model. However, there is a limited robust methodology for the rapid and efficient preparation of disease-specific astrocytic cells so far. Here, we established a polycistronic episomal expression vector, which harbors astrocyte-inducing factors with the self-cleavage 2A peptide. This system enabled to generate astrocytic cells from human neural progenitors efficiently. Furthermore, as a proof-of-concept trial, we applied this system to generate in vitro model of Alzheimer's disease. Then, we could generate astrocytic cells from Alzheimer's disease patient-derived cells and found morphological changes and enhancement of disease-relevant cellular phenotype in Alzheimer's disease patient-derived astrocytic cells. To further examine dysregulated astrocytic function of the cells, we performed functional analyses and transcriptome analysis. In the transcriptome analysis, we performed RNA-seq analysis and found that the pathways associated with inflammatory signaling were upregulated in Alzheimer's disease-patient astrocytes. These results might add new layers of pathogenesis of Alzheimer's disease, regarding neuroinflammatory response evoked by astrocytes. Taken together, our approach will pave the way to establish an in vitro system for disease-modeling of neurodegenerative disease.

F-3020

ESTABLISHING A CENTRALIZED REPOSITORY OF HUMAN PLURIPOTENT STEM CELLS FOR ALZHEIMER'S DISEASE AND RELATED DEMENTIAS

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The National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) biorepository was established in 1990 and currently houses more than 20,000 lymphoblastoid cell lines and peripheral blood mononuclear cells. Recently, NCRAD has been funded by the National Institute on Aging (NIA) to create a centralized repository of human pluripotent stem cells (hPSCs) and fibroblasts from patients with Alzheimer's Disease and Related Dementias (ADRD), mild cognitive impairment as well as healthy controls. hPSCs provide a remarkable tool to study early human development, disease modeling, and drug development. However, much variability exists between hPSC lines, which is compounded by the diversity of culture conditions that exist among laboratories. Additionally, many labs

do not have the resources to perform thorough quality control measures on cell lines or to keep up with the high demand of requests. Due to these factors, NCRAD currently works with researchers to centralize hPSC and fibroblast cell lines in one location and perform rigorous quality control to ensure cell lines can be offered in a standardized manner to ADRD researchers. hPSCs are collected from researchers around the world and are screened for multiple species of mycoplasma and other sources of bacterial, fungal, and yeast contamination. A single nucleotide polymorphism (SNP) fingerprint is obtained using a panel of 94 unique sites to assign an identity to every cell line. G-band karyotype is performed to assess genetic stability. Pluripotency is confirmed by immunocytochemistry as well as qPCR analysis. hPSCs are directed to differentiate to each of the three germ layers. Differentiation efficiency is determined by qPCR analysis of more than 20 genes corresponding to each lineage. Reprogramming factors are measured to ensure residual elements are not present. Any pathogenic mutations are confirmed by PCR amplification followed by Sanger sequencing. Lines that pass all quality control measures are made available to any researcher interested in studying ADRD. Through these efforts, NCRAD has established a standardized facility to advance the study of ADRD through the distribution of hPSCs and fibroblasts.

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F-3022

DEGENERATING NEURONS CONVERT ALS MICROGLIA FROM A NEURODEGENERATIVE TO A NEUROPROTECTIVE STATE

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder marked by severe loss of motor neurons in the central nervous system (CNS). The hexanucleotide repeat expansion in C9ORF72 is the most common genetic cause of ALS. Recent studies suggest that microglia, the resident immune cells in the CNS, modulate neurodegeneration. However, whether their behaviors are adaptive or maladaptive, and the mechanisms underlying these behaviors are not well understood due to the limited access of primary microglia from patients. Importantly, simultaneous gain- and loss-of-function mechanisms induced by C9ORF72 repeat expansion are hard to recapitulate in mice. To dissect the role of microglia in neurodegeneration, we established a human induced microglia (iMG) model using transcription factor-mediated reprogramming from patient iPSCs. iMG possess functional properties of microglia and have similar transcriptomic signatures to human primary microglia. iMG derived from C9ORF72 ALS patients display an abnormal gene expression profile that is similar to that found in microglia in multiple different models of neurodegenerative disease. In addition, C9ORF72 ALS iMG possess multiple vesicle trafficking abnormalities, a microglial phenotype that has been linked to several neurodegenerative diseases. Consistent with these findings, C9ORF72 ALS iMG induce the degeneration of control induced motor neurons (iMNs). In contrast, C9ORF72 ALS iMG surprisingly increase the survival of iMNs from C9ORF72 ALS patients, which normally degenerate more rapidly than control iMNs. Using cytokine profiling, we identified several cytokines as neuroprotective when C9ORF72 ALS iMG are co-cultured with C9ORF72 ALS instead of control iMNs. These results are consistent with a model in which the ALS causative C9ORF72 repeat expansion mutation induces microglia to adopt a neurotoxic state. However, the presence of degenerating C9ORF72 ALS motor neurons converts the innately neurotoxic C9ORF72 ALS microglia into a neuroprotective state that mediate its effects in part through cytokines release. These results suggest that in neurodegenerative diseases, microglia can initially act in a neuroprotective manner and maintaining this ability throughout the disease course could lead to new therapeutic approaches.

F-3024

ASTROCYTE CROSS-TALK WITH MICROGLIA STIMULATE PROLIFERATION AND M2-POLARIZATION OF RAT BRAIN MICROGLIA

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Astrocytes are the most abundant cells in the brain that maintain homeostasis of CNS, provide structural support as well as regulate ion, nutrient, and gas concentration. Microglia are the resident immune cells of CNS. At the injury status, microglia become activated either classically activated or alternatively activated referred as pro-inflammatory M1 or anti-inflammatory M2 type. In this study, we aimed to establish whether astrocytes and microglia can cross-talk under the resting or activated status. For this approach, microglia only or mixed glial cells were prepared from brain of postnatal day (P) 1-3 inbred Lewis rat. After the 14 day culture, microglia were isolated from both cultures using Magnetic-activated cell sorting (MACS). Astrocytes co-culture promote the cell proliferation and phenotypic polarization of brain microglia; Cd163+Cd206+ microglia among total Cd11b + brain cells were increased from 56% at P0 to 98.6% at two weeks co-culture. This positive effect of astrocytes co-culture was confirmed by treating the conditioned medium to MACS-sorted Cd11b+ brain cells and recombination of purified GFAP+ astrocyte and Cd11b+ sorted P0 or 2 weeks microglia. In a various ratio of GFAP+ astrocytes and Cd11b+ microglia co-culture, approximately ten fold more iba1+ BrdU+ cells were obtained. In an attempt to identify soluble factors present in the GFAP+ astrocyte co-culture to promote M2 polarization and proliferation of microglia, cytokine arrays was performed. GFAP+ astrocytes are expressing high

levels of activin, agrin, CINC1,2,3, CNTF, TIMP1, MCP-1, INF-γ and more cytokines. Further analysis of cross-talk between reactive astrocytes and microglia and classification of reactive astrocytes are under way. Taken together, GFAP+ astrocytes can stimulate the proliferation and M2-polarization of microglia, which suggests positive cross-talk of astrocyte under the brain injury for less tissue damage and better tissue repair.

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F-3026

ELUCIDATING AXONAL PATHOPHYSIOLOGY OF IPSCS-DERIVED MOTOR NEURONS FROM FUSED IN SARCOMA (FUS)- ALS PATIENT

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The characteristic structure of motor neurons (MNs), particularly of the long axons, becomes damaged in the early stages of amyotrophic lateral sclerosis (ALS). However, the molecular pathophysiology of axonal degeneration remains to be fully elucidated. Fused in sarcoma (FUS) is one of the most common ALS causative gene which have multifunction on neuronal axon via RNA metabolisms. We aim to elucidate the axon pathomechanism associated with FUS mutation. We constructed two sets of isogenic human-induced pluripotent stem cell (hiPSCs)-derived MNs possessing the single amino acid difference in the FUS. We identified aberrant morphology in FUS-mutant hiPSCs-derived MN axons compared with isogenic controls as a novel phenotype. Moreover, we applied microfluidic devices that enable axon bundles to be produced for omics analysis and conducted RNA profiling of isolated axons. By culturing iPSCs derived MNs on microfluidic devices, the axon specific RNA could be extracted enough to RNA sequencing. Thus, we further revealed the entire in vitro RNA profile of the human MN axon and identified a causative factor of aberrant axon morphology in FUS-mutant MNs. Intervention in the novel factor revealed the reproducibility of the morphological change in axon even in vivo. Analyzing the axonal fraction of hiPSC-derived MNs using microfluidic devices revealed a key regulator of FUS-mutant MN axon morphology.

F-3028

HUMAN IPS CELL MODELS FOR THE VALIDATION OF SMALL MOLECULE INHIBITORS OF THE TRPM4 ION CHANNEL - A NEW POTENTIAL TARGET FOR THE TREATMENT OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is the most frequent chronic inflammatory disease of the central nervous system (CNS), leading to axonal demyelination and progressive neuronal degeneration. While progress has been made in treating the inflammatory processes of MS, neuronal loss in the CNS is not well understood and there is no curative treatment available yet. The ion channel transient receptor potential melastin 4 (TRPM4) has been shown to be involved in the process of inflammation-mediated neurodegeneration. Activation and misexpression of TRPM4 contributes to neuroaxonal damage in the CNS, without modulation of the immune response in the animal model of MS (experimental autoimmune encephalomyelitis;

EAE). Furthermore, genetic knock out or unspecific blockage of TRPM4 led to an amelioration of the EAE disease course and an increased resistance of mouse neurons to glutamate-induced excitotoxicity. Previously, the suitability of TRPM4 as a therapeutic target was established and we have identified potent and selective small molecule inhibitors of the TRPM4 channel in a high-throughput screening approach. Confirmed hits have further been optimized in hit-to-lead programmes and are currently being extensively validated in various in vitro and in vivo assays using human cell lines and primary mouse neurons. We aim to demonstrate the neuroprotective effect of lead candidates targeting TRPM4 in human tissue specific cells generated from induced pluripotent stem (iPS) cells. For this purpose, we established efficient protocols for their differentiation into glutamatergic neurons. These neurons showed an upregulation of TRPM4 and NMDA receptors over time and exhibited the electrophysiological properties of functional neurons. However, 12 weeks of culturing was required to record spontaneous action potentials and synaptic activity via Patch-Clamp technique. In an alternative approach, we therefore used hiPSCs harbouring an inducible neurogenin 2 (NGN2) transgene that enables rapid conversion into defined and functional neurons. In combination with a TRPM4 knock out cell line generated using the CRISPR-Cas9 technology, we aim for validation of the potency and selectivity to prioritize compounds for consecutive preclinical development steps.

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F-3030

DEVISING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED EXCITATORY NEURAL NETWORK TISSUE FOR THE REPAIR OF DAMAGED NEURAL CIRCUITS AFTER SPINAL CORD INJURY

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We have previously developed a tissue engineering methodology to construct adult stem cell-derived neural network scaffold in vitro and used it for treating the rats with completely transected spinal cord injury (SCI). However, the existing repair strategies can hardly deliver sufficient excitatory signals across the injured area of the spinal cord, leading to unsatisfactory recovery of neural function. We therefore hypothesized that the damaged neural circuits may function properly after replenishing excitatory neurons. To test this hypothesis, we adopted the established protocols to construct induced pluripotent stem cell (iPSC)-derived excitatory neural network tissue in vitro by tissue engineering approach. Two lines of human iPSCs were induced into high purity of neural progenitor cells before seeding to a three-dimensional (3D) gelatin sponge scaffold for 3D culture lasting from 7 days to 35 days. The immunocytochemistry results suggested that over 95% of the cell population was Map2 or NF200 positive neurons starting from 7 days of culture. Approximately 80% and 15% of the Map2 immunopositive neurons expressed glutamate and ChAT, respectively. The expression of synaptic marker, synapsin-1 and PSD95 became significant at 28 days of culture. The typical action potentials of the neurons were recorded at 28 days of culture. The synapses between neurons resembled excitatory synapse features as observed by transmission electron microscopy. Detection of robust excitatory postsynaptic currents at 28 days of culture may suggest formation of an excitatory neural network in the scaffold. After 14 days of culture, the neural network tissue was transplanted into the injured area of the spinal cord in a Sprague Dawley rat transected SCI model followed by daily administration of a triple-immunosuppressive therapy. The pilot data showed a significant earlier onset of motor function recovery starting from 4 weeks after the transplantation of iPSC-derived excitatory neural network tissue, when compared with the finding from our previous studies using mixed population of neurons. Pathological analysis showed that more than 65% of the donor cells survived up to 8 weeks after transplantation. This study suggests that iPSC-derived excitatory neural network tissue may hold promise for the functional repair of SCI.

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F-3032

MODELING ASPECTS OF THE CHROMOSOME 16P11.3 DUPLICATION IN NEURAL PROGENITOR CELL, NEURONS AND ASTROCYTES FROM PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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A rare 650 kb duplication on chromosome 16p11.2 (dup16p11.2) is associated with neurodevelopmental disorders, schizophrenia, and bipolar disorder. This project aims to explore the use of induced pluripotent stem cell (iPSC) technology to study the biological impact of dup16p11.2 in neural cells and screen for therapeutic agents. Fibroblast samples were obtained from 3 carriers and 3 sex-matched non-carriers belonging to an

extended family ascertained through a proband with bipolar disorder. We also obtained additional unrelated carriers from the Rutgers University Repository. All iPSCs were reprogrammed using lentiviral methods, then differentiated into neural progenitor cells, neurons, or astrocytes. Genome-wide gene expression was measured by microarray or RNA sequencing. Several genes within the duplicated region showed increased expression in carriers compared to non-carriers. In neurons, ALDOA, KCDT13, KIF22, PPP4C, QPRT, and TMEM219 showed the greatest increase (1.5- to 2-fold). Carriers showed more neurite formation during the first two weeks of neural progenitor cell differentiation, developed fewer MAP2-positive neurons after 4 weeks. MAP2-positive neuron counts remained lower in carriers even after more than 20 weeks of culture (ratio< 50%). Consistent with these observations, gene set enrichment analysis of the many genes differentially expressed in carriers revealed significant decreases in GO terms related to neuronal differentiation (P<2.29E-14, activation z-score, -2.2), brain development (P<2.69E-19, activation z-score -1.82), synaptic transmission (P<1.26E-08, activation z -score -2.0). VPA treatment for 5 wks during neuronal differentiation led to increased counts of mature neurons in carriers. These early data show that dup16p11.2 leads to increased expression of genes within the duplicated region and a marked reduction in the differentiation and survival of neurons that is partly rescued by VPA. The dup16p11.2 also perturbed expression of large sets of genes involved in important neurodevelopmental pathways. Patient-specific iPSC are a promising approach to the neurobiology of rare copy number variants associated with neuropsychiatric disorders and may provide an efficient platform for screening novel therapeutics.

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F-3036

EXPLORING MICROGLIA REPLACEMENT AS A TOOL FOR REGENERATIVE MEDICINE

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The brain is separated from the circulating blood by the blood-brain barrier (BBB), which represents one of the most significant limitations in the treatment of brain disorders. Microglia are a myeloid population in the central nervous system (CNS) that is developmentally and functionally distinct from circulating myeloid cells. However, upon depletion of endogenous microglia, circulating myeloid cells can cross the BBB and differentiate into long-lived microglia-like cells. Thus, replacement of endogenous microglia with exogenous myeloid cells expressing therapeutic genes is an attractive way to deliver therapeutic reagents to the entire CNS. Bone marrow transplantation (BMT) studies have revealed the potential of bone marrow-derived myeloid cells to migrate into the brain and become microglia-like cells. However, microglia reconstitution after BMT is a relatively slow and inefficient process compared with donor engraftment in the bone marrow. Therefore, it is clinically important to enhance microglia reconstitution with donor-derived myeloid cells after BMT to explore the potential of microglia replacement as a tool for regenerative medicine. In the current study, we have developed an optimized BMT protocol for rapid, consistent and robust engraftment of bone marrow-derived myeloid cells in the mouse brain. We demonstrate that BMT using the alkylating agent busulfan and the CSF1R inhibitor PLX5622 leads to a high (consistently ~90%) and stable (up to 9 months) brain chimerism in mice. We also show that donor-derived cells are distributed throughout the brain of recipient mice. Although engrafted bone marrow-derived cells using this method are morphologically distinct from endogenous microglia, they express several microglia-specific proteins including TMEM119 that has been reported to be a highly specific microglia marker that is not expressed in peripheral macrophages or other immune cells. The optimized BMT protocol we developed in the current study may provide a powerful approach to efficiently treat neurological disorders with global CNS pathology such as Alzheimer's disease.

F-3038

HUMAN PLURIPOTENT STEM CELL-DERIVED PUTATIVE UPPER MOTOR NEURONS EXHIBIT PROGRESSIVE DEGENERATION ASSOCIATED WITH FAMILIAL ALS

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Amyotrophic lateral sclerosis (ALS) is caused by the progressive degeneration of both upper (cortical) motor neurons (MNs) and lower (spinal) MNs, leading to paralysis and eventual death. Using a transgenic mouse model that overexpresses mutant human superoxide dismutase 1 (SOD1G93A) progressive MN toxicity associated with ALS is accurately recapitulated. Many in vitro studies have also demonstrated that SOD1G93A astrocytes secrete neurotoxic factors which cause the death of co-cultured MNs while non-MN subgroups are spared. Therefore, we hypothesize that neurotoxicity will be observed in cortical MNs, analogous to spinal MNs. In addition, cortical inhibitory interneurons will be insensitive to the neurotoxic effects of SOD1G93A astrocytes. We used a novel differentiation

protocol to generate putative upper MNs. Using a FEZF2::GFP reporter in combination with antibodies to surface antigens specific to neuronal precursors, we isolated putative upper MNs via fluorescence activated cell sorting (FACS). We found that putative upper MNs exhibited survival deficits when co-cultured with SOD1G93A mouse astrocytes, analogous to survival deficits observed in lower MNs. Furthermore, we are testing whether various inflammatory cytokines produced by microglia are capable of inducing reactive astrogliosis, as defined by the expression of lipocalin 2 (LCN2), and assessing the resulting neurotoxicity associated with MN degeneration. Therefore, this co-culture system is a useful platform for studying the non cell-autonomous mechanisms of neurodegeneration caused by the interaction of reactive astrocytes with vulnerable neurons.

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F-3040

EFFECTS OF LIPOPOLYSACCHARIDE ON THE SELF-RENEWAL AND NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS AND NEURAL STEM CELLS

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Prenatal infections in early brain development can engender adverse neurological outcomes in children. We hypothesized that prenatal infections may affect the neural differentiation of embryonic stem cells (ESCs) and further interfere with neural development and differentiation. To mimic the most common scenario of prenatal infection, mouse ESCs and ESC-derived neural stem cells (NSCs) were treated with lipopolysaccharide (LPS). In the ESC stage, 65% ± 1.3% of toll-like receptor 2 (TLR2)-positive cells and 1.6% ± 0.5% of TLR4-positive cells in ESCs were detected by flow cytometry. Notably, the percentage of TLR2(+) cells significantly decreased to 1.6% ± 0.4% and that of TLR4(+) cells significantly increased to 7.0% ± 0.9% after the ESC-to-NSC transition at days in vitro (DIV) 7. Furthermore, LPS did not affect the viability of mouse ESCs but significantly increased the number of ESC-derived neurospheres during the ESC-to-NSC transition. When LPS was administered during the ESC-to-NSC transition, we further observed that LPS significantly upregulated the mRNA expression levels of neuronal markers (Tuj1, Map2), astrocytic marker (Gfap), and oligodendrocyte marker (O4 and Oliog2) in the differentiated neural cells at DIV14, by using quantitative reverse transcription polymerase chain reaction analyses. Whole transcriptome analysis further revealed that significant upregulation of membrane dynamics of plasma, lysosomal and vacuole membranes and intracellular protein activities are involved. Notably, administration of LPS during the NSC-to-neural differentiation resulted in the downregulation of the above neural markers through significant upregulation of cell death pathways, such as p53 signalling pathway, and ferroptosis. In conclusion, this study revealed the dynamic expression profiles of TLRs during early neural development and that the timing of prenatal infection could be crucial for determining the types and severity of neural disorders developed in adulthood. Our findings may facilitate the development of preventative and therapeutic strategies for the adverse neurological side effects of prenatal infections.

ORGANOIDS

F-3042

CAPTURING DIFFERENT DISEASE SERVERITIES OF LIS1-LISSENCEPHALY IN IPSC-DERIVED CEREBRAL ORGANOIDS

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The development of the human cortex requires a precise choreography of progenitor proliferation, neurogenesis and neuronal migration, which can be disrupted in malformations of cortical development (MCD). In the past, most studies on MCD were performed in mouse models. Critical structural differences between human and mice might, however, necessitate the use of additional model systems. In this context, pluripotent stem cell (PSC)-derived three-dimensional (3D) cerebral organoids, which faithfully recapitulate certain aspect of human brain development in vitro, have emerged as an attractive alternative. Here we use forebrain specific cerebral organoids derived from human induced (i)PSCs to address the variable phenotypic severities of LIS1-lissencephaly, which is characterized by a smooth brain and a disorganized cortex. The LIS1-protein is one component of an intracellular multiprotein complex essential for the regulation of cytoplasmic dynein, centrosomal protein localization and microtubule dynamics. When applying our cortical organoid model to iPSCs derived from LIS1-patients exhibiting different severity grades within the LIS1-lissencephaly spectrum, we found disease-related, patient-specific

phenotypes that capture the variable phenotypic severities. In particular, we observed that organoids from individuals with mild or severe disease show either mild or severe alterations in the organization of vRGCs' microtubule networks, disruption of the architecture of the cortical niche and altered expression of cell adhesion molecules. These data indicate that iPSC based 3D cortical organoids represent a sensitive tool which allows to recapitulate variable and patient-specific disease severities, and can thus contribute to an advanced understanding of developmental mechanisms and disease-related changes caused by the dysfunction of single genes.

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F-3044

SMALL INTESTINAL ENTEROIDS ON TRANSWELLS ARE IDEAL TO STUDY SWINE ENTERIC CORONA VIRUSES

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Despite several years of research efforts, the mechanisms defining enteric mucosal immunity against porcine epidemic diarrhea virus (PEDV) are poorly understood. In 2014, USDA dedicated US $26.2 million for domestic prevention of this very important swine enteric disease, which has caused devastating economic losses to the swine industry. PEDV, a member of family Coronaviridae, affects the epithelial lining of the intestine causing diarrhea and up to 100% mortality in neonatal pigs. Currently, there is a lack of well-established and reproducible intestinal infection models to evaluate the complex mechanisms underlying the mucosal immune response against PEDV and other swine enteric pathogens. Crypt stem cell–derived 3D enteroids cultures have recently emerged as a biologically and physiologically relevant ex vivo culture systems that resembles intestinal physiology in vivo. However, one of the limitations of 3D enteroids is accessing the apical surface of the enteroids during their growth in matrigel. Such methods would allow evaluation of mechanisms driving early innate immune responses towards PEDV and its receptor affinity. Thus, the objective of this study was to establish and subculture 3D porcine enteroids on transwell membranes exposing the apical surface of enteroids. We demonstrated that 3D porcine enteroids cultured in matrigel can be established on transwell culture system, replicate efficiently and can be sub-cultured and frozen for subsequent use. By day 5, the microscopic morphology of porcine enteroids on transwells (PETCs) resembled the cross-sectional epithelia of their ex vivo counterparts (duodenum, jejunum and ileum). Immuno-cytological characterization showed that PETCs expressed cell markers specific for different cell lineages of the intestinal mucosa, i.e., villin-1 (enterocyte), mucin-2 (goblet), lysozyme C (paneth), chromogranin A (enteroendocrine), Lgr5 , occludin, Ki67 and sialic acids receptors. Moreover, we demonstrated that PETCs are susceptible to PEDV infection and replication within enteroids grown on transwells. In conclusion, we have developed a novel PETCs culture system that closely resembles small intestinal epithelium of pigs and supports PEDV infection. The proposed PETC cell culture model can be extensible to study other swine enteric pathogens.

F-3046

A PERSONALIZED MODEL TO ASSESS INTESTINAL PERMEABILITY IN IBD AND VEOIBD PATIENTS USING HUMAN IPSC-DERIVED INTESTINAL AND COLONIC ORGANOIDS

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Inflammatory bowel disease (IBD) refers to a spectrum of complex polygenic disorders that are thought to result from dysregulated immune responses to commensal microbes in genetically susceptible hosts. In IBD, the intestinal epithelium is characterized by increased permeability both in active disease and remission states. The genetic underpinnings of this increased intestinal permeability are largely unstudied, in part due to a lack of scientific approach and the profound genetic heterogeneity associated with this disease. Our aim is to develop a personalized in vitro intestinal permeability model using induced pluripotent stem cell (iPSC)-derived human intestinal organoids (HIOs) and human colonic organoids (HCOs) so that genetically defined subsets of IBD patients can be selected and ultimately allows for these genetic underpinnings to be defined. To confirm our approach was suitable for a wide range of individuals, iPSCs were generated from 2 healthy controls,3 IBD and 2 very early onset-IBD (VEOIBD) patients. HIOs and HCOs

were generated from each iPSC line by directing them to form definitive endoderm, hindgut tissue and ultimately HIOs or HCOs. There was a significant increase in the apparent permeability of FD4 across epithelial monolayers derived from HIOs generated from healthy controls and all IBD patients in response to TNFα and IFNγ (both 10ng/ml). HCO epithelium was generated from a control individual and both VEO-IBD patients and there was significantly increased expression of colonic markers and significant increase in transepithelial resistance in all three lines in comparison their corresponding HIOs, confirming that colonic tissue was generated. There was also a significant increase in permeability in HCO tissue in response to inflammatory cytokines. We also demonstrate that E-cadherin expression was reduced and mislocalized in HIOs but not HCOs in response to these cyokines also. Overall we demonstrate that we can now measure intestinal permeability, and examine changes in tight/adherens junction proteins, in both the small and large bowel using iPSC-derived organoid technology. Subsets of genetically defined IBD patients can now be selected and an investigation into how single nucleotide polymorphisms associated with IBD influence intestinal permeability can now be carried out.

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F-3048

ON-CHIP CONSTRUCTION OF A PERFUSABLE VASCULAR NETWORK WITH HUMAN IPS CELL-DERIVED KIDNEY ORGANOID

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Microfluidic devices can mimic the in vivo microenvironment three-dimensionally by controlling disposition of the cells, the extracellular matrix, and the flow of the medium. We have previously reported on the construction of a perfusable vascular network system using a spheroid as a tissue model. Here, we attempt to develop a microfluidic cell culture device to construct perfusable vascular networks using a human iPS cell-derived kidney organoid, and analyze the effects that the interaction of the organoid with endothelial cells and flow-induced shear stress in blood vessels give to kidney development. Co-culturing a kidney organoid with human umbilical vein endothelial cells (HUVECs) in the device revealed that the organoid at a mid-stage of kidney development can induce angiogenesis of HUVECs. The length of the angiogenic sprouts was the longest when the media for the organoid and HUVECs were mixed. However, the elongation of angiogenic sprouts was inhibited until after seven days of culture, and the vascular network did not have a lumen. To connect a perfusable vascular network into a kidney organoid, we developed a pre-constructed vascular bed in a new type of device by introducing HUVECs and human lung fibroblasts. Perfusion culture in the vascular bed showed connection with endogenous blood vessels in the organoid and the vascular bed. Now, we are aiming to analyze the developmental stage of kidney organoids using this culture system.

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F-3050

IMPROVING CELL SURVIVAL TO CONTROL CEREBRAL ORGANOID FORMATION FROM HUMAN PLURIPOTENT STEM CELLS

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Generation of in vitro organoid models from pluripotent stem cell holds great promise for future advances in disease modeling, drug development, and tissue engineering. Cerebral organoids are complex three-dimensional (3D) structures that form by aggregation and self-organization of pluripotent cells, ultimately mimicking some aspects of the physiologically complex, multicellular, and layered architecture of the brain. However, significant variability and experimental challenges exist in organoid formation protocols. One such challenge is the large amount of cell death when pluripotent cells are dissociated and aggregated into 3D structures. Currently, the ROCK inhibitor Y-27632 is the most widely used reagent to improve cell survival. Nevertheless, poor cell survival and emergence of debris are evident even after treatment with Y-27632 suggesting that uncontrolled cell stress introduces an inherent systematic shortcoming in currently used protocols. Here, we used a novel small molecule cocktail, recently developed at NCATS, that greatly enhances cell survival during cerebral organoid formation. Improved cell survival at the onset of sphere formation generated larger, healthier and better controlled organoids. Ongoing molecular and functional experiments are aimed at demonstrating that optimal cell survival enhances morphogenesis, differentiation, and reproducibility of cerebral organoids.

F-3052

GENERATION OF OLFACTORY PLACODAL/ EPITHELIAL ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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The olfactory epithelium lies the roof of nasal cavity and is involved in smell. The organogenesis of olfactory systems were mainly investigated with in vivo animal models, and the olfactory epithelial differentiation methods from pluripotent stem cells have not been established. Here we challenged to recapitulate the organogenesis of olfactory systems in a dish. In this study, we present our Serum-free Floating culture of Embryoid Bodies-like aggregates (SFEBq)-based human olfactory epithelial organoids (hOlfOs) differentiation method and discuss the features of generated organoids. As well as lens, pituitary, ear and trigeminal ganglia, the olfactory epithelium is derived from specific region of non-neural ectoderm called placode. Previously generation of pituitary and otic organoids from pluripotent stem cells were reported, and we modified these protocols for olfactory placode differentiation. In the process of differentiation, single cell layer of non-neural ectoderm appeared at the surface of cell aggregates and gradually got thicker. The thickened epithelium took on distinctive phase-bright appearance, and the surface of epithelium was smooth as well as retinal organoids. The thickened epithelium were pseudostratified, and expressed non-neural ectoderm/placodal (Six1/Dlx5/Sox2/E-Cadherin/ EpCAM/Cytokeratins) and olfactory region (Otx2/Sp8) markers. In addition, Ebf2 (Olf2)/NeuroD1/Lhx2/Tuj1/NCAM/Calretinin+ immature olfactory sensory neurons (iOSNs) exists in these epithelium. Our preliminary data indicates that modified SFEBq method could recapitulate the self-formation of olfactory epithelium from pluripotent cells in vitro, and will provide valuable insights to investigation of olfactory system development, behavior of olfactory stem cells and genetic diseases such as Kallmann syndrome.

F-3054

MODELING HSV-1 INFECTIONS IN HUMAN CNS NEURONAL CELLS USING TWO-DIMENSIONAL AND THREE-DIMENSIONAL CULTURES DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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HSV-1 establishes latency in both peripheral nerve ganglia and the central nervous system (CNS). The outcome of acute and latent infections in these different anatomic sites appear to be distinct. It is becoming clear that many of the existing culture models using animal primary neurons to investigate HSV-1 infection of the CNS are limited and not ideal, and most do not recapitulate features of CNS neurons. Human induced human pluripotent stem cells (hiPSCs) and neurons derived from them as tools to study aspects of neuropathogenesis are documented but few have focused on modeling infections of the (CNS). Here, we characterize functional two-dimensional (2D) CNS-like neuron cultures and three-dimensional (3D) brain organoids made from hiPSCs to model HSV-1-human-CNS interactions. Our results show that: i) hiPSC-derived CNS neurons are permissive for HSV-1 infection; ii) a quiescent state exhibiting key landmarks of HSV-1 latency described in animal models can be established in hiPSC-derived CNS neurons; iii) the complex laminar structure of the organoids can be efficiently infected with HSV, with virus being transported from the periphery to the central layers of the organoid; iv) the organoids support reactivation of HSV, albeit less efficiently than 2D cultures. Collectively, our results indicate that hiPSC-derived neuronal platforms, especially 3D organoids, offer an extraordinary opportunity for modeling the interaction of HSV-1 with the complex cellular and architectural structure of the human CNS.

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F-3056

DEVELOPING A HUMAN EMBRYONIC STEM CELLS BASED HIGH-THROUGHPUT PLATFORM TO SCREEN FOR DEVELOPMENTAL TOXICANTS

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Every year, millions of infants worldwide are born with a serious birth defect, which not only raises the risk for lifelong disabilities to those who survive but also increases the economic burden to their families and society. Besides genetic or hereditary factors, many of these defects can be caused by environmental chemical exposure, such as alcohol, smoking, and drugs. While there are over 80,000 chemicals registered for use in the United States, many of them have undergone little safety testing. Therefore, a rapid and accurate method for predicting developmental toxicants in the environment to humans and understanding their toxic mechanisms is strongly desired. Pluripotent human embryonic stem cells (hESCs) possess the capacity to differentiate into any cell type which makes them an ideal in vitro model to investigate developmental toxicity. In this study, we aim to develop a cost-effective transcriptomic-based high-throughput platform using hESCs to screen for environmental chemicals and pharmaceutical compounds with embryotoxic potential. In general, three-dimensional embryoid bodies (EBs), which recapitulate many developmental processes of early embryogenesis, were formed from hESCs. 30 chemicals with known or suspected teratogenicity (i.e. thalidomide, sodium arsenate, and tretinoin) were administered to EBs for seven days at concentrations causing a minimal cell viability loss (i.e. LC10). Pluripotency and embryonic differentiation of EBs were assessed by measuring the expression of 16 hallmark genes of these processes. The impacts of tested chemicals on key signaling pathways (Wnt, Notch, Sonic hedgehog, and TGF-β) required for the early embryogenesis were investigated as well. Hierarchical clustering analysis of our preliminary data allowed us to separate embryonic toxicants apart from the negative controls. Furthermore, consistent with previous reports, our results also indicate that tretinoin, benomyl, and perfluorooctanoic acid possess neural developmental toxicity since they drastically decrease the expression of genes associated with ectoderm formation (≥2-fold). Together, these results indicate that our screening platform could be successfully applied for identifying developmental toxicants and understanding their etiology.

F-3058

A PROTEIN ASSAY TO MEASURE R-SPONDIN LEVELS IN CONDITIONED MEDIUM FOR ORGANOID CULTURES

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3D organoid culture systems are increasingly employed as powerful tools for the study of human diseases. R-spondin-1 (RSPO1) is one of the most extensively used niche factors for culturing 3D organoids and has been used to establish organoid cultures from the stomach, small intestine, colon, pancreas and liver from both mouse and human sources along with intestinal organoids from various large & small animals. R-spondin-1 conditioned medium (RSPO1 CM) is widely used as a potent and inexpensive alternative to purified recombinant RSPO1 protein. However, most protocols for the generation of RSPO1 CM do not monitor RSPO1 protein levels and thus the timing for conditioned media collection may be suboptimal. Batch-to-batch variations may also exist which may affect the quality and subsequent analyses of the different organoid culture systems. We describe a semi-quantitative assay to measure the levels of RSPO1 protein in medium conditioned with a 293T cell line stably transfected with a modified mouse R-spondin-1 gene. Results from the protein assay directly correlated with the ability of different batches of RSPO1 CM to culture and expand mouse intestinal organoids over multiple passages. With the aid of the assay, we were able to scale-up the manufacture of RSPO1 CM, ascertain the optimal time to harvest the CM, ensure batch-to-batch consistency and determine the stability of the RSPO1 CM in long-term storage conditions (>1 year). RSPO1 CM was subsequently used to develop an optimized supplement that could be used to isolate and maintain mouse intestinal organoids. We show that in the optimized RSPO1 CM supplement, relatively primary mouse intestinal organoids (<p5) initiated budding within 3 days after passage versus 10 days with an alternative commercial source of RSPO1-supplemented medium. Moreover, neglected and overgrown preparations of mouse intestinal organoids could be rescued by culture in the optimized RSPO1 CM supplement. We anticipate that these findings may be extended to other organoid systems that require R-Spondin-1.

TISSUE ENGINEERING

F-3060

EFFECT OF INCUBATION TIME ON THE VIABILITY AND FUNCTION OF POST TOOTH EXTRACTED HUMAN DENTAL PULP STEM CELLS

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Human teeth contain a variety of mesenchymal stem cell populations that could be used for cell-based regenerative therapies. Human dental pulp stem cells (HDPSCs) are stem cells present in the dental pulp, the soft living tissue within the teeth. They are multipotent, so they have the potential to differentiate into a variety of cell types and possess the potentiality or infinite applications in reconstruction, regenerative therapies and tissue repair. HDPSCs are easily accessible and histocompatible. Daily, many live teeth are extracted for many reasons, such as avulsion, exfoliation or during orthodontic treatments. These teeth are usually discarded rather than being put to use. The infinite potential of HDPSCs, their accessibility, and their multipotency to differentiate into different cell lineage makes them a promising source for tissue regeneration and stem cell banks. However, there haven't been studies about the effect of the post tooth extraction incubation time on the viability and function of HDPSCs. It is very important to establish how long pulp cells are viable and functional in order to construct a pulp cell preservation protocol for stem cell banking. Considering the above problems the aim of this study was to investigate the viability and function of HDPSCs isolated from the extracted teeth immediately and after 6 and 24 hours (hrs) postextraction. Cell proliferation was analyzed using MTT and XTT assay both of which showed slight reduction in the proliferation of the HDPCS collected after 24 hrs post extraction compared to immediate and 6 hrs group. There was no change in cell morphology and cell viability between groups as evidenced by light microscopy, crystal violet staining and trypan blue hemocytometer cell counting. The HDPSCs isolated immediately,6 and 24 hrs post extraction were characterized by flow cytometry using mesenchymal stem cells (MSC) specific and unspecific markers, the result showed that all the HDPSCs groups were more than 80% positive for MSC positive markers such as CD44,CD106, CD90 and more than 80% negative for MSC negative markers such as CD45 and CD11b. Taken together the data suggests that a delay of up to 24 hrs for tooth processing and HDPSCs collection does not inhibit the establishment of dental pulp cell cultures or affect the cell morphology and the viability of the HDPSCs.

Funding Source: University of Sharjah

F-3062

LOW OXYGEN GRAPHENE AS A PLATFORM FOR OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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The effects of low oxygen graphene can be utilized as both a cell-adhesion substrate and a delivery platform for the osteogenic differentiation of human mesenchymal stem cells (MSCs). Graphene, a 2D high surface area lattice monolayer is composed of hexagonally arranged carbon atoms, and can be functionalized by chemical modifications to generate nanocomposites with specific physicochemical properties. In this study, we first identified the mass concentration of graphene that showed a significant increase in osteogenesis, by Alizarin red staining and quantitation, and subsequently, the expression and cellular localization of extracellular matrix (ECM) glycoproteins was assessed in vitro by immunofluorescence. The expression of ECM proteins laid down by human MSCs was evaluated in undifferentiated and osteogenic cells. ECM is a network structure composed of various biomolecules, controls the cellular behavior and provides a favorable microenvironment for the growth and differentiation of cells. The results show that graphene is cytocompatible towards MSCs and the amount of graphene coated on a surface modulates cell adhesion, spreading, growth, and osteogenic differentiation. We also observed that human MSCs express various integrin subunits and lay down the matrix i.e. rich in fibronectin, suggesting the interaction between the two, to play a major role in adhesion and osteogenic differentiation of human MSCs on graphene surfaces. In this study, we will discuss (1) bone ECM composition and key integrin proteins implicated in osteogenic differentiation, (2) the use of graphene alone and graphene modified with ECM–mimetic peptides/protein fragments that will promote the

mechanical fixation of implants to bone and to enhance bone healing within large defects. This study suggests that a layer of graphene as a potential bone scaffold will be beneficial for osteoblast attachment, proliferation, and differentiation.

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F-3064

DERMAL PAPILLA CELLS-ENCAPSULATED HAIR BEADS FOR HAIR REGENERATIVE MEDICINE

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Hair loss generally occurs due to various causes such as genetics, aging, hormonal imbalances, autoimmune reactions, and anti-cancer drug medications, and this is linked to the loss of stem cells responsible for normal hair formation and hair cycling. Recently, hair regeneration by co-transplantation of epithelial-derived follicular stem cells and dermal papilla cells (DPCs) have emerged as a promising approach for treating hair loss. DPCs provide signals to hair follicle stem cells, specifying the size, shape, and pigmentation of hair shafts, and epithelial-derived follicular stem cells differentiate and eventually form a hair shaft. However, the hair induction ability of both cell types is gradually lost after isolation from in vivo tissues and during expansion culture. Latest studies have shown that three-dimensional culture of DPCs could recover expression of hair induction markers and efficiently induce hair follicles when transplanted with hair follicle stem cells. In this study, we propose an effective approach to fabricate collagen-enriched cell aggregates, named hair beads (HBs), through the spontaneous constriction of cell-encapsulated collagen drops. DPCs were encapsulated in 2-μl collagen microgels, which were spontaneously constricted and concentrated >10-fold in volume during 3 days of culture. Interestingly, HB constriction was attributed to attraction forces driven by myosin II and involved the upregulation of follicular genes. Gene expression of hair induction markers in DPCs cultured in HBs was greater than that in typical spheroid culture at 7 days of culture. The DPCs cultured in HBs were mixed with epithelial-derived follicular stem cells, and subcutaneously transplanted into a pocket surgically created on the back skin of nude mice and shown to be capable of efficient hair follicle and shaft generation at 3 weeks after transplantation. Compared to spheroid culture, hair numbers generated were more than double in HB culture. These results suggested that the HB culture provides a more suitable approach for recapitulating the in vivo dermal papilla niche.

F-3066

METABOLICALLY-DRIVEN MATURATION OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED HEART-ON-A-CHIP

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Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) are a promising in vitro testbed for drug development. However, their immature electrophysiology limits their utility. For in vitro diagnostics, approaches involving aligned 3D culture enhance hiPSC-CM structural, but not electrophysiological maturation. We hypothesized that recapitulating the heart's ability to use fatty acids as an energy source could enhance electrophysiological maturation of hiPSC-CM cultured in 3D Microphysiological Systems (MPS). We formed cardiac MPS by adding a defined cocktail of 80% hiPSC-CM and 20% isogenic stromal into microfabricated tissue chips. We assessed micro-tissue function by tracking contractile motion and calcium flu , and voltage. Cardiomyocytes in MPS aligned rapidly and exhibited robust sarcomere assembly. However, the Action Potential Duration (APD) of hiPSC-CMs within the MPS was prolonged when the system was cultured in standard cardiac media. To identify medias that could enhance electrophysiologic maturation, we used a Design of Experiments approach to study combinatorial effects of albumin, glucose, galactose, oleate and palmitate. In MPS, optimal fatty-acid based media yielded APDs similar to that of adult human left ventricle. However, the same media did not alter the APD of hiPSC-CM monolayers. Matured cardiac MPS was superior to both hiPSC-CM monolayers and

standard MPS in terms of its ability to predict safety margins for drugs that exhibit false positive toxicity (Verapamil), or false negative toxicity (Alfusozin). In silico modeling of specific ion channel contributions to action potential and calcium flux, together with gene expression analysis, suggested enhanced Ca2+ handling as the mechanism underlying action potential shortening.by fatty acid enriched media.

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F-3068

SILK-BASED SCAFFOLDS FOR SKIN WOUND HEALING

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Skin wounds have a large socio-economic effect on the society. The global wound care market is expected to increase from 18.35 million USD in 2017 to 22.01 million USD by 2022. Full-thickness wounds, especially caused by 3rd degree burns, > 10 mm in diameter require skin grafting to fully heal and prevent related complications. In spite of a range of products available, complete skin regeneration is still considered as an unmet clinical need. Till date, there is no such skin substitute which can recapitulate the anatomy and functionality of native human skin. Thus, this proposal intends to fabricate functional skin substitute that could; (i) target efficient wound healing, (ii) possess optimal mechanical properties, (iii) validation of protocols as per GMP compliance for translational purpose. Different ratios of silk are being combined with Polycaprolactone (PCL)/Pluronic F-127/gelatin to optimize inks in terms of rheology, printability, stiffness and stability for bioprinting. In vitro optimization and characterization of various bioinks and commercial bioinks (as positive control) was conducted. Confocal micrographs demonstrated that majority of encapsulated mesenchymal stem cells were live (Live/Dead staining) in the 3D stack and possessed characteristic morphology as observed using phalloidin/DAPI. Nanofibrous electrospun mats of silk-based biocomposites (in formic acid) were fabricated having nanofibers in the range of 200-600 nm. Lyophilization was also performed to obtain different scaffold geometries resulting in robust structures with pore sizes in the range of 50-500 um allowing infiltration of MSCs and subsequent ECM synthesis. Biocompatibility assessment is currently underway using human mesenchymal stem cells for cell viability assays (MTS/Live/Dead staining), proliferation and differentiation to compare the most optimal scaffold surface for regenerating the different layers of the skin. The in vivo studies conducted on excision wound rat model showed enhanced healing and regeneration in groups transplanted with the 3D printed scaffolds. This research aims to develop transplantable customized scaffolds for faster and efficient wound healing restoring the native functionality of the skin.

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F-3070

GENERATION OF HIGHLY VASCULARIZED INDUCED HEPATIC-LIKE TISSUES VIA DIRECT REPROGRAMMING

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Induced hepatic (iHep) cells generated by direct cellular reprogramming have been proposed to replace primary hepatocytes for drug screening industry and regenerative medicine. However, the practical use of a three-dimensional (3D) hepatic tissue culture comprised of iHep cells for drug screening and toxicology testing has not been explored in detail yet. In this study, we demonstrate a 3D vascularized liver organoid composed of non-virally generated iHep cells and a liver extracellular matrix (LEM) cultured in a microfluidic system. iHep cells were generated by transfection with polymer nanoparticles and plasmids expressing transcription factors important in a hepatic lineage. Here, the iHep cells were co-cultured with endothelial cells in the 3D LEM hydrogel in a microfluidic-based cell culture device with a continuous dynamic flow of media that mimicked the blood circulation. The resultant 3D vascularized liver organoids maintained under this physiologically relevant culture microenvironment exhibited improved hepatic functionalities, metabolic activity, and drug responses. Finally, we confirmed the feasibility of using the iHep-based 3D liver organoid as a high-throughput drug testing platform, as well as its use in an integrated drug testing platform comprised of multiple internal organoids. Our study suggests that a combined strategy of direct reprogramming, matrix engineering, and microfluidics could be used to develop a highly functional, drug screening and toxicological analysis platform.

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F-3072

TUNABLE PARATHYROID HORMONE-RELATED PROTEIN RELEASE FOR MODULATION OF HUMAN MESENCHYMAL STEM CELL HYPERTROPHY TOWARD STABILIZED CHONDROGENESIS

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Stable chondrogenesis of mesenchymal stem cells (MSCs) has not been achieved since their implantation leads to rapid formation of endochondral bone, which is attributed to strong hypertrophic chondrocyte phenotype expressed by the implanted cells. This study aimed to suppress cell hypertrophy via tunable delivery of parathyroid hormone-related protein (PTHrP) to human MSCs undergoing TGF-β3 induced chondrogenic differentiation. To this end, soluble PTHrP was encapsulated within poly lactic-co-glycolic acid (PLGA; PLA-to-PGA, 75:25) microspheres using a water-in-oil-in-water double emulsion method. PLGA with an ester-terminated (capped) or carboxylic acid-terminated (uncapped) end group was used to generate different PTHrP release profiles. While no significant difference in physical properties was identified between PTHrP-loaded capped and uncapped PLGA microspheres, the initial burst release of PTHrP decreased from 30% in the ester-terminated group to 10% in the acid-terminated group. Moreover, most of encapsulated PTHrP molecules were retained within acid-terminated microspheres for more than 6 days, indicating that PLGA with an uncapped end group tends to interact firmly with encapsulated biomolecules. Each of cell pellets was then prepared by mixing 106 MSCs with 1 mg of either type of PTHrP-loaded microspheres, followed by centrifugation. PTHrP-free uncapped microparticles were used as the control. MSC pellets were cultured with TGF-β3-encriched chondrogenic medium for 4 weeks. We found that PTHrP-loaded ester-microspheres promoted gene expression of type II collagen (a chondrogenic marker) whose level remained similar in the other two groups. With the delayed PTHrP release, however, acid-terminated microspheres significantly reduced synthesis of hypertrophic markers (type X collagen and MMP-13) by chondrogenically differentiated MSCs at both gene and protein levels. In summary, our findings suggest that delayed PTHrP treatment during MSC chondrogenesis can effectively suppress hypertrophy of the cells without compromising their chondrogenic potential. This work not only substantiates the importance of PTHrP release profile in modulating chondrocyte hypertrophy, but represents significant advancement toward our ultimate goal of achieving stable MSC chondrogenesis.

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ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

F-3074

BONE MARROW STROMAL CELLS AND THEIR STEMNESS PARADIGM

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The prevailing definition of a stem cell is a cell with unlimited self-renewal and potential to produce highly differentiated cells. However, after decades of debate stem cell definition remains argumentative. Even though today claimed stem cells are widely used in the clinic, most of the public is unaware of their true nature. Some clinicians promote their indiscriminate use for multiple conditions, without any scientific support. We performed a literature review about adult stem cells, how they became famous for assumed properties and their possible role in therapies. The field of adult stem cells emerged in the 1950s' in an effort to develop strategies for radiation protection with bone marrow transplantation in humans. In 1988, Friedenstein and Owen described non-hematopoietic bone marrow-derived stromal cells. In 1998, isolation of human blastocysts cell lineages served as a catalyst in this field. To avoid the controversies established by embryonic cell line use, Arnold Caplan et al. isolated human bone marrow stromal cells and named them mesenchymal stem cells (MSCs), suggesting properties not yet proven. Soon the literature regarding MSCs grew strikingly. Since then many claim MSCs have regenerative properties. However, most studies lack cell-tracing demonstrating persistence in tissue after implantation. It is vexing that a supposed stem cell isolated from a particular tissue can regenerate tissue from a different lineage. Understanding of embryonic lineages is fundamental to unravel their possible differentiation potential. Despite increasing numbers in publications and therapies, there is no standardized protocol for their manufacturing, with important implications on quality and function. Studies have shown ex vivo serial replications lead MSCs into replicative senescence, affecting their quality. At present, only therapies for bone marrow transplantation and grafting of bone for treating injuries, which rely on adult stem cells have proven to be successful. In conclusion, although MSCs have regenerative properties in certain scenarios, it is unclear whether they are true stem cells. There is still much to be learned about bone marrow stromal cells for bank purposes and their expansion for therapies before they are used in patients.

F-3076

GLOBAL TRENDS IN STEM CELL RESEARCH SINCE 1998

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Previously, we comprehensively analyzed the trends of research using induced pluripotent stem cells (iPSCs) and reported how it had made a phase transition since 2006 over a 10-year span. Including iPSCs, various stem cells that are ethically acceptable such as embryonic stem cells (ESCs) and somatic stem cells, have been used for the research. It is important for the study design to keep track of trends for whole stem cell research. Here, we broaden the range of analysis of the stem cell study as a whole, including targeting ESC and somatic stem cell studies which were carried out from 1998 through present day to determine which cells have been used for a specific kind of study. To investigate stem cell research trends, we used MeSH term “Stem cells” and “Humans” to search for publications published in the PubMed database from 1998 to 2018. From the reports obtained, secondary information, articles without abstract, or articles not written in English were excluded. Over 50,000 original articles were obtained and tagged for 7 stem cell types used and 19 research targeted disease areas, using MeSH term. The articles were also classified by country based on the first authors’ affiliations. When classified data were categorized by country, the United States was top-ranked by the total number of articels, followed by China, Japan, Germany and United Kingdom. In regard to stem cell-type analysis, the number of articles for ESC has increased from 2006. Comparatively, iPSC research has increased from 2010 and has continued to grow, surpassing the number of studies for ESC in 2016. Although the number of articles for multipotent stem cells began to increase around 2005, it has gradually declined after reaching a maximum of 300 studies in 2012. Regarding the number of articles using each stem cell-type, the United States was always ranked in first place, but the second place varied by country-specific characteristics. In addition, by using the accumulated data, we analyzed the focused disease areas of each country. This analysis revealed that ESC (since 2006) and iPSC (since 2010) have been the leading engine in facilitating and promoting various research for diseases.

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F-3078

PUBLIC ATTITUDES IN THE UNITED STATES TOWARDS HUMAN-ANIMAL CHIMERIC EMBRYO RESEARCH USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO GENERATE HUMAN ORGANS FOR TRANSPLANTATION

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Public attitudes are a contributing factor in determining how stem cell research is funded and governed. Yet research remains very limited on whether the American public supports the creation of human–animal chimeric embryos (HACEs) for basic research, drug discovery, and organ transplantation. To fill this gap and robustly assess American public attitudes on HACE research, we conducted a national survey of Americans, replicating an approach pioneered in a 2017 study of Japanese public attitudes on HACE research using iPS cells. Our new data suggest that the American public strongly supports HACE research. But we also find that this support is mediated by political ideology, religious affiliation, and the organ to which the human cells contribute. The study was conducted from April to August 2018. 328 survey participants, from 40 different states, were recruited via Amazon’s Mechanical Turk service to take a survey hosted on the Qualtrics online platform. The survey asked a series of questions to elicit participant attitudes related to HACE research. Notably, participants were asked a multi-part question to assess their support for each of several steps required for the creation of HACEs. Overall, nearly 75% of the participants reported a willingness to provide their own cells for blastocyst complementation, and over 80% of the participants felt it socially acceptable to allow for iPS cells to be injected into swine embryos. Further analysis revealed additional important trends. First, while almost 75% of the respondents accepted growing livers in swine, less than 50% of participants supported growing brain cells and less than 40% supported growing

human cells in sperm/ovum. Second, the majority of both non-religious and religious participants support human-animal chimera research. Over 90% of non-religious participants, and about 70% of religious participants, support at least some steps toward creating HACEs. These findings are timely because the NIH is currently reviewing its funding moratorium on HACE research. Given the important role of public attitudes on science policy, the results of our study will contribute to on-going debate in this area.

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CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

F-3080

EFFECTS OF TYPE I COLLAGEN ON THE GROWTH OF HUMAN DENTAL PULP STEM CELLS IN A XENOGENEIC SERUM-FREE MEDIUM FOR THE ESTABLISHMENT OF A PRACTICAL CULTURE METHOD

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Human dental pulp stem cells (DPSCs) are useful tools in regenerative medicine. We recently reported that xenogeneic serum-free culture (XFC) is preferable for the clinical applications of DPSCs because XFC prevents contamination with pathogens and can be used to obtain a large number of DPSCs in a short period of time. However, it was also found that the XFC cells develop a multilayered structure upon reaching over-confluence, and this abnormal change induces apoptosis/cell death resulting in the reduction of the number of cells. The aim of this study was to establish an appropriate XFC condition in order to suppress the unfavorable death of DPSCs. We hypothesized that over-production of type I collagen (COL1), a major part of the extracellular matrix, contributes to the formation of an abnormal multilayered structure by DPSCs. We first confirmed that the XFC cells abundantly produced COL1 protein upon reaching over-confluence. To investigate the effects of COL1 on XFC, the culture dishes were pre-coated with COL1 protein. Upon seeding the cells, the COL1-XFC cells adhered to the culture dishes significantly earlier than those cultured in fibronectin- or non-coated dishes. On the other hand, the DPSCs cultured in a serum-containing medium showed no significant difference with the different types of culture dishes. Moreover, the COL1-XFC cells showed higher growth than those cultured in fibronectin- or non-coated dishes. Interestingly, although the COL1-XFC cells formed a proper stratified structure upon over-confluence, the cells did not show apoptosis and reduction in their number. Collectively, these findings suggest that COL1 secreted by the XFC cells is involved in the stratified/multilayered formation, and that pre-coating of culture dishes with COL1 could prevent apoptosis/cell death within the stratified XFC. Therefore, the use of COL1-precoated dishes facilitates an effective large-scale expansion of DPSCs under XFC conditions. These findings might be useful in the establishment of a safe and highly predictive xenogeneic serum-free culture method for handling DPSCs.

F-3082

ALLOGENEIC CORD BLOOD CELL THERAPY COMBINED WITH ERYTHROPOIETIN IN CHILDREN WITH CEREBRAL PALSY: A RANDOMIZED PLACEBO CONTROLLED TRIAL

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Recently, stem cell therapy has been highlighted as a new treatment option for cerebral palsy (CP). In our previous study, children with CP treated by allogeneic UCB with erythropoietin (EPO) showed higher improvements in motor and cognitive aspects, yet it lacked to identify individual and synergistic efficacies of UCB and EPO. This factorial designed study aimed to identify individual and synergistic efficacies of UCB and EPO in children with CP. Children with 1) a diagnosis of CP, 2) age between 10 months and 6 years, 3) appropriate UCB units, 4) written informed consents from parents were included as study candidates. Participants were randomly assigned into four groups: A) UCB + EPO, B) UCB + placebo EPO (pEPO), C) placebo UCB (pUCB) + EPO), and D) pUCB + pEPO groups. Allogeneic UCB units were selected including at least 3 ´ 107/kg total nucleated cells, matched for at least 4 of 6 human leukocyte antigen (HLA) types A, B, and DRB1. A single infusion of UCB or pUCB was delivered intravenously and 500 IU/kg of EPO or pEPO were injected subcutaneously for 6 times every 3 days. Group A and B received oral cyclosporine. Primary outcomes such as gross motor function measure (GMFM), gross motor performance measure (GMPM), Bayley scales of infant development II (BSID-II) were assessed at baseline, 1, 3, 6 and 12 months post intervention. Baseline and post-intervention MRI and PET/CT were also acquired. All adverse events were monitored during 12 months. Eighty-eight children with CP were included as final subjects in this study (n=22, 24, 20, and 20 for Group A, B, C, and D, respectively). There were no significant differences of baseline characteristics among four groups. Group A showed meaningful improvement in the ratio of GMPM change at 12 months post-therapy compared to group D (p=0.021). Most of the parameters in four groups showed improvements in primary outcomes, although the changes were not significant. More HLA-matched UCB presented better

enhancement in change of GMFM at 1 month (p=0.036) and 3 months (p=0.050) post-intervention. Ten serious adverse events were reported, although these cases were all resolved and the distribution of events did not differ among four groups. These results suggest that UCB therapy is safe and effective treatment for children with CP, and its combination with EPO can bring more synergistic effects.

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F-3084

STRATEGIES TO IDENTIFY MESENCHYMAL STEM CELLS IN FRESH HUMAN BONE MARROW ASPIRATE CONCENTRATE LACK CONSENSUS AT THE SINGLE CELL TRANSCRIPTOME LEVEL

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Given the current regulatory landscape in the United States, the most convenient and applicable use of mesenchymal stem cells (MSCs) as a therapeutic agent to treat orthopedic conditions involves aspiration and re-injection of autologous bone marrow aspirate concentrate (BMAC) into the target site within the same surgical procedure. As MSCs are suggested to be the primary active compound of BMAC, identification and quantification of MSCs are prerequisites to define necessary BMAC doses and dose-dependent outcomes. Current strategies to identify MSCs in human BMAC have been established in cultured cell populations. However, as both surface marker and gene expression change in these cells upon culture, this study investigated whether the current strategies are well-suited to identify non-cultured MSCs in a source that is of direct clinical relevance, i.e. fresh BMAC. Running single cell RNA sequencing (scRNA-seq) on nine orthopedic patients' BMAC, we hypothesized overlap of "MSCs" identified by different strategies is relatively low. The proposed gold standard (according to the International Society for Cellular Therapy, ISCT) identified zero cells since fresh BMAC-MSCs lack expression of CD90. A few CD271+ cells were detected, but these cells clustered with B cells and monocytes. Only 116 of 386 genes that were previously reported to be expressed by both cultured and non-cultured MSCs were detected in our patient BMAC samples. 35 of 12850 BMAC cells expressed at least 9 of these 116 genes and 66% of these cells were not identified as MSCs by any of the other strategies. As a control, we pooled cultured MSCs with the patient BMAC samples and all strategies identified the cultured MSC cluster, with the ISCT guidelines being the best (1354 of 2978 cultured MSCs detected, no detections of other cells), and CD271+ being the worst strategy (14 of 2978 cultured MSCs detected, 9 of 23 CD271+ cells clustered with patient B cells and monocytes). In conclusion, novel strategies to identify MSCs in fresh BMAC are urgently needed so that orthopedic researchers can design meaningful clinical trials using autologous, minimally manipulated BMAC injections, and investigate dose-response relationships.

F-3086

INTRATHECAL INJECTIONS OF AUTOLOGOUS MESENCHYMAL STEM CELL-DERIVED NEURAL PROGENITORS IN PATIENTS WITH PROGRESSIVE MULTIPLE SCLEROSIS: RESULTS FROM PHASE I/II TRIALS

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MSC-NPs are an autologous bone marrow-derived population of cells currently under investigation as a novel MS treatment targeting CNS repair. Preclinical evidence suggests that MSC-NPs function through immunoregulatory/trophic mechanisms. The open-label phase I clinical trial in 20 patients with progressive MS demonstrated safety and tolerability of the treatment, and was associated with functional neurological improvement, particularly in ambulatory patients. Whether or not these improvements are sustained and quantifiable against a placebo control remains unknown. The objective of this study is to evaluate therapeutic efficacy of repeated dosing of intrathecal mesenchymal stem cell-derived neural progenitor (IT-MSC-NP) treatment in patients with progressive MS. Bone marrow MSCs were isolated, expanded, and cultured with neural progenitor media to generate MSC-NPs. In the phase I trial, IT-MSC-NPs were injected with 3 doses of up to 10 million cells every 3 months with follow-up EDSS assessments performed 12 and 30 months following the start of the treatment. The phase II trial is a double-blind, placebo-controlled, randomized crossover study in 50 patients with progressive MS, consisting of 6 doses of IT-MSC-NPs every 2 months. Eight of the 20 subjects in the phase I trial showed EDSS improvements (range 0.5 to 3.5 point improvement) after 12 months. The 30 month follow-up in 18 out of 20 subjects demonstrated that 7 subjects (39%) showed

continued sustained improvement in EDSS. One subject who had previously demonstrated 1.0 improvement, demonstrated slight worsening in year 2. In the remaining subjects, 33% had continued stable EDSS, and 22% showed continued disease progression. These results suggest that additional treatments may be required to sustain the full effect of IT-MSC-NP treatment. A phase II trial is underway to investigate the safety and efficacy of 6 separate IT-MSC-NP treatments compared to a placebo IT-saline control. IT-MSC-NP therapy is a promising regenerative therapy for progressive MS.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

F-3088

CULTURE AND ANALYSIS OF HUMAN SPERMATOGONIAL STEM CELLS

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Spermatogonial Stem Cells (SSCs) are essential for the generation of sperm and have therapeutic potential. In mice, it has been shown that SSC transplants can rescue fertility in infertile males, providing a possible future therapeutic option in humans. “SSC therapy” has potential therapeutic value for various forms of male infertility (which afflicts >100 million men world-wide), including as a means to restore fertility to pre-pubescent males that have been rendered infertile by chemotherapy. A major bottleneck limiting SSC therapy is the lack of reproducible methods to culture and enrich for human SSCs. Here, we report on our progress on this topic using human testes biopsies obtained from fertile donors. In organ culture, we observed a proliferative expansion of spermatogonia, including cells expressing SSC markers, and dramatic loss of more differentiated germ cells. Cultured dissociated germ cells exhibited a similar pattern of marker expression; clusters of proliferative cells were also consistently observed. FACS analysis with the human SSC marker, SSEA4, revealed that both culture conditions increased the number of SSEA4+ cells, confirming SSC expansion. Lentivirus infection with a shRNA against the germ cell gene, RHOXF2, successfully depleted RHOXF2 expression, demonstrating our ability to manipulate gene expression in these SSC cultures. To define the transcriptome of enriched SSCs cultured under different conditions, we have employed RNAseq analysis. By comparison with the transcriptomes of freshly isolated primitive spermatogonia (highly enriched for SSCs) and differentiating spermatogonia, each purified with specific antibodies, we are comparing how different culture conditions enrich for human SSCs. Our focus is on newly described stage-specific spermatogonial markers defined by our recent single-cell RNA sequencing analysis of neonatal and adult testes. Our study reveals conditions that allow for the survival and expansion of human SSCs and it provides information on growth factors and cell signaling pathways that drive the survival and expansion of SSCs for future clinical applications.

F-3090

MESENCHYMAL STEM CELLS PROTECT TESTICULAR SPERMATOGONIAL STEM CELL NICHE IN VITRO

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In childhood cancers, 46% infertility rate has seen in male patients because of the damage in germ cells during chemo or radiotherapy. In males, because the spermatogenesis starts with puberty, it is not possible to collect mature sperms from children prior to cancer therapy. To be able to provide sustainability of the fertility, viability of spermatogonial stem cells (SSC) should be provided in in vitro conditions. Normally, SSCs locate in a very complex, physical and chemical 3D niche. Therefore, it is not easy to provide the longterm viability of the SSCs by conventional cell culture methods. Multi-potent, adult and somatic mesenchymal stem cells (MSC) support several niches due to their immune regulator, proliferative and anti-apoptotic characteristic. Moreover, MSCs coming from the same origin with the Sertoli Cells which are the cells giving chemical and physical support tod the SSC homeostasis in testis. Therefore, it is hypothesized that MSC secretions ensure the viability of SSCs by supporting the Sertoli Cells. With this hypothesis, MSCs isolated from bone marrow (BM) of the mice and characterised. Then, the cells were co-cultured with the testes isolated from newborn mice for 1, 2, 4 and 6 week time periods. As control group, the testes which were cultured alone are used. After end of each period, the testes were taken out, then quantitative histomorphometric analysis was performed on HE, MT and PAS stained tissue sections under light microscope according to tubule diameter , number of living/death cells and SSCs. The SSCs have been quantitatively evaluated by flow cytometric and immune fluorescent (IF) labelling. The integrity of the seminiferous tubules in co-cultured testis group exhibited a better sustainability in comparison to the control group ($p<0.05$). A decrease was noted in the apoptotic index in MSC co-cultured testes compared to the control group according to TUNEL assay ($p<0.05$). The number of SSCs in co-culture group increased according to IF labelling. Thus, it is concluded that the co-culture with BM-MSCs contribute the protection of the

microenvironment of SSCs during the ex vivo culture period of the mouse testis. The outputs gathered from this research are important for elucidating the relation between MSCs and the continuity of SSCs in in vitro conditions.

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F-3092

MAINTENANCE OF GENOMIC IMPRINTING IN PLURIPOTENT STEM CELLS

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Genomic imprinting is essential in mammals. Dysregulation of genomic imprinting causes many major human diseases including cancer, diabetes, cardiovascular diseases and neurological disorders. Previously, we and others have shown that genomic imprinting is not stably maintained in pluripotent stem cells, in particular the nuclear transfer-derived embryonic stem (ntES) cells and induced pluripotent stem (iPS) cells. Since loss of genomic imprinting can cause cancer and other human diseases, it is important to find out how genomic imprinting is maintained in the pluripotent stem cells before they can be used for cell-based therapies. We identified a maternal-zygotic effect gene Zfp57 as a key regulator in genomic imprinting. To elucidate the molecular mechanisms for the maintenance of genomic imprinting, we have generated Zfp57 mutant embryonic stem (ES) cells by gene editing and analyzed its functions in ES cells. We found that ZFP57 maintains genomic imprinting at most imprinted regions examined in ES cells, similar to its roles in mouse embryos. We have also applied gene editing to examine the functions of DNA methyltransferases, PGC7, UHRF1 and other related factors that may be involved in maintaining genomic imprinting in ES cells. To test if the maintenance of DNA methylation imprint is a dynamic process in ES cells, we eliminated TET proteins that are known to play important roles in active DNA demethylation. Indeed, DNA methyltransferases, PGC7 and UHRF1 are important for maintaining genomic imprinting in ES cells. Our preliminary results also suggest that genomic imprinting may be dynamically maintained in ES cells that requires ZFP57 and other multiple factors involved in DNA methylation. Dissecting the functions of these proteins in genomic imprinting will shed light on the molecular mechanisms underlying the maintenance of DNA methylation imprint in ES cells. This will help to obtain therapeutically suitable pluripotent stem cells with relatively stable genomic imprinting in the future.

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F-3094

TRIM28 HAPLOINSUFFICIENCY IN MOUSE GERMLINE CAUSES INFERTILITY IN ADULT MALE

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Trim28 is a chromatin regulator required to repress retrotransposons in somatic cells and the germline. A null mutation in Trim28 causes embryonic lethality, with haploinsufficiency leading to complex metastable epigenetic phenotypes in somatic cells of mice and in men. In order to evaluate Trim28 haploinsufficiency in the germline, we created a null mutation at the Trim28 locus in mouse PGCs using Cre-LoxP recombination with Blimp1-Cre. We discovered that Trim28 haploinsufficiency beginning in the PGC stage leads to age-related changes in spermatogonial stem cell self renewal and differentiation. Specifically, we discovered a progressive loss of germ cells in seminiferous tubes of adult male mice starting from 2 months of age. By 4-6 months, the majority of seminiferous tubules were composed of sertoli cells, and devoid of germline cells. A clinical phenotype in men referred to as sertoli cell only syndrome. Fertility testing from 8 weeks of life confirmed that the Trim28 PGC haploinsufficient mice were indeed infertile. Using immunofluorescence we discovered that a Trim28 haploinsufficiency phenotype starting in the PGC stage lead to a loss of SALL4 positive spermatogonial stem cells by 4 months, and this was accompanied by exhaustion of the differentiated cells. Taken together, our data suggests that the acute mutation of TRIM28 in one allele during early PGC development results in problems with balancing spermatogonial stem cell self renewal and differentiation which eventually leads to infertility.

CHROMATIN AND EPIGENETICS

F-3096

RONIN SAFEGUARDS SISTER CHROMATID COHESION IN PLURIPOTENT STEM CELLS

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Recent advances in understanding the genomic architecture and its spatial organization in stem cells have been restricted mostly to coding regions. Despite the abundance of repetitive sequences, such as those found in the centromeric and pericentromeric domains, we do not yet understand the detailed principles governing their structural arrangement. Pluripotent stem cells are characterized by the presence of relatively few voluminous chromocenters that upon differentiation become smaller, condensed and more numerous. Exactly how and why these regions are formed, maintained and change with differentiation is an open question. Here, we report that the stem cell factor, Ronin (Thap11), is essential for the integrity of these nascent chromocenters in pluripotent stem cells and, as a result, for proper cohesion between sister chromatids. Ronin directly binds to both major and minor satellite repeats and is associated with chromocenters. Upon conditional Ronin knockout, chromocenters lose their constitutive heterochromatin signatures. The resulting phenotypes range from an increased number of chromocenters and/or chromocenter shattering to an eventual loss of chromocenters. Thereafter, sister chromatid cohesion deteriorates specifically at centromeric and pericentromeric regions, delaying mitotic progression and promoting segregation errors. Mechanistically, Ronin requires its cofactor, HCF-1, to enable its translocation to the pericentromeric heterochromatin. This allows for the binding of HDAC3, which was previously identified as a regulator of sister chromatid cohesion. Together, these data shed light on the importance of Ronin in early development and prompt us to propose that Ronin configures the unique chromocenters and other heterochromatin structures seen in pluripotency.

F-3098

UTX/KDM6A MAINTAINS IDENTITY AND FUNCTION OF HUMAN PLURIPOTENT AND NEURAL STEM CELLS

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Epigenetic mechanisms execute gene expression programs for cell fate decisions and organismal development. UTX/KDM6A is a demethylase of methylated lysine 27 in histone H3 (H3K27me). In humans, mutations in UTX have been linked to the Kabuki Syndrome and myriad cancer types. To fully understand the role of UTX in human development and disease, we aim to elucidate the mechanism by which UTX executes developmental gene regulation during the differentiation of human embryonic stem cells (hESCs) along the neural lineage. We used CRISPR-Cas9 to generate UTX-knockout (KO) hESCs, which lost pluripotency marker expression and had a morphology similar to human neural stem cells (hNSCs). Comparison of marker expression and transcriptomes (by RNA-seq) of UTX-KO cells, wild-type hESCs, hNSCs, and differentiated neurons revealed that UTX-KO cells most resembled hNSCs. Combined chromatin immunoprecipitation-deep sequencing (ChIP-seq) with RNA-seq showed that UTX targets and promotes the expression of pluripotency factors OCT4 and HMGA1 as well as suppressors of neurogenesis NOTCH1, WDR62, KDM2B and DNMT3B. Other UTX target genes modulate the self-renewal property of hNSCs. During directed differentiation toward neurons, UTX-KO hNSCs had markedly reduced neuronal differentiation but instead preferentially differentiated towards the glial lineage. These data suggest that UTX is required for neuronal differentiation and suppressing the glial lineage. We conclude that UTX binds and promotes activators and suppressors of cell lineages and is therefore able to directly regulate self-renewal as well as concomitant specification of cell lineages. Comprehensive chromatin profiling of control and UTX-KO hNSCs discovered an unexpected influence of UTX in chromatin dynamics and gene regulation. We will also report our current effort in using UTX-KO stem cells in a pharmacological screen to suppress stem cell defects.

F-3100

GLOBAL CHARACTERIZATION OF X CHROMOSOME INACTIVATION IN HUMAN PLURIPOTENT STEM CELLS

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X chromosome inactivation (XCI) is an essential epigenetic silencing process established during early embryonic development of female mammals, guaranteeing a proper dosage compensation of X-linked genes between males and females. Activation of the lncRNA XIST, which triggers XCI, occurs early during differentiation, therefore human pluripotent stem cells (hPSCs) are potentially a major model for studying this process. While XCI was extensively studied in the mouse, there are significant differences when compared to humans, both in vivo and in vitro. Previous studies demonstrated variable states of XCI in hPSCs, many of them exhibit aberrant XCI patterns also after differentiation, thus impeding the use of these cells in therapeutic applications. These studies however focused on only a limited number of cell lines grown in the same lab. Here, we performed a large-scale characterization of XCI in 785 hPSCs from various sources, using RNA sequencing (RNA Seq) data. The analysis combined inputs on XIST expression, allelic polymorphism quantification and assessing the global expression levels of X-linked genes. These genome-wide analyses provide significantly higher coverage compared to other methods which interrogate individual genes, thereby enabling a sensitive and accurate classification of XCI state. We identify a striking difference between embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) in their XCI landscape, in which most iPSCs maintain the inactive X of their parental somatic cells, whereas ESCs silence the expression of XIST and upregulate distal regions of the X-chromosome. We suggest this to be the most significant epigenetic difference between ESCs and iPSCs, globally dividing them to distinct groups based on their XCI status. We further show that this erosion is slightly reduced upon differentiation, in a XIST-independent manner. Altogether, we propose a model in which XCI variations in hPSC lines are affected by epigenetic memory of their different parental sources, as well as by dynamic changes occurring during derivation and culture of hPSCs. Collectively, our research may serve as a platform to analyze the status of XCI for the study of human development and disease modeling and to characterize the cells prior to their transplantation. *Under-revision in Cell Reports

F-3102

CANCER MUTATIONS TO THE COHESIN COMPLEX IMPAIR TRANSCRIPTIONAL INSULATION IN A MURINE EMBRYONIC STEM CELL MODEL SYSTEM

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The Cohesin complex has been implicated in transcriptional regulation through the formation and maintenance of two classes of chromatin loops. Enhancer-promoter loops drive gene expression by bringing active enhancers directly into contact with target promoters, while insulated loops restrict enhancer activity to discrete units of the genome flanked by loop boundary sequences that bind the architectural protein CTCF. In stem cells, insulated loops are essential regulators of genes that encode master transcription factor (TFs), supporting robust expression of pluripotency master TFs and maintaining suppression of developmentally-silenced lineage-specific master TFs. In cancer settings, mutational inactivation of insulated loop boundary elements has been implicated in activation or re-expression of silenced oncogenes. Notably, the genes that encode the subunits of the Cohesin complex are also recurrently mutated across many cancers, but the mechanisms through which this may drive malignancy are not understood. Here, we examine whether these mutations might cause altered cell identity through global interference with insulated loop formation or function. We used CRISPR/Cas9 genome editing to generate murine embryonic stem (ES) cell lines with cancer mutations in endogenous SMC1A and STAG2 Cohesin subunits, and assayed those cells for transcriptional changes using RT-qPCR and RNA-seq. We identified missense mutations in SMC1A and inactivating mutations in STAG2 that reduced transcriptional insulation across numerous insulated loop boundaries. Strikingly, the ES cell transcriptional program was broadly dysregulated, reducing expression of pluripotency-controlling TFs and increasing expression of differentiation TFs. Surprisingly, the SMC1A and STAG2 mutations act through distinct mechanisms to impair insulation: preliminary data suggest that SMC1A mutation reduces binding of Cohesin to chromatin, while STAG2 loss allows for normal binding and localization of Cohesin on chromatin despite reduced capacity for insulation. Together, these results indicate that proper insulated loop function is indispensable for maintenance of stem cell identity, and that cancer-linked mutations to the Cohesin complex impair the ability of cells to properly maintain transcriptional insulation.

PLURIPOTENCY

F-3104

PROTECTING EARLY EMBRYOGENESIS AND PLURIPOTENT STEM CELLS AGAINST GENETIC PARASITES THROUGH A PRIMITIVE IMMUNE SYSTEM

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Retrotransposons are expressed in early ontogeny and may induce structural rearrangements and mutations that seriously damage the genome of the nascent embryo, resulting in diseases and infertility. At present, cellular protection against retrotransposons is believed to rely solely on a cell autonomous system involving epigenetic silencing. Here, we report that pluripotent cells can sense the retrotransposable load of their neighboring cells and, if it is higher than their own, act to kill and remove them. To determine how cells detect neighbors with more retrotransposon activity, we performed transcriptome analysis; this revealed that the sensing mechanism is based on a dsRNA response that triggers a negative feedback loop. We then used super-resolution microscopy paired with lysosomal activity assays, and found that cells were employing non-professional phagocytosis, most likely induced by NF-kB signaling, to remove their unhealthy, retrotransposon-infested neighbors. Finally, experiments performed in stressed cells revealed that this defense system is conditional, as its activating threshold was subject to change. Our study shifts attention away from solely cell-intrinsic defense strategies towards considering a much more nuanced system in which cells constantly monitor one another for endogenous retrotransposon-related health. Our line of investigation will provide new targets for genetic diagnosis and interventions targeting pregnancy loss and infertility.

F-3106

GENE EXPRESSION ANALYSIS OF PAPIO ANUBIS STEM CELLS REVEALS UNIQUE METABOLIC STATE THAT APPROXIMATES HUMAN STEM CELL METABOLISM

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A major hurdle impeding clinical implementation of stem cell-based therapies is the failure to translate therapeutic approaches from typical model organisms to human patients. Validating non-human primate (NHP) models for use in stem cell-based therapies has the potential to increase successful translation of therapies to the clinic by offering a model that better represents humans. To that end, we performed RNA-Seq analysis on embryonic and induced pluripotent stem cells derived from Papio anubis (Olive baboon) and compared the data to human and mouse embryonic and induced pluripotent RNA-Seq datasets. Overall, P. anubis pluripotent cells were a closer representation of human pluripotent cells than were mouse pluripotent cells. Gene ontology analysis revealed that the most highly dysregulated genes between baboons and humans and between mice and humans were related to metabolism. We found that the oxidative phosphorylation pathway was relatively downregulated in baboon stem cells when compared to human stem cells, but significantly upregulated in mouse stem cells when compared to human stem cells. Notably, in mouse pluripotent stem cells, mitochondrial genes for subunits of the NADH+ dehydrogenase complex are actively transcribed while being suppressed in human and baboon pluripotent cells. Because a unique metabolic state is critical to maintaining pluripotency, distinct metabolic states between pluripotent cells of different species may diminish translatability of stem cell therapies from typical model organisms to human patients.

F-3108

THE BCL-2 FAMILY MODULATES STEM CELL IDENTITY BY REGULATING MITOCHONDRIAL DYNAMICS

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Over the past decades, the function of the mitochondria has evolved from being a mere powerhouse to a complex signaling hub. Mitochondrial dynamics, which includes fusion and fission, have been implicated in many diseases including aging, neurodegeneration, and tumorigenesis. Recent studies from our laboratory suggest that proteins of the BCL-2 (B-cell lymphoma 2) family, known as regulators of cell death, can also modulate mitochondrial dynamics in human pluripotent stem cells (hPSCs). The myeloid cell leukemia 1 (MCL-1) protein, a known anti-apoptotic protein of the BCL-2 family, localizes not only to the outer mitochondrial membrane, where it exerts its anti-apoptotic function, but also to the mitochondrial matrix, where its function is not clear. Inhibition of MCL-1 in hPSCs via a small molecule also leads to loss of pluripotency markers (NANOG and OCT4), independently from its anti-apoptotic function. In addition, MCL-1 inhibition results in a fused and elongated mitochondrial network, which suggests that MCL-1 is crucial in maintaining fission in hPSCs. We will also present our preliminary studies describing how other proteins of the BCL-2 family, known to interact with MCL-1, are also capable of modulating mitochondrial dynamics in homeostatic and stress conditions. Overall, our data suggest that BCL-2 family plays a major role in stem cell identity and this is accomplished by the regulation of mitochondrial dynamics.

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F-3110

PODOCALYXIN-LIKE PROTEIN 1 REGULATES HUMAN PLURIPOTENT STEM CELL SELF-RENEWAL THROUGH CHOLESTEROL BIOSYNTHESIS PATHWAY

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The transmembrane signals maintain the pluripotency of human pluripotent stem cells (hPSCs) are rarely investigated. A transmembrane glycoprotein, podocalyxin-like protein 1 (PODXL, also called PCLP1, Gp200/GCTM-2, MEP21, and Thrombomucin), aside from its well-known roles in tumor malignancy, very little is known about its functions in hPSCs. Here, we report that the downregulation of PODXL in undifferentiated hPSCs significantly altered the selfrenewal and survival abilities and lead to a decrease in c-MYC and telomerase proteins. Of note, the formation of induced pluripotent stem cells (iPSCs) colonies was hampered upon the knockdown of PODXL. Consistently, overexpression of PODXL promoted hPSC selfrenewal, the expressions of c-MYC and telomerase, and iPSC formation. In a transcriptomic analysis, forcing PODXL expression revealed that PODXL can activate HMGCR expression and thus control the cholesterol biosynthesis. Meanwhile, we found that PODXL also regulates master regulators of cholesterol synthesis genes, SREBP1/SREBP2, in accordance with alteration of c-MYC expression. Importantly, compared to fibroblasts, hPSCs are more sensitive to cholesterol synthesis dysregulation blocked by chemical inhibitors statin and AY9944, and lipid raft disruption by MβCD treatment which lead to impairments of self-renewal and survival abilities. Of note, cholesterol can fully restore PODXL knockdown-mediated pluripotency loss and apoptosis phenotype in a dose-dependent manner. Cholesterol also notably restores TERT, c-MYC, and HMGCR protein expression. Furthermore, we found PODXL regulated the sizes of lipid raft and may transmit signals through controlling CD49B (integrin α2) mobilization into lipid rafts. The knockdown of CD49B blocks ESC renewal. Our data highlight the important roles of PODXL in controlling cholesterol metabolism to achieve hPSC selfrenewal.

F-3112

HUMAN NAÏVE PLURIPOTENT STEM CELLS ACQUIRE CANCER-RELATED MUTATIONS DURING THEIR ESTABLISHMENT FROM PRIMED CELLS

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Human pluripotent stem cells (hPSCs) have been previously shown to harbor several types of genetic aberrations that accumulate during culturing. These aberrations include chromosomal abnormalities and copy number variations, which can affect the tumorigenic potential of the cells. More recently, point-mutations in the gene coding for the p53 tumor suppressor (TP53) have been found in early passaged hPSCs using whole exome sequencing (WES) And late passaged cells using RNA sequencing. These mutations were shown to gradually take over the culture, suggesting they provide a growth advantage in vitro. However, it remained unclear if in other cancer-related mutations appear in hPSC cultures. We therefore established a new strategy to compare early and late passaged hPSCs to identify mutations listed in the census Catalogue of Somatic Mutations in Cancer (COSMIC Census). We found that alongside mutations in TP53, recurrent mutations appear in at least 22 other cancer-related verified genes and that these mutations appear in both embryonic and induced-pluripotent stem cells. We then looked for mutations in these genes in both primed hPSCs and their naïve derivates from eight independent studies. Naïve cells were found to harbor 4-times more cancer-related mutations on average, significantly more than their primed counterparts. We also found indications that these mutations accumulate throughout the naïve-state induction process, suggesting this procedure imposes a substantial selective pressure. Together, our results suggest that hPSCs gain validated cancer-related mutations and that selective pressures could enhance this accumulation. These mutations should be taken into consideration in future applications, specifically in clinical ones.

F-3114

METABOLIC NETWORK-CENTRIC ANALYSIS OF SINGLE CELL RNA-SEQ DATA REVEALS NOVEL DYNAMIC CHANGES IN ENZYME EXPRESSION AND NUTRIENT UTILIZATION IN IMPLANTATION DEVELOPMENT

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Recent studies have revealed that changes in metabolism regulate cell fate decisions in a wide range of developmental processes. However, these metabolic changes are often measured in heterogeneous cell populations, potentially masking cell type-specific and rich dynamic changes. Single cell RNA-seq have been extensively used to study cell fate decisions, but few have been analyzed from a metabolic perspective. The massive amount of single cell RNA-seq data of developmental processes is both a significantly untapped resource and unprecedented opportunity to study metabolism with high cellular and temporal resolution. Using monkey pre- and post-implantation epiblast development as a model system, we combined single cell RNA-seq data with genome-scale metabolic network models to systematically reveal dramatic metabolic dynamics in this early developmental stage. Our predictions recapitulated many known metabolic shifts, such as changes in glycolysis, fatty acid oxidation, fatty acid and lipid synthesis, OXPHOS, vitamin C/L-proline ratio and the effects of aKG in naïve and primed pluripotency (corresponding to pre- and post-implantation, respectively). Furthermore, we further revealed many novel metabolic shifts, such as anti-oxidant response, amino acid utilization, co-factor and mineral uptake. We also identified novel transient dynamics of metabolic enzymes that would be missed by bulk gene expression assays, and discovered differential temporal trajectories of isozymes localized in the mitochondrial vs. cytoplasmic compartments. By analyzing trophoblast development in parallel, we also identified unique enzyme dynamics and nutrient requirements in each lineage. These computational predictions can potentially lead to culture medium metabolically optimized to maintain naïve pluripotent stem cells or trophoblast stem cells. The computational framework is generally applicable to many other developmental processes where single cell RNA-seq data is available, such as hematopoiesis, neural stem cell development etc.

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PLURIPOTENT STEM CELL DIFFERENTIATION

F-3122

DEVELOPMENT OF AN EVALUATION SYSTEM THAT CAN PREDICT THE OSTEOGENIC POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS EASILY AND PROMPTLY

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Human mesenchymal stem cells (hMSC) are promising candidate cells as raw materials for regenerative medical products. When hMSC are differentiated to produce target products, selection of hMSC strain with high differentiation potential is important, and development of an efficient screening method is required. In this study, focusing on the osteogenic potential of hMSC, we aimed to develop a system capable of evaluating bone differentiation ability at an early stage in bone differentiation induction which takes about 3 weeks. We investigated the relationship between the mRNA expression levels of 5 genes widely used as markers of bone differentiation and the actual bone differentiation potential of each hMSC strain.10 lots of hMSC derived from bone marrow of different donors were induced to osteogenic differentiate and the each degree of bone differentiation of those hMSCs was determined by measurement of calcium deposits by Alizarin Red staining. These 10 lots of hMSC contained 5 lots each of a strain with high bone differentiation ability and a strain with low. In order to investigate whether each osteogenic potential can be predicted according to the gene expression level, mRNA expression levels of five genes (Runx2, Alkaline phosphatase (ALP), Type I Collagen (COL1), Osteopontin (OPN), Osteocalcin (OCN)) which are often used as markers of osteogenic differentiation were measured before and one week after induction of bone differentiation. We found that mRNA expression level of ALP was significantly higher in hMSCs with higher bone differentiation potential than in hMSCs with lower, both before induction of bone differentiation and one week after induction. On the other hand, the mRNA expression levels of the other four genes (Runx2, COL1, OPN, OCN) were not related to the bone differentiation potential of hMSC. ALP mRNA expression level is related to the osteogenic potential of hMSC. Its potential can be predicted by the expression level of ALP even before induction of bone differentiation. By measuring mRNA expression of ALP, it was found that osteogenic differentiation ability of hMSC can be predicted at the latest at one week in bone differentiation induction which takes about 3 weeks.

F-3124

MATURATION AND ACTIVITY-DEPENDENT RESISTANCE OF HUMAN IPSC-DERIVED NEURONS TO PRO-APOPTOTIC STIMULI

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Neurons represent a highly specialized cell population of the central and peripheral nervous system responsible for communicating information throughout the body. In humans, most neurons are born during embryonic development and persist throughout the entire life span of an individual. Presumably, they rely on preemptive strategies to protect against stress and accidental cell death. During early development neurons are produced in excess numbers which are later reduced during the establishment of mature neuronal circuitries. Thus, cell death pathways should be essential at earlier stages of neuronal network formation to enable trimming of surplus connections while they might become dispensable or even detrimental once specialized mature neuronal networks have been established. We set out to decipher the pathways associated with increased resistance of mature human neurons to stress and apoptosis using human induced pluripotent stem cell (iPSC)-derived neuronal cultures. We show that during the time course of maturation, iPSC-derived neurons become increasingly resistant to several types of cellular stressors. This is accompanied by a global downregulation of initiator and effector caspases and a shift in the balance of pro- and anti-apoptotic proteins. Contemporaneously, the neurons possess an increased activation of the AKT pro-survival pathway acting upstream of the identified changes. This activation of AKT can be modified by interfering with neuronal activity indicating that recurring synaptic stimulation of neurons within a complex network reinforces their survival and boosts network resilience.

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F-3126

DYNAMICS OF CELL FATE PATTERNING IN LIVE HUMAN EMBRYONIC STEM CELLS

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Live-cell reporters of pluripotency transcription factors in human embryonic stem cells (hESCs) allows the study of protein dynamics and the critical role they play in maintaining pluripotency and differentiation. However, generating clonal cell lines expressing fluorescently tagged genes is difficult not only due to the relative inefficiency of homologous recombination, but also to the high sensitivity of hESCs to perturbations, their spontaneous differentiation, and recalcitrance to being cultured as single cells. Historically, most approaches to selecting modified hESCs clones have relied on an iterative process of enriching a population for positively expressing cells ("cell pruning"). This process is tedious, time-consuming, and limits the timely development of new reporter cell lines. Here, we describe the use of microraft array cell sorting technology and high-content imaging to identify and isolate undifferentiated reporter hESC lines. The microraft arrays consist of 12,000 microwells each containing a magnetic releasable cell culture element ("microraft"). The arrays were populated with hESCs targeted through CRISPR-mediated homologous recombination to introduce fluorescent reporters into the endogenous loci of genes of interest. Each microraft cell carrier was rapidly screened for successfully gene-edited cells via fluorescence imaging. Microrafts identified as containing potential clones were gently released from the array and collected with a magnet for expansion of their adhered cells in order to screen them for the correct insertion. This approach was applied to develop endogenous reporters for three core human pluripotency factors: OCT4, SOX2 and NANOG. Using time-lapse imaging to track the real-time expression of these factors in single hESCs, we describe the dynamics of transcription factor patterning in stem cell colonies as a function of cell growth and differentiation.

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F-3128

NOVEL CROSSTALK BETWEEN VPS26A AND NOX4 SIGNALING DURING NEUROGENESIS

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Despite numerous studies on the molecular switches governing the conversion of stemness to differentiation in embryonic stem cells (ESCs), little is known about the involvement of the retromer complex. Under neural differentiation conditions, Vps26a deficiency (Vps26a-/-) or knockdown suppressed the loss of stemness and subsequent neurogenesis from ESCs or embryonic carcinoma cells, respectively, as evidenced by the long-lasting expression of stemness markers and the slow appearance of neuronal differentiation markers. Interestingly, relatively low reactive oxygen species (ROS) levels were generated during differentiation of Vps26a-/- ESCs, and treatment with an antioxidant or inhibitor of NADPH oxidase (Nox), a family of ROS-generating enzymes, led to restoration of stemness in wild-type cells to the level of Vps26a-/- cells during neurogenesis. Importantly, a novel interaction between Vps26a and Nox4 linked to the activation of ERK1/2 depended highly on ROS levels during neurogenesis, which were strongly suppressed in differentiating Vps26a-/- ESCs. Moreover, inhibition of phosphorylated ERK1/2 (pERK1/2) resulted in decreased ROS and Nox4 levels, indicating the mutual dependency between pERK1/2 and Nox4-derived ROS during neurogenesis. These results suggest that Vps26a regulates stemness by actively cooperating with the Nox4/ROS/ERK1/2 cascade during neurogenesis. Our findings have important implications for understanding the regulation of stemness via crosstalk between the retromer molecule and redox signaling, and may contribute to the development of ESC-based therapeutic strategies for the mass production of target cells.

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F-3130

A CHEMICALLY-DEFINED AND ANIMAL-ORIGIN FREE CULTURE MEDIUM STEMFIT AS400 IS USEFUL FOR TRILINEAGE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS FOR CLINICAL APPLICATIONS

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Human pluripotent stem cells (hPSCs) such as iPS cells and ES cells are expected to be a valuable source for regenerative medicine. Although it is possible to expand hPSCs under chemically-defined and animal origin-free (AOF) medium, most of differentiation medium contains animal-derived or undefined components such as FBS and B27. Undefined and animal component-containing medium hampers clinical applications of hPSC-derived cells/tissues because of higher risks of viral infection and larger lot-to-lot variation. Therefore, it is necessary to develop a differentiation medium for clinical application without affecting differentiation efficiency. We have developed a novel differentiation nutrient supplement “StemFit AS400” (AS400), which is utilized by addition to basal medium, differentiation factors, and other reagents. All ingredients of AS400 are chemically-defined, and none of the ingredients originate from animal sources. In this study, hPSCs were spontaneously differentiated via embryonic body (EB) formation using our medium containing AS400 for 2 weeks and evaluated trilineage differentiation potential with TaqMan® hPSC ScorecardTM. We found that differentiation medium

containing AS400 supported sufficient trilineage differentiation. Furthermore, we succeeded in making neural stem cells (ectoderm), cardiomyocytes (mesoderm), and hepatocyte (endoderm) from hPSCs by using AS400 on defined and xeno-free extracellular matrix-coated dishes with comparable efficiency to conventional medium. These results suggest that AS400 is useful for trilineage differentiation of hPSCs in various cases. This nutrient supplement is expected to promote clinical applications of hPSC-derived target cells/tissues by establishing the chemically-defined and AOF culture systems of both hPSC expansion and differentiation.

F-3132

HUMAN IPSC-DERIVED M1/M2 MACROPHAGES: NOVEL OFF-THE-SHELF CELL THERAPEUTICS FOR CANCER AND REGENERATIVE MEDICINE

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Distinct populations of macrophages show significant contribution to human disease pathogenesis, as well as repair of damaged tissues. Our studies provide a method to routinely generate a large number of macrophages from human induced pluripotent stem cells (hiPSCs). Moreover, these iPSC-derived macrophages can be polarized to specific M1 and M2 phenotypes. Briefly, hematopoietic progenitor cells (HPCs) are derived from undifferentiated hiPSCs using a well-defined serum-free and stromal-free protocol. These HPCs then cultured in stage II conditions in serum-free media supplemented with M-CSF and IL-3. Using these conditions, macrophage progenitor cells (CD14, CD11b, CD36, SIRP-α and CD68) are continuously produced from a starting cell population of approximately 105 undifferentiated hiPSCs for more than 8 weeks. During this time, we collect 0.5 × 106 – 106 macrophage progenitor cells per well per week. These cells can then be further differentiated in stage III conditions to mature macrophages (hiPSCs-Mφ) in presence of M-CSF for 7 days. HiPSCs-Mφ exhibited morphology, surface marker expression (CD14, CD11b, CD36, SIRP-α and CD68) and latex bead phagocytic activity that are similar to peripheral blood-derived macrophages (PB-Mφ). We can further polarize these hiPSCs-Mφ toward M1 pro-inflammatory macrophages by culture with LPS and IFN-γ. M1 macrophages are characterized by expression of CD80, TNF- α, and IL-6. We can also produce M2 anti-inflammatory macrophages by culture with IL-4 and IL-13. M2 macrophages are characterized by expression of CD206, CCL17, and CCL22. Both hiPSCs-Mφ and PB-Mφ expressed high level of CD80 upon stimulation in pro-inflammatory conditions although the M1 differentiation was more efficient in hiPSCs-Mφ. Conversely, the M2 polarization of PB-Mφ resulted in more pronounced expression of CD206 compared to hiPSCs-Mφ. hiPSCs-M1 expressed high level of TNF-α and IL-6 while hiPSCs-M2 showed elevated level of CCL17 and CCL22 in culture supernatant. Collectively, these studies provide an efficient system for rapid generation of distinct human macrophage populations, helping to better understand their biology and facilitating their use for cell-based therapy of human diseases.

F-3134

KUF11 TREATMENT LED TO ENHANCED HEMATOPOIETIC STEM CELL DIFFEREENTIATION FROM URINE CELL- OR BLOOD CELL-DERIVED PLURIPOTENT STEM CELLS

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Autologous urine-derived stem cells (USCs), which can be noninvasively obtained from urines, are thought to be ideal for applications in regenerative medicine. Here, we developed a method for efficient isolation and culture of USCs, which showed enhanced migration and colony forming capacities. We also efficiently produced human USC-derived induced pluripotent stem cells (hU-iPSCs). Efficient maintenance of the undifferentiated status of pluripotent stem cells (PSCs) may be crucial for producing a high-quality cell source that could be successfully applied in stem cell research. Here we tried to screen various natural compounds to find the materials enhancing of the quality of human pluripotent stem cells (hPSCs). Among the tested compounds, treatment of KUF11 showed increases in cell proliferation, cell cycle (S phase), and phosphorylation of extracellular signal–regulated kinases (ERKs) signaling pathway, and survival rate. Of note, KUF-exposed hPSCs showed upregulation in the level of glutathione (GSH) and generation of high population of GSH-high cells when measured by FreSHtracer system. hPSCs treated with KUF11 showed enhanced mesodermal differentiation efficiency, in particular, into hematopoietic stem cells (HSC) differentiation. Taken together, our data afford a novel natural compound possessing high efficiency in enhancing hPSC proliferation, pluripotency, and HSC differentiation that can be applied for production of high-quality hPSC for the clinical applications in therapy and hematology in the future.

F-3136

EFFICIENT, LARGE-SCALE PRODUCTION AND FUNCTIONAL CHARACTERIZATION OF NOCICEPTORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Development of new non-addictive analgesics would greatly benefit from directed differentiation of hPSCs into relevant cell types. Here, we devised a highly efficient step-wise protocol that differentiates human pluripotent stem cells (hPSCs) exclusively into nociceptors under fully defined conditions. By manipulating critical cell signaling pathways using small molecule inhibitors, hPSCs were first converted into SOX10+ neural crest stem cells followed by differentiation into bona fide nociceptors. Time-course RNA-Seq analysis (Day 0-28) and immunocytochemistry experiments confirmed that nociceptors expressed typical neuronal markers, transcription factors, neuropeptides, and ion channels. Focusing on pain-relevant receptors expressed by hPSC-derived nociceptors (e.g. P2RX3, opioid receptors), we could demonstrate robust functional activities in multi-electrode array experiments and differential responses to nociceptive stimuli and specific drugs including natural and synthetic opioids. The nociceptor differentiation protocol was then automated by using a robotic cell culture system (CompacT SelecT™) enabling multiple high-throughput projects that require large numbers of cells. In summary, the scalable human nociceptor platform developed here will aid in the discovery of new pain medications as part of the effort to tackle the opioid crisis.

Funding Source: NIH Common Fund, NIH HEAL Initiative, NCATS Intramural Research

F-3138

HETEROGENEITY IN THE SURFACE MARKER EXPRESSION AND THE DIFFERENTIATION POTENTIAL OF HAEMATOPOIETIC STEM CELL PROGENITORS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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As human induced pluripotent stem cells (hiPSCs) can be differentiated to haematopoietic stem and progenitor cells (HSPCs) and further to erythroid cells, hiPSCs generated from patients with red cell diseases are valuable tools for studying the pathogenesis of these diseases. Two different types of iPSC derived HSPCs (iPSC-HSCs) with different expression of surface makers, CD34+CD235a+CD41+ and CD34+CD45+, have been reported in the literature, and both have been found to have the potential to differentiate to erythroid cells. We carried out a study to know whether these two types of iPSC-HSCs are generated on different stages of differentiation of iPSCs and whether there is a difference in their differentiation potential to erythroid lineage. We differentiated three iPSC lines to HSPCs using a previously reported protocol, and the suspension cells containing HSPCs were analyzed for the expression of CD34, CD235a, CD41 and CD45 on days 11, 15 and 17 of differentiation. We found that CD34+CD235a+CD41+ HSPCs were generated from the early stage (day 11) and CD34+CD235- CD45+ HSPCs from the late stage (days 15 and day 17) of differentiation. Colony forming unit assay showed that early and late HSPCs predominantly formed colonies with granulocyte, macrophage and megakaryocyte lineage, but the late HSPCs formed 3 times more erythroid colonies compared to the early HSPCs (26% Vs 7%). Erythroid differentiation of iPSC-HSPCs in liquid culture also showed 10-fold increase in the number of erythroid cells generated from the late HSPCs, compared to the early HSPCs. However, expression kinetics of the erythroid specific markers, CD36, CD71 and CD235a, showed that the early HSPCs underwent rapid differentiation than the late HSPCs. RNA sequencing analysis of the early and the late HSPCs showed significant difference in the expression of haematopoietic genes. Altogether, our results showed that two types of HSPCs are generated from iPSCs at different time points of differentiation, and they have significant difference in their erythroid differentiation potential. For studying the disease mechanisms of red cell diseases, the HSPCs that express high levels of CD45 are more suitable due to their higher erythroid differentiation potential.

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F-3140

A PLURIPOTENT STEM CELL DERIVED, 3-DIMENSIONAL MODEL OF THE HUMAN ENDOMETRIUM

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The human endometrium is a highly dynamic organ – cyclically proliferating, differentiating, and shedding in response to ovarian hormones. Precise endometrial responses are necessary to enable implantation, pregnancy, and birth. While mouse models of pregnancies exist, there are fundamental differences that make it a poor model for the human endometrium. Cells from the human endometrium can be obtained by biopsy but have limited proliferative capacities and involve an invasive procedure. Consequently, little is known about how the human endometrium differs in healthy versus diseased pregnancies and births. To address this, we developed a model of the human endometrium that combines human pluripotent stem cells (PSCs)-derived endometrial mesenchyme (EM) and endometrial epithelial cells (EEC) in a 3-dimensional organoid system. We developed a protocol for differentiation of PSCs into EM, precursors of endometrial stromal fibroblasts (ESF). The PSC progress through developmental stages: first differentiating to cells of the late primitive streak, intermediate mesoderm, Müllerian mesenchyme, and finally EM. The progression of differentiation through these stages was characterized by gene expression and immunocytochemistry. Notably, these cells expressed anti-mullerian hormone receptor 2 (AMHR2), which is specific to reproductive tract mesenchyme, and the PSC-EM demonstrated regional specificity characterized by expression of HOXA10 and HOXA11, but not HOXA9 or HOXA13. The EMs then were co-cultured with EEC organoids derived from the adult uterus. The PSC-EM progenitors interacted with the EEC organoids to form a structure in which the PSC derived cells organized in a layer surrounding the EECs. This model of the endometrium will enable the study of how these two human cell types interact and will enable the study of defects in pregnancy as well as endometrial diseases such as endometriosis and endometrial cancer.

Funding Source: March of Dimes

F-3142

THE ROLE OF ACIDIFICATION IN HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION

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Human pluripotent stem cells (hPSCs) hold profound medical promise because they can differentiate into any cell type, yet, for clinical applications, improved differentiation protocols are required. The embryogenesis process is initiated by formation of the three embryonic germ layers— mesoderm, ectoderm, and endoderm—which ultimately constitute all cell types in the body. Current differentiation strategies using chemical agents, cytokines, and growth factors produce suboptimal terminal cells of interest. Recently, the facilitating role of metabolic flux and metabolite levels in reconfiguring epigenetic regulation programs to improve hPSC differentiation has been established. Our findings show that induction of distinct metabolic programs is necessary during embryonic germ lineage differentiation; these metabolic activities result in differential extracellular acidification levels during early cell type specification. Yet, acidification has not been examined as a microenvironment stimulus controlling hPSC differentiation despite its known roles in driving differentiation during pathogenic and physiological development. Our findings suggest that low pH skews embryonic tri-lineage progenitor cell fate, possibly revealing another aspect of developmental regulation thus far unexplored. This confirms a direct connection between metabolism and acidification, and further suggests a role for pH in early cell fate determination. Success in these studies will open new pathways through pH manipulations for generating superior in vitro models of early human development for numerous promising applications in health and disease.

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F-3144

SINGLE-CELL TRANSCRIPTOME APPROACH TO INVESTIGATE THE MECHANISM OF SPECIFYING MESODERM LINEAGES USING HUMAN INDUCIBLE PLURIPOTENT STEM CELLS

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Human inducible pluripotent stem cells (iPSCs) is a useful tool for studying the development of human embryo. Mesoderm population including kidney, which organoid we have successfully generated, are all derived from primitive streak (PS) during gastrulation. Interestingly, the 2D cultured iPSCs did not uniformly differentiate into one type of mesoderm cell, instead, they consist of heterogeneous progenitor cells including ureteric bud (UB) and metanephric mesenchyme (MM) precursors expressing either anterior or posterior makers of intermediate mesoderm (IM). In the present study, we aimed to investigate the mechanism of heterogeneity in mesoderm development using directed differentiation of iPSCs. Recently, single-cell RNA sequence (scRNA-seq) already became a powerful method to describe heterogenous cell populations expressing variability of genes. We made use of this technique to trace the dynamics in genes expression. To this end, we used the culture condition that could give rise to the heterogeneous mesoderm cells containing anterior, posterior, paraxial, and lateral plate mesoderm. iPSCs undergoing differentiation were harvested every day from day 0 to 7 and performed scRNA-seq. The result showed highly

expressed CDX2, a marker for undifferentiated tail bud, indicating cells were not fully differentiated into posterior and lateral mesoderm cells by day 7. In order to obtain better mediolateral and anteroposterior balance at more differentiated stage, here, we optimized the culture condition and confirmed the induction of each mesoderm region by qPCR and immunofluorescent.

F-3146

STANDARDIZED QC WORKFLOW FOR ANALYZING THE QUALITY AND DIFFERENTIATION POTENTIAL OF HUMAN IPSCS

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Human induced pluripotent stem cells (hiPSCs) derived from human somatic have given scientists new opportunities to model and investigate human diseases, as well as to develop new therapeutics. The routine application of hiPSCs and functional cells derived from hiPSCs in high throughput applications for drug discovery will require a constant supply of pluripotent, well characterized and quality controlled cell stocks. However, the absence of standardized quality control of IPSC in many groups and execution challenges laboratory efficiency and experimental reproducibility. Here, we established a workflow to monitor the hiPSCs morphology and proliferation using longitudinal time-lapse imaging, coupled with an assessment of pluripotency through immunofluorescence staining and q-PCR analyse of pluripotency markers, assessment EB formation and differentiation to 3 germ layers by q-PCR and IF staining, assessment of karyotypic abnormalities by qPCR. Following this workflow, 10 healthy hiPSCs lines cultured in different medium (mTeSR1 and Essential 8) were monitored using this process. Cell cycle time, cell attachment, spontaneous differentiation and cell viability in passage 1 and 7 post thaw were evaluated. No significant differences were observed between two media but slightly different exist from line to line, at both early and late passage. All hiPSC lines showed a homogenous morphology with high expression of pluripotency markers SSEA-4, Nanog, Oct3-4 and also retained genomic stability. EB formation and q-PCR analysis provided further validation that these cell-lines possessed an intrinsic ability to differentiate into any of the three germ layers. Together, the workflow outlined here provides a simple, standardized characterization for routine quality control of hiPSC to ensure researchers can adapt for their own labs, to ensure high quality hiPSCs for their research and translational needs.

F-3148

FUNCTIONAL SCREEN REVEALS ESSENTIAL ROLES OF RNA BINDING PROTEINS IN THE EXIT FROM PLURIPOTENCY

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RNA-binding proteins (RBPs) play crucial roles in various cellular processes mainly through post-transcriptional control of RNAs, including polyadenylation, splicing, stabilization, localization and mRNA translation. However, the functions of RBPs in the onset of differentiation of mammalian embryonic stem (ES) cells remain largely unknown. Here, by functional screening dozens of pre-selected RBPs in ES cells, we identified a few crucial RBPs required for exit from pluripotency and further explored the underlying molecular mechanisms. Firstly, a number of candidate RBPs were picked by bioinformatics analysis based on the RBPs database of mouse ES cells and published literatures. Then, we disrupted these candidates by using CRISPR/Cas9 system in mouse ES cells respectively, and evaluated the onset of differentiation in these mutant cell lines thoroughly. A few candidate RBP-null cell clones, including Rbm34, Hnrnpll and Rbpms, showed significantly differentiation defect compared with wide-type ES cells. Further mechanism research showed that the RBPs regulate mRNA splicing and stability. The RNA-seq analysis revealed substantial changes in pre-mRNA alternative splicing (AS) at each examined time point during ES cell differentiation, revealing distinct temporal patterns of AS underlying cell-fate changes. Combined with RIP-seq data, we found the long transcript of two important transcription factors cannot be decreased accordingly without the RBP. These results indicated that RBPs played an important role in the exit from pluripotency.

F-3150

TRISOMY 12 COMPROMISES THE MESENDODERMAL DIFFERENTIATION PROPERTY OF HUMAN PLURIPOTENT STEM CELLS

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Trisomy 12 is one of the most frequent chromosomal abnormalities in cultured human pluripotent stem cells (hPSCs). Although potential oncogenic properties and augmented cell cycle caused by trisomy 12 have been reported, the consequences of trisomy 12 in terms of cell differentiation, which is the basis for regenerative medicine, drug development, and developmental biology studies, have not yet been investigated. Here, we report that trisomy 12 compromises the mesendodermal differentiation of hPSCs. hPSC sublines carrying trisomy 12 showed a lower propensity for mesendodermal differentiation in embryoid bodies cultured in serum-free medium. BMP4- or Activin A-induced exit from the self-renewal state was impaired in the trisomy 12 hPSC sublines, with less upregulation of key transcription factor gene expression. As a consequence, hematopoietic and hepatic differentiation were also impaired in the trisomy 12 hPSC sublines. We reveal that trisomy 12 disrupts the genome-wide expression patterns that are required for proper mesendodermal differentiation.

F-3152

COMPARATIVE EVALUATION OF HORMONES AND HORMONE-LIKE MOLECULES IN LINEAGE SPECIFICATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Proficient differentiation of human pluripotent stem cells (hPSCs) into specific lineages is required for applications in regenerative medicine. A growing amount of evidence has implicated hormones and hormone-like molecules as critical regulators of proliferation and lineage specification during in vivo development. Therefore, a deeper understanding of the hormones and hormone-like molecules involved in cell fate decisions is critical for efficient and controlled differentiation of hPSCs into specific lineages. Thus, we functionally and quantitatively compared the effects of diverse hormones (estradiol 17-β (E2), progesterone (P4), and dexamethasone (DM)) and a hormone-like molecule (retinoic acid (RA)) on the regulation of hematopoietic and neural lineage specification. The sex hormone E2 enhanced functional activity of hematopoietic progenitors compared to P4 and DM, whereas RA impaired hematopoietic differentiation. In addition, E2 increased CD34+CD45+ cells with progenitor functions, even in the CD43– population, a well-known hemogenic marker. RA exhibited lineage-biased potential, preferentially committing hPSCs toward the neural lineage while restricting the hematopoietic fate decision. Our findings reveal unique cell fate potentials of E2 and RA treatment and provide valuable differentiation information that is essential for hPSC applications.

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F-3154

PROTEIN DISCOVERY PLATFORM USING DEER ANTLER AS A MODEL OF MAMMALIAN ORGAN REGENERATION

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Animal models can provide important insights into how to activate and control stem cells during tissue repair and regeneration. The robust regenerative capacity of some lower-order animals, such as planarian and zebrafish, has been well documented. Stem cell niches in mammals that drive complete organ regeneration are rare. Deer antler, however, is the only known mammalian model of complete organ regeneration based on stem cells. Antler neural crest-derived stem cells can be used as a model system to study the maintenance of a stem cell niche and activation and differentiation of stem cells. This model allows the discovery of proteins and mechanisms underlying the activation of stem cell niches involved in regeneration of a complex organ. In the present study, label-free quantification was used to investigate the protein profiles of antler stem cells under different stages of activation and included: dormant pedicle periosteum (N=3), growth centre (N=3), middle beam periosteum (N=3), and, as a control tissue, deer facial periosteum (N=3). PEAKS and Ingenuity Pathway Analysis software were utilized to interpret the proteomics data. Pleiotrophin (PTN), one of the key regulators during antler regeneration obtained by in-situ hybridization, with its main receptors were studied using immunohistochemistry (IHC). PTN's expression levels in antler stem cells with/without osteogenic media were measured by ELISA. Moreover, the location of the three commonly-accepted markers of mesenchymal stem cells (MSCs) - CD73, CD90 and CD105 - was also examined by IHC. Our research firstly confirmed the central role of stem cell activation in development of this mammalian organ by localizing the MSCs' markers within the antler growth centre. The functions played by PTN during rapid antler regeneration on both tissue and cell levels were preliminarily investigated. In addition, this protein discovery platform revealed that multiple biological processes and signalling pathways involving functional molecules participate in antler regeneration. This work sheds new light on the underlying mechanisms in mammalian stem cell-driven organ regeneration and provides further directions for mammal organ regeneration research.

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PLURIPOTENT STEM CELL: DISEASE MODELING

F-3158

THE EFFICIENT PRODUCTION OF FUNCTIONAL CHOLANGIOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

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Cholangiocytes, the epithelial cells that form the bile ducts of the liver express the cystic fibrosis transmembrane conductance regulator (CFTR), the chloride channel mutated in cystic fibrosis (CF). Dysfunction of CFTR in in the cholangiocytes of CF patients leads to impaired secretion and deposition of bile resulting in cholestasis. The ability to generate functional cholangiocytes from human pluripotent stem cells (hPSCs) offers the opportunity to model CF disease and to test the function of new CF drugs. We have previously described a protocol for the generation of hPSC-derived cholangiocytes in 3D organoids (Ogawa et al. Nat. Biotechnol. 2015) that yielded mature cholangiocytes, but was not amenable to cell expansion or high throughput drug screening. To improve the differentiation efficiency in monolayer cultures, we screened cytokines and small molecules based on flow cytometric analysis with the DHC5-4D9 antibody that stains mature cholangiocytes in the adult liver. We identified a combination of factors that promoted the development of hPSCs-derived DHC5-4C9+ cholangiocytes that express high levels of CFTR and contain primary cilia. Cholangiocytes generated from three different CF patient iPSC lines generated with this approach showed differential rescue of CFTR function in response to CFTR modulators using a high throughput FLIPR assay indicating that cells produced under these conditions can be used for drug efficacy studies. 3D cysts/ organoids were efficiently generated from monolayer cultures to apply the CFTR mediated swelling assay. As the secretory activity of mature

cholangiocytes is regulated by Ca2+ signaling, we next analyzed intracellular Ca2+ flux in the hPSC-derived cells to determine if they display this capacity. hPSC-derived cholangiocytes showed Ca2+ Influx in response to ATP or flow stimuli. Finally, to evaluate their potential to generate biliary structures in recipient livers, iPSCs-derived cholangiocytes were transplanted into TK-NOG mice. Six weeks following transplantation, multiple ductal structures comprised of human cholangiocytes were detected throughout the liver, indicating that these cells have some capacity to regenerate the biliary tree. Future studies will focus on evaluating the function of the engrafted ductal structures in these animals.

F-3160

MODELING ISCHEMIC HEART CONDITION WITH HUMAN EMBRYONIC STEM CELLS

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Ischemic heart disease is characterized by reduced of blood and oxygen supply to the heart muscle and it is the most common cause of death in most western countries. In 1986, Charles Murry discovered a phenomenon called pre-conditioning that causes adaptive changes in heart cells and protects them from extended ischemia damages. Here we aimed to use human pluripotent stem cells (hPSCs) in order to generate a human cell based model for ischemic heart disease and pre-conditioning. The first stage in our study was to differentiate hPSCs into beating cardiomyocytes. Next, we tested the effect of hypoxia on the differentiated cardiomyocytes cells. For this purpose, we grew the cells under hypoxic conditions for 4 hours (0% oxygen) or for 24 hours (1% oxygen). We found that the hypoxic conditions lead to the around seven fold increase in the secretion of the enzyme creatine kinase (Which is one of the markers to detect muscle damage and Myocardial infarction) compared to control cells (under normal oxygen conditions). Consequently, we showed that our system cells behave in the same way as cardiomyocytes from animal models and, in general, mimic the ischemic heart diseases. We will use this model to examine how to induce cardiomyocyte pre-conditioning by pharmacological materials in order to “rescue” the cells from an extended hypoxia. We hope that this system will serve in the future as a tool for screening various materials for pre-conditioning induction in people at high risk for ischemic heart diseases.

F-3162

KCNQ2-ENCEPHALOPATHY IN A DISH: MECHANISTIC INSIGHT THROUGH 2D AND 3D NEURONAL MODELS

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Mutations in the gene KCNQ2 are associated with both severe KCNQ2-encephalopathy (KCNQ2-E), characterised by neonatal seizures and developmental delay, and a self-limiting epilepsy syndrome called Benign Familial Neonatal Epilepsy (KCNQ2-BFNE), where development is completely normal. Interestingly, dominant negative (DN) or gain-of-function (GOF) variants are the underlying cause of KCNQ2-E, whereas haploinsufficiency results in KCNQ2-BFNE. KCNQ2 encodes for a subunit of the M-channel which is responsible for the resting membrane potential and controlling neuronal excitability. While the role of KCNQ2 in epilepsy is evident, as dysfunction of the M-channel affects neuronal excitability, its role in neurodevelopment is less well understood. To understand how KCNQ2 affects neuronal development, we generated 2D and 3D neuronal cultures (brain organoids) derived from human induced pluripotent stem cells (hiPSC). We compare 1 known GOF (R201H) and 3 known DN KCNQ2-E variants (D290G, A294V and R560W), as well as 2 KNCQ2-BFNE variants (K327G, R547W). By studying 2D neuronal co-cultures of excitatory and inhibitory neurons, generated via a fast overexpression protocol we aim to (1) discover a read-out system for both the epileptic and the neurodevelopmental features of KCNQ2-E, and to (2) develop a platform that can be used for future drug screening. The brain organoids are used to (3) study the effect of the mutations on the more heterogeneous and complex neuronal network, to discover the affected cell types and to find possible new drug targets. Currently used therapy for KCNQ2-E consists of symptomatic treatment, mainly directed at the (often difficult to treat) seizure activity. So far, no therapies influencing the developmental outcome of KCNQ2-E exist. To develop such a therapy, it is of utmost importance to shed light on the mechanism underlying the neurodevelopmental delay observed in these patients.

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F-3164

MODELLING NON-ALCOHOLIC FATTY LIVER DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of disorders caused by accumulation of excess dietary lipids within the liver. NAFLD is the fastest growing chronic liver disease and without intervention can progress to liver cancer and liver failure. Poor prognosis and limited therapeutic options for patients is hindered by the absence of robust, human specific platforms for modelling NALFD. To address this need we have created an in-vitro human model of NAFLD using hIPSCs derived from patients with familial NAFLD. In this study, we have improved our differentiation protocol to differentiate hIPSCs into hepatocytes that are more similar to primary human hepatocytes than foetal hepatocytes. We demonstrate higher expression of cytochrome P450 enzymes as well as many genes involved in a broad range of metabolic processes including lipid biosynthesis and metabolism. We have then applied this more advanced platform to model NAFLD by inducing lipid accumulation in healthy and familial-NAFLD-hepatocytes using varying concentrations of palmitic acid. Palmitic acid replicates a high fat, western diet and we observed both control and NAFLD-hepatocytes demonstrate key features of hepatic steatosis such as accumulation of lipids within the cytoplasmic space. Familial-NAFLD-hepatocytes when compared to healthy hepatocytes treated with palmitic acid show unique differences in their inflammatory and fibrotic gene expression profiles that may represent biomarkers for people with genetic predisposition to NAFLD. Familial-NAFLD-hepatocytes also show unique activation of signalling kinases that may represent novel interventions for NALFD. Finally, we also observe significant differences in metabolic profiles and mitochondrial functionality between healthy and NAFLD-hepatocytes that may represent new mechanisms that drive the spectrum of clinical observations in the pathogenesis of NALFD. Our study demonstrates the potential of NAFLD-hepatocytes to provide mechanistic insight and discovery of novel biomarkers and drug targets for NAFLD.

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F-3166

REPERTAXIN ATTENUATES THE COLITIC PHENOTYPE IN HUMAN-DERIVED ULCERATIVE COLITIS ORGANOIDS

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Ulcerative colitis (UC) is a relapsing inflammatory colonic disorder characterized by bloody diarrhea associated with sloughing of the colonic mucosa. Current ex-vivo models of UC inadequately recapitulate the complexity of the pathogenesis. We established an ex-vivo human derived organoid model (iHCOs) to compare the developmental process of UC to normal colon (NL). This model has both epithelial and mesenchymal components reflecting major phenotypic characteristics of UC. We hypothesize that the inflammatory phenotype of UC iHCOs affects their developmental pattern, specifically the aberrant formation of the adherens junction (AJ) vs. tight junction (TJ) structure in the epithelial barrier. Yamanaka factors were applied to reprogram NL and UC isolated fibroblasts into induced pluripotent stem cells (iPSCs) followed by directed differentiation to iHCOs. Downstream effects of the pro-inflammatory phenotype in UC iHCOs originating from the interaction of CXCL8-CXCR1/2 axis were studied in the context of development. Furthermore, the effect of repertaxin, a CXCR1/2 antagonist (3 weeks of exposure) on the development of UC iHCOs was investigated. We established robust conditions to differentiate iPSCs to iHCOs reflecting the major phenotypes in the tissue of origin including an elevated secretion of CXCL8, a pro-inflammatory chemokine, in the mesenchyme compartment of UC iHCOs (7.167x NL; $p < 0.005$). The transcriptome of UC vs. NL iHCOs revealed an inflammatory signature consisting of the AJ vs.TJ structure in the epithelial barrier. In response to repertaxin, UC iHCOs revealed more NL phenotype including morphology, cell junction and a decrease in the aberrant regeneration pattern of the epithelium (average of 20 µm decrease in epithelium thickness and a 65% decrease in the number of organoids vs. control; $p < 0.0001$). UC iHCOs recapitulate the phenotype of their primary tissues and are functionally responsive to CXCL8-CXCR1/2 axis inhibition. The creation of human derived organoids model will permit investigation not only of the developmental, pharmacologic, and genetic aspects of UC, but also, the role of inflammatory mediators in the microenvironment.

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F-3168

ESTABLISHMENT OF A DRUG SCREENING PLATFORM FOR FAMILIAL HYPERTROPHIC CARDIOMYOPATHY IN HIPSCS USING CRISPR/CAS9

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Programmable nucleases such as CRISPR/Cas9, ZFN, TALEN, and Cpf1 are versatile tools for editing specific genomic sites in hiPSCs. In particular, CRISPR/Cas9 efficiently enable the introduction of genetic changes into hPSCs in a site-specific manner, including correction of pathogenic mutations in patient-derived hPSCs and introduction of specific mutations into normal hPSCs. These approaches enable the generation of genetically matched, isogenic iPSC lines. In our study, we established a Familial Hypertrophic Cardiomyopathy (FHC) model from normal hiPSCs using a CRISPR/Cas9 genome editing tool. About 80% of for FHC is caused by genetic mutations in MYH7 and MYBPC3. In particular, an intronic 25bp deletion in MYBPC3 gene is a prevalent genetic mutation in Southeast Asia. It is estimated that 4% of the Southeast Asians carries this pathogenic mutation. To better understand and develop drugs for FHC, we generated FHC-hiPSC harboring an 25bp deletion in intron 32 of MYBPC3 gene by introducing carefully designed dual sgRNAs, Cas9 protein, and 195mer ssODN in normal hiPSCs. About 4% of CRISPR/Cas9 transfected hiPSCs showed bialleclic 25bp deletion in MYBPC3 gene by targeted deep sequencing and Sanger sequencing. These gene-edited hiPSCs exhibited normal pluripotency and stemness compared with genetically matched normal hiPSCs. Based on these results, we are going to find effective drugs from various clinically available drug libraries by differentiating MYBPC325bpΔ-hiPSCs into cardiomyocytes.

F-3170

TARGETED PRECISION THERAPIES FOR INFANTILE PARKINSONISM

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Most inherited neurodegenerative disorders are incurable, and often only palliative treatment is available. Precision medicine has great potential to address this unmet clinical need. We explored this paradigm in Dopamine Transporter Deficiency Syndrome (DTDS), caused by bi-allelic mutations in SLC6A3, which encodes the dopamine transporter (DAT). Patients present with early infantile hyperkinesia, severe progressive childhood parkinsonism and raised cerebrospinal fluid dopamine metabolites. The absence of effective treatments and relentless disease course frequently leads to death in childhood. Using patient-derived induced pluripotent stem cells (iPSCs), we generated a midbrain dopaminergic (mDA) neuronal model of DTDS. Patient-derived neurons exhibited impaired DAT activity and apoptotic neurodegeneration associated with TNFα-mediated inflammation and dopamine toxicity. Whilst DAT activity was ameliorated with the pharmacochaperone pifithrin-μ, the effect was mutation-specific. In contrast, Lentiviral gene transfer restored DAT activity and prevented neurodegeneration in all patient-derived mDA lines. To progress towards clinical translation, we utilized the knockout (KO) mouse model of DTDS, which recapitulates human disease, with reduced survival and parkinsonism features, including tremor and bradykinesia.

Neonatal intracerebroventricular injection of adeno-associated virus (AAV) vector provided neuronal expression of human DAT which rescued motor phenotype, lifespan and neuronal survival in the substantia nigra (SNc) and striatum.

F-3172

GENERATION AND FUNCTIONAL CHARACTERIZATION OF HUMAN MICROGLIA FROM INDUCED PLURIPOTENT STEM CELLS

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Microglia are immune-competent cells residing within the brain that play a critical role in maintaining immunological balance for normal brain function. As phagocytic cells, they are activated following clearance of pathogens and secreted proteins and respond by either exacerbating or dampening a pro-inflammatory environment. Primary human microglia are difficult to acquire and stably culture in vitro. We generated and characterized functional human induced pluripotent stem cell-derived microglia (iCellÂ® Microglia) from episomally reprogrammed iCell Hematopoietic Progenitor Cells (proprietary technology) under defined conditions. iCell Microglia express CD45, CD11b and CD33, and consistent with a microglial phenotype, they also express PU.1, CX3CR1, IBA, TREM-2 and P2RY12. The purity for all these markers is greater than 80% and the retain the expression of all these markers post cryopreservation. iCell Microglia were able to phagocytose opsonized bacteria and fibrillar AÎ² within a span of 2-60hrs and reveal a ramified morphology when treated with 5ÂµM Thiazovivin. Additionally, iCell Microglia secrete cytokines and chemokines including TNFÎ±, IL-8, IL-10, CCL2, CCL4, CCL3, CCL4, CXCL10, CXCL11, CXCl1, CXCl2 and CXCL10 when stimulated with LPS and interferon gamma. iCell Microglia will serve as a great tool for disease modeling and drug testing for neuroscience research.

F-3174

USING HIPSC DERIVED ASTROCYTES TO INVESTIGATE APOE EFFECTS IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the sixth leading cause of death in the United States and poses an increasing burden on the healthcare system. Following the first report of AD in 1907, numerous clinical trials addressing the classic amyloid hypothesis have failed to identify a cure. It is essential to understand the role of genetic risk factors such as the cholesterol transport protein apolipoprotein E (ApoE) in the more prevalent sporadic form of the disease. ApoE is highly expressed by astrocytes in the human brain, where it is found as the E2, E3 and E4 isoforms, with ApoE3/3 being the most common genotype. The less common E4 allele greatly increases the risk of developing sporadic AD and reduces the mean age of onset from 84 to 68 years, whereas the E2 allele has a protective effect. The mechanism of this effect remains unknown and ApoE has been implicated in amyloid peptide aggregation and clearance defects in animal models. Although the mouse model of AD has provided invaluable mechanistic insight and led to the identification of early biomarkers of the disease, translation into human therapy has been unsuccessful. With the advent of iPSC technology, it is possible to model AD using reprogrammed patient cells and study disease progression as it relates to the complex human genetic landscape. We differentiated six (two non-demented control, familial and sporadic AD) patient derived iPSC lines into neural progenitor cells (NPC) and subsequently into astrocytes on laminin and VDP, a synthetic substrate that supports the maintenance of cell types of the CNS. Our robust protocol generated mature astrocytes that secreted ApoE, exhibited calcium transience and were responsive to inflammatory stimuli. These astrocytes took up fluorescently labeled Aβ-42, indicative of their role in Aβ peptide clearance. They retained their astrocytic identity and functionality following cryopreservation. Additionally, the differentiation protocol was scaled up to a bioreactor system to generate mature functional astrocytes. We are currently studying the isoform specific effects of ApoE in astrocytes and neurons generated using isogenic lines derived from a familial AD patient. We plan to study the mechanism of ApoE isoform specific effects in co-culture systems of astrocytes and neurons derived from patient iPSCs.

F-3176

ALTERED NEURAL PROGENITOR PROLIFERATION ASSOCIATED WITH COPY NUMBER VARIANTS IN PSYCHIATRIC DISORDERS

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Copy number variations (CNVs) of certain chromosomal regions are closely associated with neurodevelopmental and neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia. Recent evidence suggests that alterations in neural progenitor cell (NPC) proliferation associated with particular CNVs may underlie the cause of abnormal brain development. However, we still have limited knowledge of the cellular and molecular profiles of NPCs during neurodevelopment that adds to the complexity of these diseases, which can affect multiple cell types in different regions of the brain. In order to investigate the cellular and molecular mechanisms underlying neuropsychiatric disorders, we have used NPCs and neurons derived from human induced pluripotent stem cells (hiPSCs). Here, we generated NPCs and neurons from patients diagnosed with a psychiatric disorder and particular CNVs to better model these disorders and identify molecular targets for intervention. We have found that proliferation rates of NPCs can be closely tied to specific CNVs, allowing us to group patients by particular disease phenotypes to help gain insight into the disruption of cellular and molecular pathways during development. Interestingly, these differences in NPC proliferation pathways mechanistically converge with neuroimmune mechanisms. To identify how these signaling roles play in cell survival and clearance, we have co-cultured our derived NPCs and neurons with immune-derived cells and assessed differences in cellular elimination. Many forms of psychiatric disorders such as ASD and schizophrenia are associated with abnormal synapse connections which could allude to improper elimination of NPCs that lay the groundwork for the brain architecture. Our study will be the first to systematically investigate novel neuroimmune-related mechanism(s) tied to ASD and schizophrenia as well as correlate a clinical phenotype to a cellular phenotype.

F-3178

A HUMAN IPS CELL FUNCTIONAL MYOGENIC MATURATION SYSTEM ENABLING ACUTE AND CHRONIC DISEASE MODELING OF DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is characterized by progressive muscle weakness and degeneration. There are currently no available treatments for the disease. Although the lack of dystrophin protein causes the disease, the mechanisms underlying its pathogenesis still remain unclear. While induced pluripotent stem cells (iPSCs) are a powerful tool for understanding the pathogenesis of intractable diseases, currently iPSCs-based disease modeling of muscular dystrophy is critically lacked. Such a model is essential to understand the cellular and molecular events during muscle weakness and degeneration. However, in vitro culture system enabling skeletal muscle cells to be mature enough to function is quite limited. In this study, we developed a MyoD expression-induced myogenic culture system combined with replating technique and electrical-field stimulating (EFS)-mediated muscle training on a 12kPa soft gel. Myotubes trained under this condition showed progressed myogenic maturation characterized by increased cell fusions and expression of slow and fast muscle markers. In addition, cells started contracting (excitation-contraction coupling) at day 14, and the sarcomere formation was organized by day 16. Furthermore, we developed an acute and chronic disease model of DMD using patient-derived iPSCs. In the acute model, dystrophic cells showed a significant reduction of muscle performance as well as induction of inflammatory responses and apoptosis. In the chronic model, dystrophic cells showed comparable muscle performance until day 20 compared to normal cells. However, the muscle performance of dystrophic cells started to drop down after day 22, indicating progressive muscle weakness and fatigue. Finally, we stablished a new model for culturing contracting skeletal muscle cells and recapitulated dystrophic phenotypes using patient-derived iPSCs in vitro. Further investigations will identify how muscle weakness and degeneration are initiated and progressed under the dystrophic condition, and design a strategy to develop a novel drug to treat the disease.

F-3180

STRUCTURE-FUNCTION STUDY OF NEUROGENIN3 DISEASE CAUSING ALLELES DURING HUMAN PANCREAS AND INTESTINAL DEVELOPMENT

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The transcription factor Neurogenin3 (NEUROG3) is required for formation of all endocrine lineages of the pancreas and intestine. Patients with homozygous or compound heterozygous NEUROG3 mutations are born with congenital malabsorptive diarrhea due to complete loss of enteroendocrine cells (EECs), whereas the impact on endocrine pancreas development varies in an allele-specific manner, ranging from neonatal diabetes to

later onset in life. These findings suggest a context dependent requirement for NEUROG3 in pancreas versus intestine that can only be accurately studied in an endogenous context. We utilized a NEUROG3-/- human pluripotent stem cell line to functionally analyze NEUROG3 mutants in the context of developing human pancreatic and intestinal tissue. NEUROG3-/- cultures failed to form any pancreatic or intestinal endocrine cells, demonstrating its requirement for human endocrine development. Endocrine specification was fully rescued by physiologic expression of wild type NEUROG3, and we used endocrine cell rescue to test the activity of mutants' effect on pancreatic and intestinal endocrine development. Most disease-associated NEUROG3 alleles had similar hypomorphic or null phenotypes in both tissues, whereas the S171fsX68 mutation had reduced activity in the pancreas but was largely null in the intestine. Molecular and biochemical studies revealed NEUROG3 variants have distinct molecular defects that affect protein stability, phosphorylation, heterodimer formation with E-proteins, and/or DNA binding. Moreover, we found that the NEUROG3 protein was highly unstable in the intestinal epithelium relative to the pancreas, possibly explaining the enhanced sensitivity of intestinal endocrine cell defects relative to the pancreas in patients with disease associated NEUROG3 alleles. In addition to revealing the molecular and developmental defects in patient-derived NEUROG3 mutations, these studies emphasize that studies of human mutations in the endogenous tissue context may be required to accurately assess structure-function relationships.

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F-3182

HUMAN INDUCED PLURIPOTENT STEM CELL DERVIED CARDIOMYOCYTES AS DRUG SCREENING PLATFORM OF CARDIAC LAMINOPATHY

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Lamin A (LMNA) is an essential component in nuclear matrix served to maintain chromosome and genome integrity. We hypothesize that premature heart block and DCM phenotype of lamnopathy is due to retarded mechano-sensitivity of lamin in nuclear structure via actin-polymerization pathway. We aim to investigate the arrhythmogenic effects of mechanosensitive ion channels in cardiac laminopathy by human induced pluripotent stem cell-cardiomyocytes (hiPSC-CMs) bearing LMNA mutations. hiPSC of a patient bearing LmnaR225X/WT, an isogenic corrected, LmnaWT/WT and mutated lines, LmnaR225X/R225X were created by CRISPR-Cas9. To investigate mechanical-related regulation, the hiPSC-CMs were treated with either F-actin depolymerization small molecules, latrunculin B (0.5 μM), cytochalasin D (10 μM), or myofilament desensitizer, blebbistatin (250 nM). To study subsequent force-frequency relationship (FFR) of electrical-contraction (EC) coupling, calcium transients and contractility were recorded under electrical field stimulation or stretching condition. Apart from action potential (AP) recording to study proarrhythmic risk related to APD prolongation, we further extrapolate our study to develop mechanism-based drug interventions using multielectric array (MEA) and optical mapping of cell monolayers in a high throughput way. Disorganization of F-actin in LmnaR225X/WT-hiPSC-CMs, interconnecting nuclear lamina to sarcomere and cell surface, reduced nuclear integrity and disrupted uniform force generation making the cells being overstretched. Latrunculin B and blebbistatin could resume the compromised EC coupling in the LmnaR225X/R225X cells with upstroke velocity significantly boosted by ~75% (n=5). Furthermore, electrical instability as reflected by loss in rate dependency (1-2 Hz) of the mutated group could be rescued by another small molecule for actin depolymerization, cytochalasin D (n=10). The ranolazine-sensitive APD30 and APD50 shortening by 57.5% and 27.4% (n=5) respectively in LMNA-mutated cells indicated the overactivated forward mode of the actin-dependent ion channels, sodium-calcium exchanger (NCX1). The channel might be overstimulated by signal from nuclear lamina leading to sodium influx, which triggers premature heart beats.

F-3184

THE EFFECTS OF HYPOXIA ON DIFFERENTIATION AND MATURATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITORS

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Disease specific human induced pluripotent stem cells (hiPSCs) have been expected as useful disease models for pathophysiological analysis and drug discovery. However, hiPSC-derived neural cells are often immature for the detection of phenotypes of adult onset neurological diseases. In this study, to obtain iPSC-derived mature neural cells, we examined the effects of hypoxia on differentiation and maturation of hiPSC-derived neural progenitors. First, hiPSCs (201B7) were differentiated into motor neuron (MN) progenitors under hypoxia (2% or 5%) or normoxia (20%), and were examined for neural induction. As a result, hiPSCs showed massive cell death under hypoxia and did not show any positive effects of hypoxia on neural induction. Then, we differentiated hiPSC-derived MN progenitors under hypoxia, and examined their differentiation and maturation. Hypoxia induced significant downregulation of the markers of immature neural progenitors, Nestin and Sox1, and upregulation of the markers of mature MNs, ChAT, Synaptophysin and PSD95, in an oxygen concentration dependent manner. We also examined functional maturation of iPSC-derived MNs by multielectrode array (MEA). Motor neurons differentiated under hypoxia showed significantly more mature electrical activity compared with those differentiated under normoxia. Finally, we investigated whether the hypoxia facilitate the detection of the phenotypes of disease specific iPSCs of adult onset neurdegenerative diseases. iPSCs established from patient of spinal bulbar muscular atrophy (SBMA) and control iPSCs were induced to MN progenitors, and differentiated under hypoxia or normoxia for up to four weeks. In comparison with control iPSC-derived MNs, SBMA iPSC-derived MNs cultured under hypoxia showed significant increase in the expression of CALCA and in the phosphorylation of c-Jun, known phenotypes in SBMA model mice, while those cultured under normoxia showed less or insignificant differences. Together, it is suggested that hypoxia is effective for promoting differentaition and maturation of iPSC-derived neural cells, and detecting phenotypes of disease specific iPSCs of adult-onset neurological disorders. Thus, hypoxia is applicable to varieties of pathophysiological analysis and drug screening using disease specific iPSCs.

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F-3186

LOSS-OF-FUNCTION DUE TO MODY1/HNF4A MUTATION ABROGATES HUMAN PANCREAS AND LIVER DIFFERENTIATION FROM MODY1-IPSCS

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Maturity onset diabetes of the young 1 (MODY1) is caused by autosomal dominant mutations in the HNF4A gene. HNF4A is a key determinant dictating foregut endoderm development which eventually forms the liver and pancreas. Based on knowledge from rodent development, we hypothesised that mutations in human HNF4A gene implicate foregut endoderm development that subsequently leads to the eventual loss of insulin secretory ability of the pancreatic β-cells in MODY1 patients. To understand the pathogenesis of MODY1 in humans, we differentiated patient-derived iPSCs into foregut endoderm/ hepato-pancreatic progenitors (HPP) and hepatic or pancreatic β-like cells in a stepwise manner. We observed that mutant HNF4A protein is sequestered in the cytoplasm in MODY1-HPP. The downregulated gene expression and the inability of mutant HNF4A protein to enter the nucleus suggest that these MODY1 patients could have a reduced HNF4A gene dosage. Genome-wide transcriptomic analysis revealed a diversion towards hindgut lineage evident by upregulation of numerous HOX genes in MODY1-HPP, while HNF4A and foregut genes were downregulated. HNF4A haploinsufficiency also led to the downregulation of several key hepatic and pancreatic markers. We then demonstrated that MODY1/HNF4A mutation resulted in a loss of transcriptional activation on selected hepatic and pancreatic gene promoters. Collectively, our patient-derived iPSC MODY1 model revealed that HNF4A haploinsufficiency results in downstream gene dysregulation and impairs foregut development and its derivatives. These effects may propagate to long-term pathological consequences observed in MODY1 patients.

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F-3188

PATIENT IPS CELL DERIVED NEURONAL CELLS AS DISEASE MODELS FOR POMPE DISEASE

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Pompe disease is an autosomal recessive disease caused by a deficiency of acid alpha-glucosidase, a lysosomal enzyme involved in the breakdown and recycling of glycogen. Although the clinical manifestations largely reflect on the skeletal and cardiac disorders, glycogen accumulation in the brain, brainstem nuclei and anterior horn cells has also been observed in the autopsy studies. To facilitate drug discovery and model disease pathophysiology, we generated neural stem cells (NSCs) and neuronal cells from Pompe patient-derived induced pluripotent stem cells (iPSCs). The NSCs exhibited characteristic disease phenotypes with deficiency of acid alpha-glucosidase, accumulation of glycogen and neutral lipid within cells. Using this NSCs disease model, we observed the reduction of lipid accumulation by the treatment of two small molecular compounds: hydroxypropyl-β-cyclodextrin (HPβCD) and δ-tocopherol. Our data demonstrate that the patient-derived NSCs can be used as a cell-based disease model to study disease pathology and perform compound screening for drug development.

REPROGRAMMING

F-3190

A TRANSIENT SWITCH IN THE SWI/SNF SUBUNITS BCL11A AND BCL11B ORCHESTRATES REPROGRAMMING TOWARD PLURIPOTENCY AND MALIGNANCY

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A key challenge for developing organisms is to establish cell identities by restricting plasticity. In contrast, such plasticity is regained during the conversion of somatic cells into induced pluripotent stem cells (iPS) by Oct4, Sox2, Klf4 and c-Myc (OSKM). Malignant transformation also implies cellular identity loss and the acquisition of embryonic features, leading to the hypothesis that oncogenes such as c-Myc and K-Ras induce a direct reprogramming process that accompanies the acquisition of the tumorigenic phenotype. For these reasons, a better characterization of cellular identity loss occurring during iPS cells generation and malignant transformation might have profound implications for both regenerative medicine and cancer biology. The aim of this study is to perform a comparative and comprehensive analysis of the early steps of pluripotent reprogramming, induced by OSKM, and malignant transformation triggered by c-Myc and Kras in MEF. RNA-seq analysis of FACS sorted reprogramming intermediates led us to demonstrate that, in the early days of both iPS and malignant cells emergence, loss of cellular identity is correlated with a switch in the expression of the transcription factors Bcl11b and Bcl11a, components of the SWI/SNF complex. Gain- and loss-of-function approaches revealed that this balance controls cellular identity loss and the emergence of pluripotent and malignant reprogrammed derivatives. Moreover, the use of Bcl11-reporter mice led us to (i) define a molecular roadmap of reprogramming and (ii) characterize novel and rare “intermediate states” emerging in the early days of both processes at the transcriptomic and epigenomic levels. Collectively, this project shed light on molecular mechanisms that constraining reprogramming and therefore iPS cells generation and tumorigenesis.

F-3192

MANIPULATING CELL FATE TO IMPROVE TISSUE REGENERATION DURING AGING

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Aging is a complex process modulated by genetic and epigenetic factors and marked by progressive appearance of age-related pathologies and decrease of cell and tissue regenerative capacity. Our objective is to delay these effects by inducing global rejuvenation or increasing the organismal regenerative capacity. In 2007, Dr. Yamanaka showed that human fibroblasts can be converted into pluripotent stem cells by inducing the expression of four transcription factors. Ourselves, we showed in 2011 that senescent cells which are accumulating in aging organisms can be reprogrammed and regained a rejuvenated physiology and metabolism. Our hypothesis is that in vivo induction of a transient reprogramming process could erase the marks of cellular aging and improve tissue regeneration to restore altered cell physiology and delay tissue aging and deleterious consequences. For this, we use a transgenic mouse model to control the induction of the expression of the reprogramming factors in order to reprogram the cells of the whole organism. These mice also recapitulate the human phenotype of Hutchinson-Gilford progeria syndrome, mimicking accelerated physiological aging. Our results showed a significant increase in life expectancy and improvement in tissue integrity related to activation of tissue regeneration. The impact of controlled transient reprogramming on age-related pathologies has also been monitored and a protective role in osteoarthritis and osteoporosis has been observed in these animals at eight months of age. Also, a two-week low-dose treatment, at two months of age, allows for increased life expectancy in later ages while decreasing phenotypic markers associated with aging suggesting a memory effect of transient reprogramming maintained along the entire life. Cellular and molecular mechanisms were examined in vitro. We highlighted, in the context of transient reprogramming, the implication of Foxo3a, an actor of the stress response, the involvement of DNA repair mechanisms and the activation of autophagy. We also initiated a global analysis of gene expression on cells isolated from our murine models, and experiments to determine the role of endogenous stem cells. This work demonstrates how transient cellular reprogramming reduces the negative impacts of aging at the organismal level.

F-3194

INFERRING INDIVIDUAL CELL TYPE CHIP-SEQ PROFILES FROM POPULATION CHIP-SEQ AND SINGLE CELL RNA-SEQ DATA

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Histone modification profiles are informative for understanding gene regulation for a given cell type. However, current histone modification profiling technologies yield averaged profiles of a mixture of cells that mask individual cell type-specific profiles. This is a major shortcoming given that cellular variability is inherent in most cell populations. The current method for single cell ChIP-seq has low per-cell sequencing coverage and exhibits a signal-to-noise ratio too high for meaningful interpretation. Here, we present a method that predicts chromatin maps at the single cell type level by jointly modeling population epigenetic data with single cell transcriptomics. We train a machine learning model to learn relationships between gene expression and chromatin features using a compendium of data containing histone modification maps and gene expression profiles. To deconvolve the histone profiles of a complex mixture of cells we integrate two types of models that account for within- and across-cell type relationships. The within-cell type model leverages information about expression of nearby genes, and the across-cell type model predicts ChIP-seq profiles from the expression of all genes. Using these relationships, we can infer histone modification profiles at the individual cell type level using single cell expression data. This allows us to annotate difference in cis-regulatory sites, such as enhancers, between cell types from complex population of cells. We apply this framework to the context of reprogramming somatic cells into induced pluripotent stem cells (iPSCs), a process limited by low conversion efficiency potentially due to epigenetic barriers that are difficult to overcome. It is unclear which chromatin changes allow some cells to progress towards pluripotency. Our preliminary results suggest our model can predict cell type-specific enhancers within somatic and iPSC populations solely from single cell gene expression data. Utilizing these different data types will provide a foundation for understanding the regulatory program at the single cell or subpopulation level.

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F-3196

ENHANCED CARDIAC REPROGRAMMING BY ELECTRICAL STIMULATION USING MICROPILLAR ARRAY

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Cardiac diseases including cardiomyopathy, arrhythmias, and myocardial infarction are life-threatening. Cellular reprogramming technology has been attracting great attention for patient-specific cell therapy and drug screening. In particular, direct reprogramming can readily turn somatic cells into other lineage cells in a short period without using stem cells. However, there is a need to be improved in terms of conversion efficiency and maturation. In this study, we applied electrical cues to promote cardiac reprogramming and generation of induced cardiac spheroids. We confirmed upregulated cardiac gene expressions in cardiac reprogramming as 3D spheroids, compared with reprogramming under 2D condition. Induced cardiac spheroids also showed autonomous beating, which could be facilitated by isoproterenol. Direct electrical stimulation using micropillar array to induced cardiac spheroids could further promote differentiation and maturation of cardiac lineage cells in the spheroids. Our study provides a new engineering platform for electrical stimulation to improve direct reprogramming, which would be applied for understanding disease mechanism and testing potential drugs.

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F-3198

INDUCED SENSORY HAIR CELLS: UNDERSTANDING AND ENHANCING REPROGRAMMING EFFICIENCY, SPECIFICITY AND MATURITY

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Deafness affects 360 million people worldwide, the leading cause of which is loss of sensory hair cells in the cochlea. Hair cells are scarce and fragile, making research studies difficult. Here we used direct cellular reprogramming to generate induced sensory hair cells (iHCs) in vitro. Direct reprogramming is an indispensible technique for studying rare and inaccessible cell types but comes with its own limitations of efficiency and heterogeneity. We used a combinatorial analysis of hair cell specific transcription factors (TF), and identified an optimal cocktail of four TFs for reprogramming mouse fibroblasts towards a hair cell fate. RNA sequencing of iHCs indicates a robust recapitulation of the transcriptional profile of primary hair cells. Further bioinformatics analysis revealed that iHCs bypass a progenitor stage, yet can still up regulate 72% of primary hair cell genes. Interestingly the genes that fail to activate fall into known developmental pathways of hair cell differentiation (ie. Notch signaling). To understand if these unique differences are attributable to failure to activate primary hair cell enhancer networks we performed ATAC sequencing. Despite bypassing normal developmental pathways, the iHCs open >90% of the primary hair cell enhancers. This correlated with significant up regulation of the putative gene targets of these enhancers. Taken together these results indicate a need to examine whether the failed developmental pathways are limiting the reprogramming efficiency, specificity, and maturity of iHCs. To address this question we are using an inducible polycistronic vector for reprogramming to ensure proper stoichiometry of the TFs, single cell sequencing to better illustrate the temporal changes and maturity achieved in reprogramming, and ChIP sequencing on the fibroblasts that fail to reprogram in order to profile histone marks that may be acting as barriers to reprogramming. Ultimately as we push towards a bona fide iHC model the goal is to provide the biological material necessary to perform high throughput studies for protective and regenerative initiatives of sensory hair cells.

F-3200

EPIGENETIC MECHANISM OF DIRECT CONVERTING ADULT HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS INTO IMSCS USING A NOVEL REPROGRAMMING SYSTEM

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Bone marrow and adipose tissue are the major sources in obtaining mesenchymal stem cells (MSCs); unfortunately, the acquisition process requires invasive surgery. Previously we reported that human cord blood hematopoietic progenitors (CB-CD34+ cells) could be directly converted into iMSCs with a single factor, OCT4. To expand this finding, we attempted to generate patient-specific iMSCs from adult peripheral blood mononuclear cells (PBMCs) using a modified oriP/EBNA1-based episomal vector (EV) system. After screening multiple combinations of factors, we identified a cocktail containing five factors (including OCT4 and SOX9) that was highly efficient in reprogramming adult human PBMCs into iMSCs with trilineage differentiation potential in vitro. More importantly, SOX9, unlike SOX2, restricts cell fate to MSC without going through the pluripotent stage, evidenced by 0% TRA-1-60+ cells during the reprograming. Inclusion of SOX2 led to 1-2% TRA-1-60+ cells, even when cultured in the MSC medium. Factor omission studies showed that without OCT4, MSC-like colonies were observed and the reprogramming efficiency was reduced by

only 20-30%. However, four factors (without OCT4) generated iMSCs showed significantly impaired multilineage differentiation potential. In particular, the expression of osteogenic related genes like GBLAP, SP7, and ALP was reduced by 40-80%. To understand the epigenetic mechanism of iMSC reprogramming involving OCT4, we analyzed chromatin accessibility by ATAC-seq, DNA methylome by RRBS and transcriptome by RNA-seq and single-cell RNA-seq. We found that without OCT4, the 4F-derived iMSCs were hypermethylated ($P<0.0001$) at the transcription start sites, suggesting that OCT4 is critical for reshaping the DNA methylation pattern during reprogramming. ATAC-seq results confirmed a distinctive chromatin accessibility pattern when omitting OCT4. Overall, we developed an efficient EV-based reprogramming system containing the factors for generating integration-free patient-specific iMSCs from PBMCs, and there was an epigenomic reprogramming orchestrating with transcriptomic changes during the cellular reprogramming. Our data also suggest that transient expression of OCT4 may play a critical role in promoting the direct reprogramming by reshaping the global epigenome.

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F-3202

BI-POTENT PROGENITOR CELLS GENERATION THROUGH SMALL MOLECULE-MEDIATED HUMAN HEPATOCYTES REPROGRAMMING

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Given the shortage of organ donors, cell-based regenerative medicine can prove useful and ground-breaking in terms of gene and/or stem cell therapy for patients suffering from end stage liver disease. Unlike mouse bipotent progenitor cells that have been proved to provide ease of isolation and long-term expansion from terminally differentiated mouse hepatocytes, the challenge remains with adult human hepatocytes. Using hepatocytes from diseased and healthy livers in combination with two small molecules and growth factors, we report a new technique to generate patient-specific hepatic progenitor cells from human hepatocytes. Three days after the treatment of hepatocytes with small molecules and growth factors, a key driver of hepatic progenitor cell activity, generated small polyglonal cells with expansion properties which co-expressed hepatic progenitor cells and lineage specific marker genes. These chemically derived human hepatic progenitor cells (hCdHs) showed a retention in normal phenotype and karyotype when passaged to at least 10 passages. They were also able to differentiate into functional hepatocytes and biliary epithelial cells in vitro. Furthermore, molecular similarity between hCdHs and human hepatoblasts was seen through a next-generation sequencing analysis. Finally, the transplantation of hCdHs into immunocompromised mice with diseased liver showed effective restoration and repopulation capacity of hCdHs. In conclusion, hCdHs provide a safe novel tool that permits expansion and genetic manipulation of patient-specific hepatic progenitor cells to study regeneration and repair of diseased liver.

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F-3204

REPROGRAMMING HUMAN SOMATIC CELLS DIRECTLY TO NAIVE IPSCS USING NON-MODIFIED MRNAS AND MIRNAS

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Pluripotent stem cells (PSCs), including induced PSCs (iPSCs), hold great potential for both basic research and biomedical applications due to their unlimited self-renewal and differentiation potential into multiple somatic cell lineages. Human epiblasts in the embryo, the founder of the fetus and the origin of PSCs, develop continuously from inner cell mass to a single-layered epithelial disc, which is primed for gastrulation. Human PSCs representing the more primed post-implantation epiblast have been well studied. We have recently reported generation of human naïve stem cells from dissociated inner cell masses and primed human PSC lines. These cells have molecular signatures related to emerging naïve epiblast. Here we report establishment of transgene-free human naïve iPSCs by direct somatic cell reprogramming using an RNA-based reprogramming method

that combines a novel cocktail of synthetic, non-modified reprogramming [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28 (OSKMNL)] and immune evasion mRNAs [E3, K3, B18-R] with reprogramming-enhancing mature, double-stranded microRNAs. This unique combination of different RNAs results in a fast, highly efficient and robust reprogramming protocol that can generate not only iPSCs directly from somatic cells but also naïve iPSCs. mRNA is the most favorable technology for reprogramming of somatic cells into naïve iPSCs since it does not integrate into the genome and therefore overcomes many limitations for the translational of the overall iPSC technology for clinical applications. We validated this technology on multiple adult human fibroblasts and endothelial progenitor cells (EPCs) derived from adult blood. Clonal naïve RNA-iPSC lines can be expanded in culture for more than ten passages and retain a normal karyotype. They express a panel of naïve specific transcription factors and surface markers. Direct production of transgene-free naïve iPSCs offers a new platform to evaluate differentiation propensity of naïve versus primed pluripotency.

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F-3206

BETA-HYDROXYBUTYRATE PREVENTS VASCULAR SENESCENCE THROUGH HNRNP A1-MEDIATED UPREGULATION OF OCT4 IN VASCULAR CELLS

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Here we report that β-HB promotes cellular quiescence in vascular cells, which significantly inhibits both stress-induced premature senescence and replicative senescence through p53-independent mechanisms. Further, by using a ligand fishing pulldown approach, we identified heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) as a direct binding target of β-HB. This binding of β-HB to hnRNP A1 markedly enhanced hnRNP A1 binding with Octamer-binding transcriptional factor (Oct) 4 mRNA. The binding of hnRNP A1 with Oct4 mRNA stabilizes Oct 4 mRNA and Oct4 expression. Finally, we found that both fasting and intraperitoneal injection of β-HB in vivo upregulates Oct4 and Lamin B1 in both vascular smooth muscle and endothelial cells in mice. We conclude that the ketone body β-HB exerts anti-aging effects in vascular cells by upregulating an hnRNP A1-induced Oct4-mediated Lamin B1 pathway.

TECHNOLOGIES FOR STEM CELL RESEARCH

F-3208

THE PROGENIES OF HUMAN OR CHIMP PLURIPOTENT STEM CELLS DISTURB INTERSPECIES CHIMERA DEVELOPMENT

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We have been working on generating human organ in animal body by injecting pluripotent stem cells (PSCs) to organ deficient animal embryos. Previously, we generated mouse pancreas in mouse-rat chimera by injecting mouse embryonic stem cells into apancreatic rat embryos. Diabetic mice transplanted with generated mouse pancreatic islets showed normal blood glucose level throughout their life. Despite the various advantages of this approach, difficulty to create human-animal chimera prevent human application. To solve this problem, we used chimp iPS cells, as an alternative to human PSCs, and screened chimera forming cells with xenogeneic animals. Chimp-mouse and chimp-pig chimeric fetus were obtained by preventing apoptosis in chimp iPS cells, however, all of developed interspecies chimeric fetus showed low chimerism compared to rat-mouse chimeras. Consistent to our results, several reports succeeded to generate human-animal chimeric fetus also showed low chimerism. The reason why chimp (or human) PSC derived chimeras showed poor chimerism is remain unknown. Here we created chimp-mouse chimeras by forced expressing BCL2 in conventional chimp iPSCs and analyzed at various developmental stages. Highly chimeric embryos disappear before E8.5 and only poorly chimeric embryos could develop further, which is unlikely to rat-mouse chimeras. Chimp-mouse highly chimeric embryos showed severe malformation even from egg cylinder-stage, and then resulted in degeneration or abortion at later stages. Consistently, chimp-pig chimeric fetus developed to later stage showed lower chimerism than degenerated fetus. It was also consistent with human iPSC-derived chimeras with mouse embryos. These observations suggested that the reason only poorly chimeric fetus were observed was that human (or chimp) iPSC-derived progenies disturb host animal's development and highly chimeric embryos could not survive. Some sort of modification would be required to harmonize human PSC-progenies in mouse or pig embryo development and obtain highly chimeric animals, which maximize the chance to generate whole human organ in animal body.

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F-3210

MULTIPLEXED AUTOMATED IMAGING ASSAYS FOR COMPOUND TESTING USING INDUCED PLURIPOTENT STEM CELL-DERIVED CELL MODELS

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There is an increased need for expanding variety and complexity of cell-based assays for biologic research and drug discovery. Stem cell-derived cells and tissues become an increasingly attractive alternative to traditional in vitro and in vivo testing in pharmaceutical drug development and toxicological safety assessment. In this study, we used human iPSC-derived cardiomyocyte and neuronal cell models to develop functional and morphological readouts for testing effects of different compounds in a multi-parametric assay format. The effects of selected compounds on cardiac physiology were monitored by measuring spontaneous contractions of stem cell derived cardiomyocytes. Kinetic patterns were characterized by measurements of calcium ion flux using time-lapse recording of the fluorescence intensity of calcium ion sensitive dyes. In addition, the effects on cell viability and mitochondria integrity were monitored at the end-point using additional readouts. We used automated cell imaging and analysis with the ImageXpress Pico Imaging System to simultaneously determine calcium oscillation frequency, cell viability, cytoskeletal integrity, apoptosis, and mitochondrial function. We demonstrated the effects of several cardio-active and cardio-toxic compounds on amplitude and the frequency of calcium oscillations and determined EC50 values for the effects on calcium oscillations, as well as EC50 values for cell viability and mitochondria potential. For neuro-spheroids automated imaging was used to evaluate the number of live cells and monitor calcium oscillations using time-lapse imaging. Multiplexed assessment of different readouts provides additional insight of the mechanisms of action of various compounds. The methods were characterized using a set of cardio-active drugs, neurotransmitters, and selected neurotoxic or cardiotoxic compounds. Overall, our results demonstrate how a variety of assays can be utilized for quantitative screening of chemical effects in iPSC cardiomyocytes and neuronal models and enable rapid and cost-efficient multidimensional biological profiling.

F-3212

DEVELOPMENT OF NOVEL QUALITATIVE AND QUANTITATIVE METHODS TO ASSESS MATURATION STATUS OF CARDIOMYOCYTE DERIVED FROM MOUSE PLURIPOTENT STEM CELLS

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Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) are a promising cell source for research and medical applications. Although PSC-CMs are efficiently obtained from cardiac differentiation using conventional protocols, but these cells solely show fetal-like phenotype and arrest at embryonic state of maturation instead of developing to adult cardiomyocytes (CMs). Presently, generation of fully matured CMs in a reasonable time is still a challenge. A primary limitation in the production of fully mature CMs is a lack of applicable high-throughput technologies providing distinguished parameters for determining how CMs mature and measuring the maturity of CMs. To this end, we aimed to develop novel qualitative and quantitative methods to assess the effects of hormone agonists on CM maturation. For a qualitative method, we generated a maturation reporter line, that is a PSC-line with the RFP gene knocked-in so that it is fused to a sarcomere gene and upregulated postnatally. Unless cells differentiated to mature CMs, no RFP could be detectable. After 2 weeks of CM differentiation, weak RFP became visible in approximately 40% of PSC-CMs. In this research, we first examined if hormone agonists could enhance CM maturation using the reporter line. We found that hydrocortisone added to the culture increased Myom2-RFP intensity, whereas the RFP signal was significantly reduced in triiodothyronine (T3)-treated condition. These data indicated that these agonists affected the maturation of PSC-CMs. For a quantitative method, we collected a reference transcriptome dataset from embryos to adults with a cost-effective transcriptome method with next generation sequencing. Next, we compared transcriptome of the treated PSC-CMs to that of in vivo counterparts and quantitatively assessed the maturation status according to microarray-based method as we demonstrated in our previous paper (Uosaki, Cell Rep, 2015). We found that T3 enhanced CM maturation to the maturity equivalent to that of postnatal day 7 to 14 and promoted isoform switches of sarcomere proteins, as well as increased expressions of CM maturation markers. In this study, we highlight T3 as a hormone that potentially enhances CM maturation.

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F-3214

HARNESSING HIERARCHICAL NANOTOPOGRAPHIES TO DIRECT STEM CELL FATE

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Manipulation of cell fate is a critical process in regenerative medicine and cell-based therapies. Strategies and methods to maintain stemness of stem cells and direct them into specific cell types are ongoing challenges in cell biology. To date, a number of studies have reported that biophysical stimulation in the form of surface nanotopographies can influence stem cell attachment, proliferation, and differentiation. In addition, specific surface nanotopographies can enhance efficiency of cell reprogramming and maintain stemness of stem cells. While biochemical cues are generally effective, biophysical cues have other advantages such as scalability, cost effective, longer lifetime, and easily to be defined. In our group, we dedicated

to fabrication of various surface nanotopographies including nanogrooves, nanopillars, nanopores, and binary colloidal layers using different nanotechnologies including electron beam lithography, reactive ion etching, soft lithography, and self-assembly. Having these cell culture tools we were able to direct stem cell fate into desired outcome. We believe that combining biochemical and biophysical stimulation has the greatest potential to generate functionally mature cells at a scalable and inexpensive way for diverse applications in regenerative medicine and cell therapy.

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F-3216

EXPANSION OF MESENCHYMAL STEM CELLS ON MICROCARRIERS WITH OPTIMIZED PHYSICAL AND CHEMICAL PROPERTIES

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Mesenchymal stem cells (MSCs) are attractive avenues for cell therapeutics due to their immunomodulatory capabilities and ability to secrete trophic factors. However, current culture techniques inhibit their translation into clinical medicine in part due to the cost of growth media. Serum supplementation of cell culture media can be significantly reduced with three-dimensional bioreactor culture, easing MSC culture into clinical medicine by reducing cost. Previous work has shown that gelatin-derived materials can be generated with tunable mechanical properties and the capability to release growth factors in a time-dependent manner. Therefore, the purpose of these experiments is to optimize expansion of induced pluripotent stem cell-derived MSCs (iPS-MSC) on gelatin-methacrylamide (GelMA) microcarriers. Monodisperse GelMA microcarriers with tunable stiffness are mass-produced using a custom, inexpensive microfluidic device that operates under biocompatible conditions and can be run in parallel for scale-up. Using various bioreactor configurations, future experiments on GelMA microcarriers include coupling and encapsulating bioactive factors for release, and quantifying expansion and downstream therapeutic properties of iPS-MSCs on microcarriers with various parameters. Finally, a parametric computer model will be prepared to optimize the expansion of iPS-MSCs while minimizing the amount of media supplementation and volume requirements. Collectively, this proposed method aims to reduce cell culture costs by limiting the amount of required supplements and materials without sacrificing cell quality.

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F-3218

DISCOVERING THE ROLE OF LONG NON-CODING RNAS DURING PANCREATIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS USING DCAS9 TECHNOLOGY

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From ultra-deep RNA sequencing studies, non-coding RNAs (ncRNAs), including long non-coding RNAs (lncRNAs), have been identified throughout the human genome. lncRNAs are thought to play roles in embryonic development, dosage compensation, disease regulation, immune response and cell regulation. Through a small number of studies on specific lncRNAs, it has been demonstrated that lncRNAs can operate through diverse mechanisms. However, only a small fraction of known lncRNAs have been thoroughly studied and characterized. Recent advances in CRISPR technology has enabled programmable control of gene expression for studying gene function. The nuclease dead CRISPR-Cas9 (dCAS9) enzyme can be directed to the promoter of a specific target genes to either block the initiation of the transcription (CRISPRi), or, when fused to a transactivator domain (e.g. VPR), to promote transcriptional initiation (CRISPRa). However, many of the existing CRISPRi/CRISPRa systems do not work well in hESCs due to gene silencing. This study aims to optimize these systems in hESCs to determine the functions of lncRNAs during pancreatic differentiation. Using a TALEN assisted gene-trap, doxycycline-inducible dCAS9 and dCAS9/VPR constructs were integrated into the “safe harbor” AAVS1 locus. hESC clones carrying the correct integration of the construct into the AASV1 safe harbor without random integration events were identified. Induction of dCas9 mRNA and protein expression in undifferentiated and differentiated hESCs was used to select optimal clones. Constructs for constitutive expression of guide RNAs (gRNAs)

targeting the promoters of lncRNAs and transcription factors (TFs) differentially expressed during pancreatic differentiation were introduced into these clones. Using this system, we are able to overexpression and knock-down selected target transcripts in undifferentiated hESCs. Ongoing studies are designed to disrupt or promote gene expression at specific time points during differentiation and study the effects on the differentiation process. In addition to delineating the functions of the specific lncRNAs and TFs targeted in these experiments, this study aims to develop a more robust system for programmable manipulation of gene expression in hESCs, particularly during directed differentiation.

Funding Source: California Initiative for Regenerative Medicine

F-3220

HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELL EXPANSION IN XENO-FREE CONDITIONS FOR CLINICAL-GRADE APPLICATIONS

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The ability to rationally propose xeno-free culture environments to expand human multipotent mesenchymal stromal cells (hMSCs) for clinical-grade applications would represent a significant progress in stem cell-based therapies. Nevertheless, rational selection of the appropriate source of nutrients and growth factors for cell maintenance and proliferation requires a deeper understanding of hMSC response to the molecules present in supplemented media. In particular, fetal bovine serum (FBS) has been broadly accepted as a growth factor source for hMSCs. However, its use is problematic because of its xenogeneic origin and biosafety concerns, especially, for cell-based therapies. A number of alternatives to FBS have been explored, including human platelet-derived growth factors (PDGF). Although several studies have evaluated the impact of PDGF on hMSC proliferation and differentiation, few studies have assessed their influence on relevant hMSC processes, such as metabolism and gene expression. Thus, we began to address this challenge by culturing human adipose-derived MSCs (hAD-MSCs) in media supplemented with 10% PDGF or FBS in order to characterize them and evaluate its effect on cell metabolism and gene expression of associated regenerative factors, during cell expansion. Following confirmation of the expression of stem cell surface markers, hAD-MSCs were differentiated into osteogenic and adipogenic lineages. We found a significant osteogenic differentiation in the presence of PDGF without using differentiation medium ($p<0.05$). Likewise, compared to FBS, PDGF induced a significant two-fold increase in the specific growth rate ($p<0.02$). Nevertheless, in terms of cell metabolism profile, no significant differences were found between both culture conditions. Furthermore, significant differences in collagen I and angiopoietin 2 expression were observed between both conditions ($p<0.01$). The present results demonstrate that PDGF influence hAD-MSC behavior; in particular, their metabolic profile suggested that the required carbon source for cell growth could be different from glucose that may be present in the media. Our results advance our understanding of the processes associated with MSC responses for clinical applications.

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F-3222

ANALYZING HUMAN PLURIPOTENT STEM CELL FATE DYNAMICS BY LIVE, MULTI-REPORTER HIGH CONTENT MICROSCOPY

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Stem cells integrate a variety of dynamical inputs – the activity of multiple transcription factors, morphological and proliferative properties, cell-cell interactions and others – in order to allow the formation of correctly differentiated, organized and stratified tissues. Dissecting how those various inputs together impact on the fate of individual cells has been challenging, due to the limited number of reporters allowing simultaneous monitoring of those inputs within live cells. To overcome this limitation we have developed ORACLE, a novel and versatile fluorescent tool allowing live observation of multiple cell fate reporters at the single-cell level. ORACLE is a genetically-encoded two-colour fluorescent reporter - whose expression can be controlled by transcription factor promoters of choice - that localizes to the nuclear envelope, making it distinguishable from and compatible with conventional nuclear-localized reporters. We validate the tool with constitutive and differentially regulated promoters, and show that ORACLE can be used with to monitor the dynamical changes of multiple transcription factors within individual human ESCs, as they undergo differentiation. We further demonstrate

that co-expression of ORACLE with fluorescently-tagged histone H2B or the FUCCI cell cycle reporter allows to directly observe how transcription factors and dynamical proliferative features co-vary in individual cells through time, which has been impracticable until now. Thus, ORACLE provides an important new tool to improve live interrogation and dissection of the dynamics of cell fate establishment with single cell resolution.

F-3224

RAPID EXPANSION OF MESENCHYMAL STEM CELLS USING OPTIMIZED MEDIA SUPPLEMENTED WITH HUMAN PLATELET LYSATE PLTMAX® OR PLTGOLD®, SUITABLE FOR CGMP EXPANSION AT LARGE SCALE

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Mesenchymal Stem Cells (MSCs) are a promising candidate for tissue engineering and regenerative medicine applications. They are currently being used in hundreds of clinical trials and have been safely administered to thousands of patients with an expanding body of evidence of therapeutic efficacy. Mill Creek Life Sciences' PLTMax®, derived from normal human donor platelets, arose as an alternative to FBS to be used as a supplement for in vitro expansion of human cells used in clinical applications. It is the only media supplement to date demonstrating clinical cultures of MSCs with long term genetic fidelity, rapid expansion and potent clinical activity. PLTMax® is being used worldwide in over 30 clinical trials including Phase I, II and III. Our latest product PLTGold®, is a second-generation human platelet lysate that does not require the addition of heparin to remain clot free, thus providing a fully xenogeneic free alternative supplement. In traditional monolayer expansion systems, we have demonstrated growth of adipose derived and bone marrow derived MSCs using an optimized, fully cGMP compliant culture system. We have found that MSC NutriStem® XF Basal Medium (Biological Industries USA, Inc, Cromwell, CT) supplemented with PLTMax® or PLTGold® exceeds the performance of other conventional media products, obtaining up to 5x108 cells (5 times more cells than with other commercially available products) in only 5 passages. Furthermore, when we use MSC NutriStem® XF Complete Medium supplemented with PLTMax® or PLTGold®, we obtained up to 2x1010 cells (200 times more cells than with other commercially available products) in only 5 passages. Cells grown in these media maintained MSC phenotype and capacity to undergo differentiation. The effective transfer into the clinic of allogenic cell therapies using MSCs will depend predominantly on the development of large scale and cost effective manufacturing platforms that allow production of functional cells at the scale required to meet clinical demand. We will also present the extension of these studies using this optimized method in to evaluate 9 different microcarriers. Together, we will present an optimized protocol for the establishment of large scale expansion of MSCs in bioreactors.

F-3226

DEVELOPING SAFE AND IMMUNE-TOLERATED CELL THERAPY FOR TREATING NEUROLOGICAL DISEASES

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Employing genome-editing strategies, we addressed the safety concerns associated with cell-based regenerative medicine through the development of the FailSafe (FS) Cell System. This proprietary technology inserts a suicide gene into a cell division essential locus, allowing selective elimination of proliferative cells through the administration of a pro-drug, whilst also protecting the suicide gene from inactivation. To develop economically feasible 'off-the-shelf' cell products, we developed a strategy for induced allogeneic cell tolerance (iACT). In this system, expression of eight transgenes involved in immune tolerance and rejection allows FS mESCs to form teratomas in MHC-mismatched recipient mice. In the current study, we sought to demonstrate the application of our technology for treatment of neurological diseases. By incorporating expression of firefly luciferase, we used bioluminescence imaging (BLI) to monitor the survival and proliferation of FS mESCs after stereotaxic injection into the mouse brain. Small numbers (1x10^4) of FS mESCs injected into the lateral ventricles were detectable by BLI. Weekly monitoring of live animals and changes in the BL signal over time identified those recipients in which the FS mESCs grafted and proliferated. Histological analysis of brain tissue confirmed that the grafted cells developed into teratomas containing structures derived from the three primary germ layers. To assess whether proliferating cells could be eliminated from the brain, we administered the pro-drug either at the time of transplantation to prevent teratoma formation, or at a delayed time point to allow for terminal differentiation of grafted cells. Longitudinal BLI studies and histological analyses confirmed that our suicide system was effective in eliminating proliferating FS cells from the brain, leaving the non-proliferating component intact. Moreover, proliferating FS mESCs engineered with iACT could be eliminated from the brain by administration of the pro-drug. We are now testing this approach in an allogeneic transplant setting. Ultimately, our FS and iACT technologies will contribute to the development of safe and immune tolerated cell therapies for treating neurological disease.

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F-3228

TWEEN-80 IS RESPONSIBLE FOR THE DELETERIOUS IMPACT OF COMMERCIAL CHEMICALLY DEFINED LIPID CONCENTRATE ON HUMAN PLURIPOTENT STEM CELL CULTURE

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Chemically defined lipid concentrate has been widely used as lipid supplement to culture numerous cell types such as CHO, hybridoma, endothelial cells, cardiomyocytes and various stem cells. Here, we report that the addition of lipid concentrate leads to the cell death of human pluripotent stem cells during passaging and proliferation in chemically defined E8 medium. Systematic analysis of individual components in the lipid concentrate reveals that surfactant Tween-80 is the main contributor to the cell death. Tween-80 permeabilizes cell membrane and disrupt the cellular integrity. However, its toxicity can be suppressed by such as albumin, but not by other polymers such as Pluronic F-68. Tween-80 significantly promotes trophoblast differentiation. We further demonstrate that we could increase the concentration of lipids to support cell growth by removing Tween-80.

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F-3230

INTEGRATED COLLECTION OF STEM CELL BANK DATA BY MIACARM

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Last decade has witnessed the fast growth in scale and number of cell lines in stem cell banks in all over the world. Although the worldwide stem cell banks may provide us with proper cell lines for cell therapies, the reproducibility and data exchange among stem cell banks are constrained by the lack of a standardized format. To solve this problem, MIACARM (Minimum Information About a Cellular Assay for Regenerative Medicine) was first developed and released in 2016 as a guideline for standardizing items and formatting cellular assay data produced from stem cell banks all over the world. Based on the MIACARM data model, a user-friendly integrated collection database was developed for cross search among 6 databases, UK Stem Cell Bank, RUCDR, hPSCreg, SKIP, Riken, and eagle-i. Presently, a total of 14,314 registered stem cell lines are available by the online search of http://icscb.stemcellinformatics.org/, where researchers can easily approach the standardized information of stem cells or stem cell lines from cell banks throughout the world. Advantages by adopting MIACARM include the deposition of minimum information and assay metadata to reduce missing and unnecessary information about the cells, the progress toward reproducible experiments, and the retrieval of stem cell lines by omnibus search across all cell banks and registries in the world. Also, MIACARM based integrated database will be promising and flexible to incorporate with newly developed cell banks with increasing number of cell lines in future. Currently, basic structure of MIACARM includes two levels (I and II) with five module structure (project, source cell, assay, experimental technology and data). MIACARM-I contains the guideline for basic cell research, which includes omics assays using human cells. MIACARM-II contains the guideline for stem cell based regenerative medicine, which includes terms related to stem cell and source cell quality checks. We further plan to extend MIACARM to be able to characterize cells differentiated from the stem cell lines (MIACARM-III).

F-3232

INVESTIGATION OF MURINE DENTAL PULP STEM CELLS WITH 3D IMAGING OF TOOTH AFTER TISSUE CLEARING

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To achieve a comprehensive understanding of tissue-resident dental stem cells and regeneration mechanism, we develop 3D imaging protocol for tooth using tissue clearing and light sheet microscopy. Mouse molars were partially decalcified in 10% EDTA (pH8.0) for 2 weeks with or without formic acid, followed by CUBIC or bone CLARITY tissue clearing protocol using X-CLARITY. Cleared molars were subjected to the primary antibody staining for 10 days and the secondary antibody staining for 10 days. After antibody staining, molars were stained with the hoechst 33342 for overnight and finally immersed in

the refractive index matching solution (RIMS). Samples were imaged with ZEISS Lightsheet Z.1 microscope and 3D images were rendered using Arivis software. Addition of formic acid produced opaque area along dental pulp and tissue clearing with CUBIC protocol was not efficient, thus either protocol was not adopted. After 10% EDTA and bone CLARITY process, molars were divided half with razor and subjected to staining with antibodies of BMI1 or SOX2 among stem cell markers and b-actin for counterstaining. Followed by the corresponding secondary antibody staining and RIMS immersion, molars were imaged with light sheet microscope. The result showed the presence of dentin layer and adequate staining of beta-actin in dental pulp area, which suggests the successful penetration of antibody and light sources for imaging. Interestingly, both BMI1 and SOX2 staining were localized to root area with increasing signal to root apex and decreasing signal toward pulp chamber. These results suggest the higher stem cell activity at root canal in dental pulp in comparison with crown chamber. We established 3D imaging protocol for tooth using bone CLARITY tissue clearing and lightsheet microscope, which provides a platform for the analysis of cellular and molecular mechanism of dentin and pulp regeneration.

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F-3234

A SMALL MOLECULE COCKTAIL PROMOTES CELL SURVIVAL AND TRANSLATIONAL APPLICATIONS OF HUMAN PLURITOTENT STEM CELLS

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Access to human induced pluripotent stem cells (iPSCs) has created novel paradigms for drug discovery and regenerative medicine. However, the poor survival of iPSCs during routine passaging and after cryopreservation poses major challenges to the establishment of well-controlled workflows to produce and store iPSCs on a large scale, and to the development of efficient genome editing protocols based on optimized single-cell cloning procedures. The ROCK inhibitor Y-27632 has been widely used to improve cell survival, but significant amounts of cell death remain evident in many iPSC applications. Here, we developed a four-component small-molecule cocktail named “CEPT” that dramatically improves iPSC viability. Testing 12, 744 compounds in quantitative high-throughput screening (qHTS), we first identified 114 hits that improved iPSC survival. Advancing 29 hits to combination screening based on their diverse modes of action, we discovered compound C and compound E as a synergistic pair (CE) that improved iPSC survival during routine passaging by approximately 50% as compared to Y-27632. Despite the dramatic effect of CE during routine passaging, its benefit was only modest when iPSCs were seeded at a low density (25 cells/cm2) or in a 1 cell/well condition. Therefore, we designed another combination screening assay to search for additional compounds that, when applied together with CE, can further enhance iPSC survival at low cell density conditions. Screening 8,011 compounds using the new assay, we found that CE together with two additional compounds (compounds P and T) dramatically improved iPSC survival at low cell density conditions, increasing single-cell cloning efficiency to ~65% as compared to the ~10% with Y-27632. We then extensively tested CEPT and demonstrated that this cocktail was highly efficient in improving cell survival during routine cell passaging, embryoid body formation, single-cell cloning following genome editing using CRISPR/Cas9, the establishment of new iPSC lines, as well as cell recovery after cryopreservation. Hence, the versatility of CEPT provides a powerful chemical platform for establishing efficient protocols and may become a widely used approach in drug development and regenerative medicine.

F-3236

MECHANOBIOLOGICAL REGULATION OF PLURIPOTENCY IN MOUSE EMBRYONIC FIBROBLASTS

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Producing induced pluripotent stem cells (iPSCs) from mature somatic cells is a promising strategy for creating new therapies for many diseases. However, most strategies for creating iPSCs often require genetic manipulation, creating concern for their

use as therapeutic cell lines. In this study, we examined whether mechanical force could play a role in enhancing the generation of iPSCs without genetic modification. We recently developed a high throughput biaxial stretching device that is capable of applying dynamic and complex mechanical strain on 576 cell cultures wells simultaneously to perform mechanobiological screening assays. Using this device, we applied mechanical stretch to mouse embryonic fibroblasts expressing an Oct-4 reporter construct. We first performed a dose response to mechanical load by applying a range of mechanical loads from 2.5-17.5% strain using the system. All mechanical strains induced some increase in Oct-4 and we observed a maximum increase of 2.5-fold in cells treated with 17.5% strain at 0.1 Hz. MEFs stretched under these conditions also expressed five-fold increase in Sox2 expression and three-fold increase in SSEA1. We also performed a drug screen in combination with mechanical loading and found several compounds that synergistically increased Oct-4 expression in MEFs with mechanical load over seven days of treatment. Further gene expression analysis through RT-PCR confirmed increase in several pluripotency markers such as Sox2, SSEA1, Nanog, and related genes. Overall, we demonstrated a new non-viral methodology to prime MEFs by efficiently identifying optimal mechanical and pharmacological treatments.

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F-3238

UNCOATED AND STARCH-COATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FOR EFFECTIVE STEM CELL LABELING AND TRACKING

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Experimental stem cell therapy protocols require cell tracking and monitoring in vivo.to determine cell differentiation, proliferation and migration. Superparamagnetic Iron Oxide (SPIO) nanoparticles have emerged as one of the contrast agents that can be detected by magnetic resonance imaging (MRI). We hypothesized that starch-coated SPIOs could be effectively used as bio-compactible labeling nanoparticle for adipose tissue-derived stem cells (ASCs). ASCs were labelled with the following forms of SIPOs: ferric oxide (Fe3O4), ferrous oxide (Fe2O3), or cobalt-nickel ferrite (CoxNi1-x Fe2O4) nanoparticles. The particles were either coated or uncoated with starch. Proliferation, viability, migration and vasculogenic differentiation of labeled cells were evaluated. Our results showed that uncoated and starch-coated Fe2O3 nanoparticles were more efficient in labeling ADSC as confirmed by transmission electron microscopy (TEM). Both uncoated Fe2O3 and starch-coated Fe3O4 nanoparticles were biocompatible as determined by lack of cell toxicity to ASCs and their significant proliferation. Both uncoated and starch-coated Fe2O3 showed a protective effect against apoptosis. Migration scratch-healing assay revealed that cells labeled with starch-coated Fe2O3 and Fe3O4 exhibited a significantly higher migration rate after 48 hrs. The angiogenic potential of ASCs was more robust in cells labeled with starch coated Fe2O3 and starch coated Fe3O4 than the CoxNi1-x Fe2O4-loaded cells. Our data show that starch-coated Fe2O3 and Fe3O4 can be safely used to track stem cells, as shown by their biocompatibility, and enhancing cell proliferation, migration and angiogenic potential.

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F-3240

H-Y- INCOMPATIBLE MOUSE EMBRYONIC STEM CELL TRANSPLANTATION CAUSES REJECTION OF NUCLEUS MATCHED CELLS

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Pluripotent stem cells (PSCs) are promising candidates for cell-based regenerative therapies. To avoid rejection of transplanted cells, gene engineering approaches, immunosuppression, or cell banks are discussed. In this context, reducing the immunogenicity of the donor cell is crucial, but little is known about the contribution of minor histocompatibility antigens

(mHA) mismatched grafts in stem cell immunobiology. For whole organ transplantation, donor H-Y antigens, a class of mHAs encoded by the Y chromosome, has revealed worse graft outcomes for male to female transplants of heart, liver, and kidney. In addition, male recipients of female hemotopoietic stem cell grafts are more likely to develop graft versus host disease (GvHD). In this study, we investigated the immunogenicity of the H-Y antigen in a murine nucleus matched, male-to-female embryonic stem cell (ESC) transplant setting. We demonstrate in vitro and in vivo immune responses in a H-Y mismatched model, that is mainly T-cell mediated, resulting in limited survival of H-Y mismatched murine ESCs in syngeneic female mice. In addition, using the acquired tolerance concept, we show that tolerance towards H-Y can be induced in the neonatal phase. Furthermore, our data indicate that the H-Y immunogenicity is preserved upon differentiation of ESCs into endothelial cells. The findings presented in our study are therefore of significant clinical importance since they reveal the need to match beyond HLA towards mHA mismatches and define optimal donor cells with caution.

Funding Source: NA

F-3242

GENOME-SCALE CRISPR-CAS9 KNOCKOUT SCREENING FOR GENES REGULATING GLUCOSE UPTAKE IN HESC-DERIVED VASCULAR SMOOTH MUSCLE CELLS

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By causing damage on micro- and macrovascular system, hyperglycemia is an independent risk predictor for cardiovascular morbidity and mortality, as well as for total mortality, independent of the subsequent development of overt diabetes mellitus. However, paucity is known about genes responsive to the high glucose stimulus in vascular system. Here, we used a genetically encoded glucose sensor in the genome-scale Crispr Cas9 knockout (GeCKO) screening for genes regulating glucose uptake in human embryonic stem cell derived vascular smooth muscle cells (hESC-VSMC). The gene candidates identified by the GeCKO screening were used in the second pooled screening to prioritize the top ones critical for regulating glucose uptake in hESC-VSMC. Through a detailed mechanistic study of the top genes for glucose uptake, we intend to gain insight about how high blood glucose level or hyperglycemia affects the VSMC function and contributes to macrovascular complications.

LATE-BREAKING ABSTRACTS

F-4002

SUBCELLULAR DYNAMICS OF RNAS AND MICRORNAS IN MOUSE EMBRYONIC AND TROPHOBLAST STEM CELLS

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Subcellular trafficking of RNAs plays a pertinent role in regulation of key biological processes, such as maintenance and progression of cell identity and function. Mouse embryonic stem cells (ESCs) and trophoblast stem cells (TSCs), as representatives of early cell fate decision – the inner cell mass (ICM) and the trophoectoderm (TE), respectively – rely on reciprocal molecular signaling between coding RNAs and regulatory RNAs for self-maintenance and proliferation. Understanding the spatial dynamics of RNA localization and interaction in ESCs and TSCs gives insight on pertinent processes involved in cell fate determination. Here, a biochemical fractionation of cytosolic and nuclear compartments of mouse ESCs and TSCs is coupled to gene expression profiling by high-throughput RNA- and smallRNA-sequencing. To ensure proper quantitative analysis between cytosolic RNA (cytRNA) and nuclear RNA (nucRNA), a mass-balance scaling factor is applied to normalize against RNA concentration and cell equivalence. Next, bioinformatics analysis of coding and noncoding RNAs shows asymmetric expression of pluripotency genes across ESCs and TSCs, suggesting our methodology of cell fractionation and data normalization is suitable for comparative analysis. Differential expression analysis of compartments revealed that nucRNA in both ESCs and TSCs show enrichment of terms associated with RNA processing, transport, and splicing. Uniquely, TSCs nucRNA was enriched in regulatory noncoding RNA. Furthermore, genes related to RNA and DNA processing show higher retention of introns relative other genes; a phenomenon conserved across cytRNAs and nucRNAs in both cell lines. A parallel pipeline for microRNAs (miRNAs) show differentially expressed miRs in both the cytosol and the nucleus of ESCs and TSCs, suggesting a nuclear role of mature miRs associated with ICM and TE phenotype. This high-throughput, compartment-specific method of surveying differentially expressed RNAs – including introns – allows a lead to a deeper understanding of regulatory dynamics governing ICM and TE fate.

F-4004

ACID-EXTRUDING AND -LOADING MECHANISM IN HUMAN STEM CELLS AND CANCER CELLS

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Pluripotent stem cells (PSCs) were high proliferation and rely on glycolysis. In tumor, the up-regulation of acid-extruders, such as Na+-H+ exchanger (NHE), was to adapt the higher production of metabolic acid from glycolysis, and its further created a reverse pH gradient: microenvironment acidification and intracellular alkalization. Thus, it's easy to associate that PSCs have the higher activity of acid-extruders. Some studies had shown that acidic culture medium retained the pluripotency during differentiation. However, acidic environment also reduced the quality of PSCs during the cultured process, through inhibited the growth and increased the apoptosis. Until now, pHi regulating mechanism in human stem cells is still unknown. The aims of this study were to investigated the role of pHi regulation in the different stem and cancer cells. The hiPSCs (HPS0077), human adipose-derived stem cells (hADSCs) and OEC-M1 was kindly provided from Dr. Dai and Dr. Lee. The change of pHi was detected either by microspectrofluorimetry method. Weak acid-base pre-pulse were used to induce the intracellular acidification and alkalization. Our present results showed that the buffering capacity were increased with the intracellular alkalization in hiPSCs. NHE, Na+-HCO3- cotransporter (NBC), Cl-/ OH- exchanger (CHE) and Anion exchanger (AE) were activated for the pHi regulating mechanism in all cell lines, while V-ATPase was only activated for the acid-extruding in HeLa and A549. Interestingly, the unknown Na+-independent acid extruder(s) and unknown Cl--independent acid-loader(s) could only detected in hiPSCs. The resting pHi value was found to be 7.7 in hiPSCs, which was much higher than that of other cells (7.46, 7.13, 7.49, 7.4 and 7.39 in hDPSCs, hADSCs, OEC-M1, HeLa and A549). The strengthening of buffering capacity and the weakening of acid extruding mechanism, including intracellular acidification and decreasing of activity of acid-extruders, was observed during the loss of pluripotency. Summary, our study provided a model and the dynamic changes of pHi regulating mechanism at different pluripotent state in hiPSCs. Moreover, the pHi in hiPSCs was much higher than the others cancer and adult stem cells. Key words: hiPSCs; hMSCs; cancer cells; acid-extruder; acid-loader; buffering capacity.

F-4006

USING ACTIVITY-BASED CHEMICAL PROTEOMICS TO UNCOVER ESSENTIAL REGULATORS OF PLURIPOTENCY

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Pluripotent stem cells exist in a spectrum of states with distinct cellular and molecular features, including differential signaling, transcriptomic and chromatin landscapes, X-inactivation state and developmental properties. For instance, mouse embryonic stem cells (ESCs) represent a naïve pluripotent state of the pre-implantation embryo, while mouse epiblastic-derived stem cells (EpiSCs) resemble the developmentally more advanced primed state of the post-implantation embryo. Better understanding these pluripotent states will yield insights into the mechanisms underlying earliest embryonic development. Functional genomic studies, such as genome-wide RNAi screens, have identified transcription factors and chromatin complexes as regulators of pluripotent states, but may not capture regulatory events at the protein-level, including regulation by enzymatic activities and post-translational modifications. Activity-based protein profiling (ABPP) in conjunction with chemical proteomics has emerged as an unbiased proteome-wide approach to identify functional and small molecule-targetable proteins in disease states. We apply the quantitative isotopic Tandem Orthogonal Proteolysis (isoTOP)-ABPP method to compare the cysteine (Cys) reactivity profiles of ESCs and epiblast-like cells (EpiLCs) to capture the molecularly different naïve and primed states, as Cys residues are important for protein stability, enzymatic activity and cellular redox reactions. Overall, we identify 3872 reactive Cys from 2192 proteins. Of these Cys, 170 show elevated labeling specifically in ESCs and 160 in EpiLCs. The Cys preferentially labeled in ESCs are enriched on proteins involved in carbon and amino acid metabolism and fatty acid degradation, while EpiLC-specific Cys labeling occurs in proteins involved in ribosomes and lipid biosynthesis. We also identify proteins with reactive Cys of unknown functions in the regulation of pluripotency. Using electrophilic compounds that target Cys in specific proteins, we observe that compound treatment inhibited the maintenance of pluripotency in ESCs. Together, these results suggest that activity-based chemical proteomics identifies novel classes of pluripotency regulators, which can be targeted by small molecules to manipulate the pluripotent state.

Funding Source: This research is supported by UCLA Broad Stem Cell Research Center.

F-4008

L-PROLINE INDUCES MESC DIFFERENTIATION TO A SPATIALLY DISTINCT EPL-CELL POPULATION THROUGH A COMPLEX SIGNALLING REGULATORY NETWORK TO PROMOTE NEURAL DIFFERENTIATION

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The amino acid L-proline has novel growth factor-like properties during development - from improving blastocyst development to driving neurogenesis. Addition of 400 μM L-proline to self-renewal medium drives mouse embryonic stem cells (mESCs) to a transcriptionally distinct pluripotent cell population - early primitive ectoderm-like (EPL) cells - which lies between the naïve and primed states. Both EPL cells and EpiSCs express 'primed pluripotency' genes such as Fgf5, Dnmt3b and Otx2. EPL cells have high expression of neurogenesis genes, and map to the anterior aspect of the ~7.0 dpc embryo – the site of the prospective neurectoderm. Conversely, EpiSCs have a propensity to differentiate to mesendoderm, upregulate expression of cardiovascular development genes, and more closely map to the distal-posterior aspect of the embryo. While both these cell types retain pluripotency, these gene expression changes indicate EPL cells and EpiSCs are predisposed to different lineage commitments. Here, we use EPL cells to understand the molecular mechanisms underpinning pluripotency and neural induction. In mESCs, L-proline acutely increases phosphorylation of MAPK, mTOR and PI3K signalling-pathway intermediates. Inhibition of these signalling pathways individually had little effect on cell fate. When these inhibitors were used in combination, more robust changes were seen in gene expression and emergent properties including colony morphology, cell number, proliferation and apoptosis. This data was analysed using statistical modelling to identify synergistic effects between inhibitors – where two pathways converge to produce a change larger than either individually, or interaction effects - where two pathways converge and produce an effect equal to either pathway alone. Further analysis using linear regression was used to determine which signalling pathways make the largest contribution to changes in gene expression and emergent properties. This statistical model showed that a decrease in expression of the naïve pluripotency marker Rex1 or an increase in the primed pluripotency marker Fgf5 could be explained by a single pathway (MAPK and mTOR, respectively). Changes in the other 12 parameters were attributed to signalling pathways acting together - indicating a complex, self-regulating signalling network.

F-4010

GENERATION OF CLINICAL-GRADE FUNCTIONAL HEPATOCYTES FROM HUMAN EMBRYONIC STEM CELLS IN XENO-FREE CONDITIONS

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As one of the most important organs in the body, the liver is susceptible to many major diseases, which are the main causes of morbidity and mortality. Patients with acute liver injuries or end-stage liver diseases have no choice but to receive a liver transplant. However, the source of donor livers is restricted. In recent years, hepatocyte transplantation has been attempted to treat liver failure clinically. However, the use of primary or fetal hepatocytes has been restricted due to the lack of available healthy donors as well as limited cell proliferation, functional deficits, the risk of immune rejection and the concern of ethical issues. Hepatocytes have been successfully generated from human pluripotent stem cells (hPSCs). However, the cost-effective and clinical-grade generation of hepatocytes from hPSCs still need to be improved. In this study, we reported the production of functional clinical-grade hepatocytes from clinical-grade human embryonic stem cells (hESCs) under GMP requirements. During hepatoblast differentiation, dimethylsulfoxide (DMSO) and four small molecules (transferrin, Vc-Mg, insulin and sodium selenite) were used instead of cytokines and FBS/KOSR. Then, hepatoblasts were differentiated into hepatocyte-like cells (HLCs) that had a typical hepatocyte morphology and possessed characteristics of mature hepatocytes, such as metabolic-related gene expression, albumin secretion, fat accumulation, glycogen storage and inducible cytochrome P450 activity in vitro. HLCs integrated into the livers of Tet-uPA Rag2-/- Il2rg-/- (URG) mice, which partially recovered after transplantation.

Furthermore, a series of biosafety-related experiments were performed to ensure future clinical applications. This efficient platform could facilitate the treatment of liver diseases using hESC-derived HLCs transplantation.

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F-4012

THE TCA CYCLE METABOLITES REGULATE HUMAN EMBRYONIC STEM CELL SURVIVAL AND MITOCHONDRIAL DYNAMICS DURING NAÏVE PLURIPOTENCY

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During early embryonic development, embryonic stem cells (ESCs) shift naïve pluripotency into primed epiblast stem cells (EpiSCs). The conversion of the metabolic demand between oxidative phosphorylation and glycolysis can impact on the transition from naïve to primed pluripotency. The TCA cycle metabolite α-ketoglutarate (αKG) can maintain naïve pluripotency, whereas aerobic glycolysis-derived cytosolic acetyl-CoA contributes primed pluripotency through epigenome remodeling. However, whether other TCA cycle metabolites can affect the pluripotent status have not been comprehensively characterized yet. Here we identify citrate, malate, oxaloacetate and succinyl CoA of the eight intermediates of the Krebs cycle led to naïve human ESC death. The cellular death attributed to the formation of autophagosomes and mitochondria with degenerate cristae manifested by transmission electron microscopy. Isocitrate but not αKG promoted hESC differentiation and rescued the cellular death caused by oxaloacetate both in naïve hESC and mouse early embryos. In vitro manipulation of the pluripotency from naïve hESC into primed hEpiSC, the mitochondria changed from punctate into the filamentous network. The primed hEpiSC counteracted the oxaloacetate-caused cell death by restoring cristae structure and elongating mitochondrial configuration. These results suggest that mitochondrial membrane structures and dynamics correlate with pluripotent status and TCA cycle metabolites have distinct functions in regulating naïve hESC pluripotency and survival.

F-4014

THE REGULATION OF HEDGEHOG SIGNALING PATHWAY BY PUM1 IN MOUSE EMBRYONIC STEM CELLS

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The PUF (Pumilio and FBF) family of RNA-binding proteins are evolutionally conserved post-transcriptional regulators. Through binding to recognition sites in target mRNAs, they can regulate gene expression by promoting mRNA degradation or inhibiting translation. PUFs have been shown to be required for germline stem cell maintenance in D. melanogaster, C. elegans, and the planarian S. mediterranea, but little is known about their function in mammalian stem cells. Pumilio1 (PUM1) and Pumilio2 (PUM2) are two murine members of the PUF family proteins. Our previous study showed that Pum1 deficiency leads to smaller body size and multiple developmental defects, indicating Pum1 may play important roles in mouse embryogenesis. Here we report that knockdown of Pum1 in ESCs does not affect their self-renewal, but results in defects in embryoid body differentiation, especially in the mesodermal lineage. Furthermore, PUM1 can negatively regulate Gli1 expression and Hedgehog (HH) signaling pathway activity both in mouse embryos and in ESCs. Thus, we hypothesize that PUM1 affects ESC differentiation and mouse embryonic development at least in part by influencing the HH signaling pathway. By investigating the regulatory mechanism of PUM1 on the HH signaling pathway, our study will shed new light on how PUF proteins and the HH signaling pathway regulate ESCs and embryogenesis in mammals.

F-4016

SEARCHING FOR A PREDICTIVE BIOMARKER TO SELECT HUMAN INDUCED PLURIPOTENT STEM CELLS WITH HIGH CARDIAC DIFFERENTIATION POTENTIAL

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Selection of human induced pluripotent stem cell (hiPSC) lines with high cardiac differentiation potential is important for regenerative therapy and drug screening, but current pluripotency markers such as OCT-4, LIN28, and NANOG cannot be used to distinguish the direction of differentiation. The purpose of the present study was to identify a biomarker for predicting efficient cardiac differentiation that can be used for selecting individual hiPSC lines by comparing the gene expression profiles of undifferentiated hiPSC lines with varying cardiac differentiation potential. We used three platforms of gene expression analysis, namely, cap analysis of gene expression (CAGE), mRNA array, and microRNA array to efficiently screen biomarkers related to cardiac differentiation of hiPSCs. Subsequently, we subjected all hiPSC lines to cardiac differentiation. Differentiation capacity was quantified based on the percentage of cardiac troponin T positive cell populations using flow cytometry and cardiac related genes using qPCR. As a result, we observed that a difference in the purity of cardiac troponin T among hiPSC lines ranging from 7.7%±2.6% in RIKEN-12A to 92.3%±1.2% in 409 B2. Differential global gene expression within the undifferentiated hiPSCs state revealed 22 distinguishing candidate genes among hiPSC lines with caridiac propensity. Of the candidate genes, PF4 was validated as a biomarker expressed in undifferentiated hiPSCs with high potential for cardiac differentiation in 13 additional hiPSC lines. In addition, we found that pretreatment with PF4 enhanced the cardiac differentiation potential of hiPSCs, suggesting that PF4 could promote cardiac differentiation. These observations suggest that PF4 gene expression, which could vary not only between hiPSC lines but also between pharmacological conditions, reflect the potential of hiPSCs to differentiate into cardiomyocytes and can thus be used as a quality control marker of hiPSCs. In conclusion, differential global gene expression of hiPSC lines with high and low differentiation capacity showed that cardiac differentiation potential may be predicted by measuring the expression of PF4, suggesting that these genes may be helpful in selecting hiPSCs lines for regenerative therapy and drug screening.

F-4018

NAÏVE VERSUS PRIME HUMAN STEM CELL FOR LIVER ORGANOID DIFFERENTIATION

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Organoid based approaches hold promise for advancing human health and disease biology, drug development, and regenerative medicine. For example, we have differentiated human induced pluripotent stem cells (hiPSCs) to make multi-lineage human liver organoids (HLOs) that are capable of modeling steatohepatitis in vitro. However, there is a considerable variability in deriving these HLOs in terms of cell type differentiation ability as well as organoid forming efficiency across hiPSC lines, even among clones derived from the same individual. We reasoned this at least in part is associated with epigenetic variabilities between lines. To overcome these challenges, we have developed a novel method to produce putative "naïve" human cells from "primed" hiPSCs. Naïve stem cells normalizes many of the epigenetic changes that occur during natural development and that can alter gene expression. After culturing hiPSCs with naïve mouse embryonic stem cells (mESCs) for 5 days and sorting out the mouse cells the hiPSCs undergo a morphological change from 2D flat shaped colonies to round, domed shaped colonies that become more stable with increased passaging. These human putative "naïve" cells express naïve markers by qPCR and immunostaining such as DPPA3, KLF17, TFCP2L1, and TFAP2C, and have downregulated primed markers such as DUSP6. This method is consistent across several hiPSC lines, and is accompanied by diverse chromatin accessibility changes. We are currently working to 1) re-prime these putative "naïve" cells and differentiate these cells into HLOs or 2) use embryoid body differentiation, to compare the HLO efficiency to the same cell line that has not been previously converted into a putative "naïve" status. To compare HLO efficiency between naïve and primed iPSC, we will quantify organoid number, levels of liver specific markers, hepatic production and drug metabolism enzyme activity. This work will help to provide valuable insights into how epigenetic changes can affect differentiation and development in HLOs and beyond.

F-4020

AN INTEGRATED GENOMIC APPROACH TO STUDY ASTROCYTE-INDUCED MATURATION IN HUMAN INDUCED GLUTAMATERGIC NEURONS

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Astrocytes are known to modulate many aspects of neuronal development, including their maturation, axon outgrowth and synapse formation. Whilst most studies of neurons are based on non-human models, the development of rapid neuronal reprogramming protocols has provided a unique opportunity to study the biology of human neurons. We have previously demonstrated that gene targeting the components of a genetic switch into genomic safe harbour sites enables optimised expression of NGN2 in hiPSC (OptiOx) and yields homogenous cultures of pure cortical glutamatergic neurons (hiNeuron) in less than four days. Here we aimed to gain a wholistic insight into how astrocytes modulate the function of human excitatory neurons. To assess electrophysiological activity, on the 3rd day of reprogramming, primary rat astrocytes were added to the hiNeuron cultures maintained on multi-array-electrodes (MEAs). Serial MEA recordings demonstrated spontaneous electrophysiological activity of hiNeurons as early as 11 days after induction. At 21 days post induction, synchronised burst patterns were detected across the network. In the absence of astrocytes hiNeurons cell bodies tended to cluster together and limited spontaneous electrophysiologic activity was recorded. The presence of astrocytes therefore profoundly affected neuronal function. In order to study genome-wide effects of astrocytes on hiNeurons, three independent biological samples were collected of hiNeurons cultured in the presence and absence of astrocytes at day 4, 14, and 21 following induction and submitted to bulk RNA-seq, bulk ATAC-seq, and single cell RNA-seq. Bulk sequencing data was de-convoluted according to species to separate human neuronal reads from those of rat astrocytes. The presence of astrocytes induced profound transcriptional and epigenetic changes in hiNeurons. Sc-RNA seq demonstrated that hiNeurons in the presence of astrocytes are distinct from hiNeurons in the absence of glia. In conclusion, mixed-species in vitro cultures of astrocytes and hiNeurons are a useful tool to study human neuronal biology. The integration of RNA-Seq, ATAC-Seq, and scRNA-Seq provides unique and wholistic insights how the presence of astrocytes affects the function of human neurons.

F-4022

TRANSIENT AND STABLE OVER-EXPRESSION OF MIR690 ACCELERATES OSTEOGENIC DIFFERENTIATION IN EMBRYONIC STEM CELLS

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Osteogenesis is a complex and critical process for the proper development of vertebrates. The complexity of osteogenesis resides in the multiple pathways that embryonic stem cells (ESCs) can take to differentiate into osteoblasts and the intricate gene regulatory network, which controls this lineage development. Specifically, osteogenic differentiation can be induced from mesoderm or neural crest cells; both of which can differentiate into mesenchymal stem cells and then into osteoblasts. This overlap of differentiation into mesenchymal cells creates a convenient point at which to study osteogenesis, but reduces the focus on the intermediate stages of differentiation. Additionally, the role of microRNAs (miRNAs) in these stages also appears to be crucial for the fate of specific progenitors, making them an important piece of the puzzle. Our lab has shown that overexpression of a specific miRNA, mir690, upregulates osteogenesis through the direct targeting of β-catenin. Additionally, the increased osteogenesis achieved from miR690 overexpression during differentiation is a time dependent process, best achieved with a transfection during days 5-7 of differentiation. Furthermore, both the stable and transient transfection of mir690 result in morphological differences as well as a shift in the calcification time line possibly stemming from a shift in the overall cell population origin. Additionally, we have analyzed the role of mir690 in DGCR8 KO cells, which have been shown to maintain a higher level of pluripotency even during standard differentiation cultures, and we have shown this cell line to have a reduced ossification potential. However, the transfection of mir690 into DGCR8 KO cells also showed an increase in calcification when transfected during the same differentiation window as the wildtype cells. Lastly, increased osteogenesis was accompanied by an earlier increase in alkaline phosphatase activity as well as specific osteoblast genes. Together, our data indicate that miR690 supports osteogenesis both when stably and transiently transfected.

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F-4024

REDUCED LEVELS OF MIR28 AND MIR377 EFFECT OSTEOGENESIS IN HYPERGLYCEMIC CONDITIONS IN MOUSE EMBRYONIC STEM CELLS

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The International Diabetes Federation stated in 2017 that approximately 425 million adults were living with diabetes and by 2045 this will rise to 629 million. Diabetes is a metabolic disease that is characterized by the presence of elevated glucose levels, and sadly the rates of diagnoses will continue to increase worldwide. Diabetes has been shown to cause numerous health problems, one being improper bone development, which could lead to other bone diseases such as osteoporosis or even bone fractures. Our lab previously showed that two miRNAs, miR28 and 377, were significantly regulated during osteogenesis of mouse embryonic stem cells (mESCs) and directly target AMP kinase (AMPK), which is typically activated by a hyperglycemic stress response. To uncover the downstream effectors of miR28, miR377 and AMPK that control lineage fate early on during specification, mRNA expression of neural crest, paraxial mesoderm and lateral plate mesoderm, which all are potential precursor to bone cells, were examined in both glucose concentrations of 5mM (low) or 25mM (high). Furthermore, miR28 was found to target FOXO1, a transcription factor downstream of AMPK, which may transcriptionally mediate the metabolic response. Ultimately, these results indicate that osteogenesis is reduced in the presence of high glucose and that manipulation of candidate miRNAs may represent a feasible treatment option to prevent developmental consequences of hyperglycemia.

F-4026

UNEXPRESSED PROTEIN TAGGING AS A TOOL TO OPTIMIZE CHARACTERIZATION IN HUMAN STEM CELLS USING CRISPR/CAS9

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Methods to confirm iPSC cell differentiation to a desired lineage are often complex and time-consuming. To streamline this process, we are developing methodology to fluorescently tag genes that are unexpressed in the iPSC state, but expressed in the desired differentiated cell line. The expression of the marker after differentiation allows for rapid visual screening, which will minimize cost and time associated with clonal isolation of properly differentiated cells, and optimization of factors relevant to characterization. As a proof of concept, we have currently tagged two genes with Emerald GFP (EmGFP) as the differentiation reporter using the CRISPR/Cas9 ribonucleoprotein (RNP) system, donor DNA, and Neon electroporation. We targeted genes indicating differentiation of iPSC cells to a neuronal lineage using beta III tubulin (TUBB3), and an astrocyte lineage using glial fibrillary acidic protein (GFAP). The donors were linear dsDNA with short homology arms coding for EmGFP and puromycin driven by the EF1 promoter. Proper integration of the donor DNA was confirmed via junction PCR and sequencing. Next, edited cells were enriched using a puromycin selection. Surviving cells were expanded in Essential 8 media for 5 days, when individual colonies harboring the knock-in had grown large enough to be picked and isolated for clonal selection. At this point, no EmGFP expression was observed, as expected. Junction PCR and sequencing was then repeated to confirm the presence of the tags in the clonal samples. The resulting TUBB3_EmGFP iPSC cell line was induced to differentiate into neurons, whereas the GFAP_EmGFP iPSC cell line was induced to differentiate into astrocytes. Since TUBB3 and GFAP only express in mature neurons and astrocytes respectively, upon differentiation the cells visibly expressed the EmGFP, demonstrating that cellular fate can be successfully and easily tracked via visualization of EmGFP expression. Further improvements can be made to this cell differentiation tracking system by tagging multiple proteins with different colored reporters to potentially visualize different stages of differentiation of lineages.

F-4028

ROBUST AND EFFICIENT GENERATION OF HUMAN MACROPHAGES USING INDUCED PLURIPOTENT STEM CELLS FOR DRUG DISCOVERY

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Robust and efficient generation of human macrophages using induced pluripotent stem cells (iPSCs) for drug discovery. After decades of research there are still persistent failures to translate preclinical drug candidates into clinical success, highlighting the limited effectiveness of disease models currently used in drug discovery. iPSC technology has provided new tools to improve drug discovery efforts from efficacy and toxicity testing to novel target identification and understanding disease mechanisms. Macrophages perform key functions in regulating homeostasis, immune response and tissue repair. Equally, their dysfunction may drive pathogenesis of inflammatory and degenerative diseases, making them a key therapeutic target. Currently, macrophages are differentiated from peripheral blood monocytes (PBMCs), which is costly at scale and results in poor reproducibility due to donor variability. Moreover, advances in gene editing of human macrophages have been hampered due to viral delivery systems and their limited proliferative capacity. We have developed a robust cellular platform to generate human macrophages using innovative iPSC technology. This provides a cost-effective, reproducible and biologically relevant large-scale source of human macrophages for use in drug discovery. We have identified a simple three-step method which enabled the generation of a continuous source of human macrophages within twenty days of initial differentiation. Careful biochemical, functional and cellular assays were performed for comparative analysis with blood-derived macrophages and other commercially available iPSC-derived macrophages. In addition, we have successfully implemented highly efficient precision gene editing in both iPSCs and iPSC-macrophages by using CRISPR RNP. This ambitious methodology demonstrates comparable results to current primary cell-based models and adds value to existing strategies by reducing donor variability, reducing timelines and ultimately decreasing costs. Moreover, the use of engineered iPSC-macrophages provides great opportunity to accelerate early-stage drug development pipelines by generating physiologically and clinically relevant models.

F-4030

INTERROGATING THE THERAPEUTIC POTENTIAL OF TRPC6 FOR ALZHEIMERS DISEASE IN NEURONS FROM PATIENT SPECIFIC IPSCS

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The patient-specific induced pluripotent stem cells (iPSCs) offer an unprecedented opportunity to model and study Alzheimer's disease (AD) in live neurons. Then, the key question in the field is subsequent applications of iPSC-derived cellular models. In this study, the possible role of transient receptor potential canonical 6 (TRPC6) in AD pathogenesis was interrogated in iPSC-derived live AD neurons. The iPSCs generated from peripheral blood cells of sporadic AD patients efficiently differentiated into mature cortical neurons that displayed higher levels of AD pathological markers Aβ and phospho-tau, but lower levels of TRPC6, than those of control neurons. Treatment of AD neurons with TRPC6 peptides or agonist inhibited the elevation of Aβ and phospho-tau. Our results in AD neurons confirm that the compromised expression of TRPC6 substantially contributed to Aβ pathology of sporadic AD, which fit well with our previous findings in mouse models. The inhibition of Aβ elevation suggested that targeting TRPC6 could help to develop novel therapeutic strategies for the treatments of AD.

F-4032

A HUMAN EMBRYONIC STEM CELL REPORTER LINE FOR CARDIOTOXICITY PLATFORM

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Human embryonic stem cells (hESCs) can be used to generate scalable numbers of cardiomyocytes for studying cardiac biology, disease modeling, drug screens, and potentially for regenerative therapies. Directed differentiation protocols for cardiomyocytes using hESCs are well established, but methods to isolate highly pure population of cardiomyocytes are limited. Reporter cell lines can be valuable for purification and visualization of cells for such applications. We used CRISPR/Cas9 in hESCs to place an mCherry reporter gene into the MYH6 locus, facilitating a simple method to purify cardiomyocytes. MYH6:mCherry positive cells express atrial and ventricular markers and display a range of cardiomyocyte action potential morphologies. At 20 days of differentiation, MYH6:mCherry+ cells show features characteristic of human cardiomyocytes and can be used successfully to monitor drug-

induced cardiotoxicity and oleic acid-induced cardiomyocyte arrhythmia. The MYH6:mCherry hESC reporter line should serve as a useful tool for disease modeling and drug development relevant to cardiomyocyte biology.

F-4034

IN VITRO DISEASE MODELING OF THE FTDP-17 TAU R406W MUTATION USING PATIENT-DERIVED IPSCS

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Frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) is a neurodegenerative disease caused by mutations in the microtubule-associated protein tau (MAPT) gene, which encodes the tau protein. Among the MAPT mutations, the R406W mutation is a unique missense mutation whose patients have been reported to exhibit Alzheimer's Disease (AD)-like clinical phenotypes independent of Aβ accumulation. To date, there is no treatment known to be effective for FTDP-17 patients, including those with the R406W mutation. The objective of this study is to establish a suitable model for studying the abnormalities induced by R406W mutant tau and elucidating the pathological role of tau in neurodegenerative diseases, as a basis for drug screening. iPSC lines were established from patients heterozygous for the MAPT R406W mutation (MAPT R406W/+). These lines were gene-edited using CRISPR/Cas-9 to establish WT isogenic lines (MAPT+/+), or homozygous mutants (MAPT R406W/R406W). iPSCs were then induced into a homogenous population of cortical neurons with more than 85% neuronal purity by dissociating cerebral organoids. In these neurons, R406W tau were less phosphorylated than WT tau by several kinases and were increasingly fragmented by calpain. Furthermore, the mutant tau protein was mislocalized to the dendrites and induced axonal phenotypes, including morphological changes and mitochondrial transport defects, which could be rescued with microtubule stabilization. In summary, we elucidated the abnormalities induced by the R406W mutant tau using iPSC-derived neurons. These findings provide new mechanistic insight into tau pathology and a potential for therapeutic intervention.

F-4036

DUCHENNE MUSCULAR DYSTROPHY: A DEVELOPMENTAL DISEASE

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DMD boys are currently diagnosed around 4, an age at which muscles have already suffered from the pathology. Meanwhile, no treatment is available yet to stop this degenerative disease. Efficacy of developing therapies aiming at restoring the expression of dystrophin in muscle stays too low to really be beneficial. All of this stresses the need of better defining the first steps of DMD in Human to be able to 1) find earlier and more specific biomarkers to increase diagnosis sensitivity; and 2) develop alternative therapeutic approaches by finding targets that compensate the lack of dystrophin in addition to restoring it. To identify theses biomarkers and therapeutic targets, we have modelled DMD in vitro using both human primary adult myoblasts and human pluripotent stem cells differentiating into myotubes. In our previous study, we identified Dp412e, an embryonic isoform of the dystrophin leading us to investigate further DMD onset during development. Comparisons at 7 cell differentiation time points of 3 healthy and 3 DMD human cell

lines by omics (bulk transcriptomes, single-cell transcriptomes, miRnomes as well as proteomes) demonstrate that 1) we have access to both embryonic/foetal and adult myotubes ; 2) embryonic/foetal myotubes exhibit described DMD phenotypes (eg. dystrophin-associated protein complex destabilization, disrupted Ca2+ homeostasis and reorganisation of extracellular matrix); 3) our in vitro model properly recapitulates myogenesis, with a mesoderm entry at D3 followed by a specification toward skeletal muscle, up to myotube formation by D25; 4) DMD cells show marked dysregulations from D10, before the expression of skeletal muscle transcription factors is even detected at D17, with a clear downregulation of mitochondrial genes and 5) our cell model is well adapted for identifying drugs using high-throughput screening. Altogether, our data identify human pluripotent stem cells as a suitable cell model to study muscle development in the context of genetic diseases, and use it as a tool for therapy development. They strongly argue for an early developmental manifestation of DMD and lead us to rethink dystrophin functions during development.

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F-4038

IDENTIFICATION OF KCC2 EXPRESSION ENHANCING COMPOUNDS AS A BASIS FOR TREATMENT OF RETT SYNDROME

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A critical need in the drug development process is the ability to identify chemicals that regulate specific molecular targets in disease-relevant cell types in order to rescue functional deficits caused by faulty gene expression. The neuron-specific protein K+/Cl- co-transporter 2 (KCC2) has emerged as a promising therapeutic target for treatment of a number of human brain disorders including epilepsy, schizophrenia, spinal cord injury, and Rett syndrome (RTT), a severe neurodevelopmental disorder. Due to the lack of a neuron-based high-throughput screening (HTS) assay, it has been challenging to discover chemical compounds that enhance the expression of the KCC2 gene. In this study, we report the development of a robust high-throughput drug-screening platform through genome editing in human embryonic stem cells, which allows for the rapid assessment of KCC2 gene expression in human reporter neurons. We have identified a group of compounds from an unbiased screen of over 900 small molecule chemicals that enhance KCC2 expression termed KCC2 expression-enhancer compounds (KEECs). The identified KEECs include FDA-approved drugs that are inhibitors of the FLT3 or GSK3β kinase pathways, and activators of the SIRT1 or TRPV1 pathways. We demonstrate that treatment with these hit compounds increases KCC2 expression in human WT and isogenic Methyl CpG binding Protein 2 (MECP2) mutant RTT neurons, and rescues the deficits in GABA reversal potential, excitatory synaptic transmission, and morphological development of RTT neurons to levels equivalent to WT neurons. Moreover, injection of KEECs KW-2449 or Piperine into a Mecp2 mutant mouse model of RTT ameliorates disease-associated respiratory and locomotion phenotypes. The small molecule compounds described in our study could potentially benefit various brain diseases through a novel mechanism of enhancing KCC2 expression.

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F-4040

NOVEL IMPRINTED SINGLE CPG SITES FOUND BY GLOBAL DNA METHYLATION ANALYSIS IN HUMAN PARTHENOGENETIC INDUCED PLURIPOTENT STEM CELLS

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Genomic imprinting is the process of epigenetic modification whereby genes are expressed in a parent-of-origin dependent manner; it plays an important role in normal growth and development. Parthenogenetic embryos contain only the maternal genome. Parthenogenetic embryonic stem cells could be useful for studying imprinted genes. In humans, ovarian mature cystic teratomas originate from parthenogenetic activation of oocytes; they are composed of highly differentiated mature tissues containing all three germ layers. To establish human parthenogenetic induced pluripotent stem cell lines (PgHiPSCs), we generated parthenogenetic fibroblasts from ovarian teratoma tissues. We compared global DNA methylation status of PgHiPSCs with that of biparental human induced pluripotent stem cells by using Illumina Infinium Human Methylation 450K array. This analysis identified novel single imprinted CpG sites.

We further tested DNA methylation patterns of two of these sites using bisulfite sequencing and described novel candidate imprinted CpG sites. These results confirm that PgHiPSCs are a powerful tool for identifying imprinted genes and investigating their roles in human development and diseases.

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F-4042

AN EFFICIENT PLATFORM TO EDIT COMPLEX PLURIPOTENT STEM CELLS FOR DISEASE MODELLING

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Human Pluripotent Stem Cells (hPSCs) including human Embryonic Stem Cells (hESCs) and human induced pluripotent stem cells (hiPSCs) offer a unique in vitro platform for the generation of large quantities of cells for disease modelling, drug screening and ultimately cell based therapy. Development of efficient targeted genome editing/alteration technologies is essential to explore the full potential of stem cells. The CRISPR-Cas9 system has emerged to be a powerful tool in this field due to its effectiveness and robustness in genome engineering at low cost. The system has been used to modify genomes of various species including microbial, plant, animal, and human cells. Genome editing/correction in healthy or patient-derived iPSCs offers one of the most reproducible and relevant approaches for personalized therapy in regenerative medicine. hPSCs are suitable for gene editing as they can be easily engineered and expanded from a single cell state without losing their pluripotency and unique ability to differentiate into various lineages and specialized cells, however this can be challenging due to the variability between different hESCs and hiPSCs. A common and efficient method to develop an hiPSC disease model is to generate a CRISPR mediated knockout of disease relevant genes through non-homologous end joining (NHEJ) and explore the pathogenic mechanism in the derived cells. Isogenic hiPSCs can also be generated using Homology Directed Repair (HDR) in order to correct a genotype, introduce disease relevant mutation or endogenously tag a protein and monitor its expression through differentiation. Here we show, through our iPSC platform created in 2015, how we adapt and characterise commercially available and patient derived PSCs to chemically defined media conditions, select optimal targeting strategies and apply the CRISPR-Cas9 technology to generate disease relevant models with a large range of editing. Moreover, we have developed a good understanding of our customer needs, thanks to our collaboration with biotech companies specialised in hiPSC reprogramming, allowing us to generate a panel of unique gene-engineered cell types, which can then generate functional tissue for disease modelling as well as drug discovery and screening.

F-4044

INVESTIGATION OF TDP RISK OF AZITHROMYCINE USING CARDIOMYOCYTE DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELL

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In the field of cardiovascular safety pharmacology, hERG assay and telemetry are used according to the International Council for Harmonisation (ICH) guideline. However, there are some medications, which were discontinued due to adverse reactions to the heart in clinical application. Therefore, to address this issue the Comprehensive in Vitro Proarrhythmia Assay (CiPA) project has been launched to develop a new method using cardiomyocytes derived from human induced pluripotent stem cell (hiPSC-CM). In this study, we established a new method that can predict the cardiovascular adverse effects of drugs in human using hiPSC-CM with reference to the CiPA. Azimilide, terfenadine, and verapamil were selected as reference drugs which were known to cause high, intermediate and very low TdP risk, respectively. Azithromycin and diphenhydramine were selected as test group because of their cardiac adverse events. To evaluate the functions of heart after administration of these drugs, the field potential duration (FPD) and spike amplitude

were investigated by using microelectrode array (MEA). Azimilide and terfenadine increased FPD by 32.7%, 18%, and verapamil decreased FPD by 15%, while azithromycin and diphenhydramine decreased FPD by 74% and 7%, respectively. Spike amplitude was decreased by 73% for azimilide, 55% for terfenadine, 11% for verapamil, 39% for azithromycin and 11% for diphenhydramine. In addition, we analyzed proteins such as Troponin, ROS, and ion channels as biomarkers to predict cardiac function. Azimilide, azithromycin, and diphenhydramine showed about two fold increases in ROS. Troponin was increased in a dose-dependent manner in all groups. These results showed that azithromycine has a cardiac arrhythmia potential at a moderate level and diphenhydramine has a low level. Taken together, it was possible to evaluate a potential of cardiac adverse events of new molecules using hiPSC-CM in new drug development.

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F-4046

HIGH-THROUGHPUT MICROFLUIDIC PLATFORM FOR DRUG SCREENING OF VASCULARIZED 3D TISSUES

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3D tissues such as spheroids or organoids derived from human pluripotent stem cells (PSCs) represent a new type of three-dimensional in vitro model for understanding organ development, disease mechanism, and drug testing. Despite the success in generating 3D cultures resembling different tissue types (brain, heart, intestine, liver, lung, and kidney), these mini-organs show limited growth potential and an immature phenotype due to lack of vascularization. Several groups have attempted to improve vascularization of organoids by transplanting them into a host (i.e. mouse, chick). However, the low predictivity of animal models, boost the development of in vitro alternative strategies. In this regard, microfluidic techniques are increasingly recognized as important toolbox able to add physiologically relevant cues to traditional cell culture models. We recently described the use of the Organoplate® for generating 3D perusable angiogenic vessels. Here, we present the use of a high-throughput 'grafting' platform which allows vessels co-culture with 3D tissue aggregates and tissue vascularization. One unit of the Mimetas Organoplate® Graft is made of two microfluidic channels in which endothelial cells can be patterned against ECM through the use of the PhaseGuide® technology. Gradient of pro-angiogenic factors (VEGF, PMA, S1P, and FGF-b) allows the formation of a perfused vascular bed on top of which tissue fragments (i.e. organoids or spheroids) can be added to enable vascularization. Tissue dependent vessels remodeling and stabilization can be monitored overtime by real time imaging and barrier integrity. When liver spheroids are used, vessels became leak-tight to dextran 150 kDa after 14 days of co-culture. Moreover, expression of CD31+ cells around and in within the spheroids proves that endothelial cells migration and tissue envelopment occurred during co-culture. The high number of units (up to 64 chips in 384 well format) enables functionality studies and compound screening in a robust and automated way. We propose the use of the Organoplate® Graft as a vessels grafting platform for multiple 3D tissues allowing drug screening and disease modeling in a more physiological environment.

F-4048

ENHANCED OSTEOGENIC CONVERSION OF HUVECS WITH OCT4-30KC19 CELL PENETRATING PEPTIDE AND BMP4 GROWTH FACTOR

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Osteoblasts mineralizes bone matrix thereby play an essential role in bone remodeling. Recently, functional osteoblasts were successfully transdifferentiated from dermal fibroblasts through ectopic expression of RUNX2, OSTERIX, OCT4 and L-MYC. However, this method viral transduction of cells which provides risks such as unwanted genetic change and teratoma formation. Herein, we developed protein-based strategy to induce transdifferenatiaton of endothelial cells into osteoblasts via nuclear delivery of OCT4 protein combined with BMP4 treatment. For the nuclear delivery of OCT4 protein, we created OCT4 recombinant protein fused with 30Kc19, which is derived from silkworm hemolymph of Bombyx mori that has cell-penetrating, protein stabilizing, non-cytotoxic and anti-apoptotic properties. Our results show enhanced osteogenic induction of HUVECs. Cells treated with OCT4-30Kc19 protein combined with BMP4

showed increased gene markers, BSP, OPN and COL I, and strong alizarin red s staining in in vitro. In addition, results from in vivo mouse cranial defect experiment demonstrate successful bone regeneration of HUVECs treated with both OCT4-30Kc19 and BMP4. Ultimately, using protein-based transdifferentiation method allows bypassing cell delivery of genetically modified cells which may induce unintended genetic change and remains as challenge to be used in clinical applications.

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F-4050

CELL RESPONSE TO MECHANO CUE AT EARLY PHASE OF CELL REPROGRAMMING VIA SELF-MODULUS SUDDEN CHANGE

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This study is to report a deterministic sudden change of cell modulus and an F-actin re-polymerization at the early phase of cell reprogramming. The study has been carried out through investigating the adult mice fibroblasts direct reprogramming into neurons via the forced expression of three transcription factors: Ascl1, Brn2, and Myt1l (ABM). After active above exogenous transcription factors via Dox, cell modulus was measured by atomic force microscopy (AFM) and quantitative deformability cytometry (q-DC) every 24 hr, and F-actin was stained by FITC-Phalloidin. Results showed that cell modulus significantly decreased in 24 hr after Dox initiate cell reprogramming and F-actin re-polymerized to a bird's nest structure at the same time. Additionally, it is believed that the process could affect the reprogramming efficiency when somatic cells response to mechano cue. Then, ABM-infected adult mice fibroblasts cultured on PA gels with different stiffness (1kpa, 20kpa, and 40kpa) before and after cell modulus changes, and reprogramming efficiency was quantified by neuron-specific Class III β-tubulin (Tuj1) staining. It is found that 20 kPa and 1kPa PA gels increased the reprogramming efficiency when ABM-infected cells cultured on gels before and after cell modulus change, respectively. This study implies that a maximum mechanical interaction between somatic cell and matrix can occur to efficaciously regulate cell reprogramming efficiency at the early phase of reprogramming when cell modulus is comparable to the matrix stiffness.

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F-4052

ROLE OF INTRACELLULAR STRUCTURES IN THE DIRECT CONVERSION OF FIBROBLASTS INTO NEURONS

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Direct reprogramming is the process of converting from one cell type into a very distantly related cell type without proceeding through an intermediate proliferative stem-cell like stage. Previous studies have shown that somatic cells can be directly reprogrammed into specific neuronal subtypes using different combinations of transcription factors and microRNAs and in addition, biochemical factors can aid in reprogramming these cells into induced neuronal (iN) cells. However, how intracellular structures may regulate iN reprogramming through mechanotransductive pathways is not well understood. Here we show, for the first time, that disruption of actin-myosin contractility via treatment with blebbistatin can enhance the efficiency of iN conversion. The derived iN cells displayed a typical neuronal morphology, expressed neuronal markers and exhibited functional neuronal properties. The involved mechanism relied on the modulation of gene expression patterns by blebbistatin. We found that blebbistatin downregulated fibroblast markers while concurrently upregulated neuronal genes. Furthermore, our findings suggest that focal adhesions and the nuclear lamina play a critical role in iN conversion. In essence, our findings highlight that the disruption of intracellular mechano-structures can regulate the direct reprogramming of fibroblasts into iN cells. By investigating signaling pathways, these novel findings can provide insights into the mechanisms that determine cell fate with potential applications in neurological disease modeling and drug discovery.

F-4054

DIRECT REPROGRAMMING OF HEPATOCYTES INTO PANCREATIC BETA CELLS BY CRISPR-DCAS9 MEDIATED MULTIPLEX ENDOGENOUS GENES ACTIVATION

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Direct reprogramming of autologous cells from diabetes patients to insulin producing cells is a new method for pancreatic cell replacement therapy. At present, trandifferentiation among mature cells is achieved mainly by introducing foreign genes into the starting cells. However, since the chromatin of the endogenous transcription factor is in an inhibitory state, the exogenously introduced transcription factor is transiently expressed, resulting in inefficient and poor maturation of the directly reprogrammed beta cells. Here, we constructed a CRISPR/dCas9 based new Casilio system that targeted Tgif2, Pdx1, Ngn3 and Mafa, which can continuously activate these four endogenous specific transcription factors. We found that when activating single gene by Casilio with targeted gRNA pools, endogenous gene expression levels can increase 10 times. When activating all four factors simultaneously, gene expression increased substantially and can maintain higher levels until 7 days. When these four factors were activated simultaneously in Ins1-EGFP reporter HepG2 cells, Ins1-EGFP+ cells can be found at 24 hours, and peaked at 72hrs. The expression of various islet beta cell specific genes in these cells was detected at the mRNA level and protein level. In conclusion, we provide a new approach for the treatment of diabetes by direct reprogramming of liver cells to insulin producing cells through CRISPR-dCas9.

Funding Source: Project 81800686 supported by National Natural Science Foundation of China

F-4056

CONVERSION OF MOUSE FIBROBLASTS INTO OLIGODENDROCYTE PROGENITOR LIKE CELLS THROUGH A CHEMICAL APPROACH

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Transplantation of oligodendrocyte progenitor cells (OPCs) is a promising way for treating demyelinating diseases. However, generation of scalable and autologous sources of OPCs has proven difficult. Here we found that chemical condition M9 could specifically initiate neural program in mouse embryonic fibroblasts, and induce the generation of colonies that underwent mesenchymal-to-epithelial transition at the early stage of reprogramming, which represent unstable and neural lineage-restricted intermediates that have not established a neural stem cell identity. By modulating the culture signaling recapitulating the principle of OPC development, these intermediate cells could be reprogrammed towards OPC fate. The chemical-induced OPC-like cells (ciOPLCs) resemble primary neural stem cell-derived OPCs in terms of their morphology, gene expression, and the ability of self-renewal. Upon differentiation, ciOPLCs could produce functional oligodendrocytes and myelinate the neuron axons in vitro, validating their OPC identity molecularly and functionally. Our study provides a non-integrating approach to OPC reprogramming that may ultimately provide an avenue to patient-specific cell-based or in situ regenerative therapy, and conceptually extends our previously established Cell Activation and Signaling Directed-reprogramming paradigm to a chemical-based lineage-specific manner.

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F-4058

CYTOSINE BASE EDITOR GENERATES SUBSTANTIAL OFF-TARGET SINGLE NUCLEOTIDE VARIANTS IN MOUSE EMBRYOS

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Genome editing holds promise for correcting pathogenic mutations. However, it is difficult to determine off-target effects of editing due to single nucleotide polymorphism in individuals. Here, we developed a method named GOTI (Genome-wide Off-target analysis by Two-cell embryo Injection) to detect off-target mutations by editing one blastomere of two-cell mouse embryos using either CRISPR-Cas9 or base editors. Comparison of the whole genome sequences of progeny cells of edited vs. non-edited blastomeres at E14.5 showed that off-target single nucleotide variants (SNVs) were rare in embryos edited by CRISPR-Cas9 or adenine base editor, with a frequency close to the spontaneous mutation rate. In contrast, cytosine base editing induced SNVs with over 20-fold higher frequencies, requiring a solution to address its fidelity.

F-4060

OPTIMIZATION OF HOMOLOGY-DIRECTED REPAIR USING CRISPR/CAS9 SYSTEMS

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Repertoire of genomic alterations via CRISPR/Cas9 genome editing depends on the efficiency of dsDNA breaks and the subsequent DNA repair events, which consist of two distinct pathways: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). HDR enables precise genome modifications which can be used to mimic disease relevant genotypes or to integrate functional domains of interest into the genome. However, the efficiency of HDR via genome editing remains quite low, mostly ranging from 0.1 to10%. Although several strategies have been claimed to enhance

HDR efficiency, there are still concerns about the variability and reproducibility. We surveyed factors influencing HDR efficiency and explored the possibility of optimizing conditions by combinatorially modulating them. Highly efficient NHEJ without a donor is pre-requite for the success. Chemical modifications of the gRNAs and the donor template seem to have significant roles in increasing HDR by stabilizing the molecules. Some small molecule NHEJ antagonists successfully increased HDR in multiple cell lines. Notably, the choice of CRISPR system delivery appears to have the most profound effect on the HDR efficiency.

F-4062

COST-EFFECTIVE DIFFERENTIATION OF HUMAN IPS CELLS TO PANCREATIC BETA-CELLS BASED ON AGGREGATE SUSPENSION CULTURE WITH DIALYSIS OPERATIONS

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The production of insulin-secreting pancreatic β-cells from human induced pluripotent stem cells (hiPSCs) is a promising strategy to solve the donor shortage problem in cell transplantation therapy for diabetes. However, the production cost of pancreatic β-cells is extremely high, because the high-priced cytokines are necessary for induction of hiPSCs' differentiation to pancreatic β-cells. Furthermore, the cytokines remaining in the used culture medium and possible autocrine factors secreted by cells are removed along with toxic metabolites when the culture medium is exchanged daily. In this study, in order to reduce the production cost of pancreatic β-cells, we investigated the feasibility of dialysis suspension culture system in differentiation induction of hiPSCs into pancreatic β-cells. This culture system enables to minimize the use of growth factors by their retention and full utilization of autocrine factors in the cell culture compartment. As the first step, we examined the feasibility in the most expensive Stages 1 and 6 among the 6 stages of the differentiation protocol. Stage 1 is the induction Stage for definitive endoderm and Stage 6 is the maturation stage of endocrine progenitor cells to pancreatic β-cells. For the dialysis suspension culture, we developed a simple culture device consists of flat-type dialysis membrane-loaded culture insert for the cell culture compartment and a deep well for the dialysate compartment. Such a dialysis culture remarkably improved the cultural environment with continuous glucose supply and lactate removal. Furthermore, it was possible to produce cells without lowering the gene expression level with a smaller amount of growth factors, possibly through by retaining and reusing the growth factors and the autocrine factors in the culture medium, Particularly in Stage 6, the gene expression levels of insulin were increased. These findings demonstrates that dialysis suspension culture system is a very promising method for cost-effective and scalable production of pancreatic β-cells for future implantation-based treatments of diabetic patients.

Funding Source: This research was partially funded by AMED-Kyoten B (Japan).

F-4064

SIMPLIFYING DEPOSIT OF IPSC LINES INTO THE EUROPEAN BANK FOR INDUCED PLURIPOTENT STEM CELLS

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The European Bank for induced Pluripotent Stem Cells (EBiSC, cells.ebisc.org) is a centralised, non-profit repository and distribution hub ensuring long term storage of deposited hiPSC lines and their distribution to academic and commercial researchers worldwide. In a second project phase launched in March 2019 (EBiSC2), EBiSC supports researchers through provision of high quality iPSC lines by allowing researchers to deposit iPSC lines into EBiSC and distribute the lines on their behalf. EBiSC has now established simplified procedures to ease deposition to increase deposit of further existing iPSC lines and thus ensure researchers can access these vital resources long term. In early cases, Depositors occasionally used consent templates which limited downstream use of donated Biosamples, leading to re-consenting or inability to proceed with iPSC line deposit. Reliance on manual data collection was also time-consuming and error prone, especially for large cohorts of iPSC lines. Lastly, institutions needed support to

finalise EBiSC deposit agreements. Using input from ethical and scientific leaders, EBiSC developed consent templates and a flexible yet robust review process that maximises utility of donated Biosamples whilst providing suitable research and ethical provenance to meet regulatory standards. Use of the human Pluripotent Stem Cell Registry (hPSCreg.eu) for direct data input by depositors bypasses reliance on burdensome methods for manual data collection and enables mass data uploads through direct database data transfer. Direct guidance for legal representatives is established to progress completion of deposit agreements. Using these experiences, EBiSC is now guiding hiPSC research projects from the outset in establishing ethical and legal frameworks compliant with deposition into a public repository. With these developments and collaborations over the past 5 years, EBiSC has successfully safeguarded iPSC lines generated by projects such as StemBANCC and HipSci, created mutual benefits across consortia, promoted scientific excellence and reduced duplication of efforts and public funds. A second project phase, EBiSC2, strives to supply iPSC related services such as cell line generation and gene editing, Quality Control, automated data input and streamlined governance processes.

Funding Source: The EBiSC and EBiSC2 projects have received funding from the Innovative Medicines Initiative Joint Undertaking (JU) and EFPIA under grant agreement No 115582 and No 821362 respectively.

F-4066

GENE EDITED INDUCED PLURIPOTENT STEM CELL-BASED THERAPIES IN PATANT APPLICATIONS

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Patent application tends were investigated for induced pluripotent stem cell (iPSC)-based therapies in PCT applications from 2006 to 2018. The number of patent applications for iPSC technologies was 1966 with 3% of applications for gene edited iPSC-based therapies. Patent applications for iPSC-based therapies described zinc-finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR/Cas) system in claims were extracted. The first patent application for gene edited iPSC-based therapy using CRISPR/Cas system was filed in 2013, then the number of applications has been increasing dramatically. In contrast, the first patent applications using ZFN and TALEN systems were filed in 2010 and 2012, respectively, however, the patent applications using these systems after 2015 also included CRISPR/Cas system. The patent applications for gene edited iPSC-based therapies were categorized into 14 disorder groups: neurodegenerative/neurodevelopmental, blood, metabolic, immune, bone/cartilage, ophthalmological, skin disorders, trisomy syndrome, pain, and infertility. Most patent applications were using CRISPR/Cas system for 10 disorder groups, which mostly included neurodegenerative/neurodevelopmental, blood, and metabolic disorders. The patent applications for gene edited iPSC-based therapies were filed by applicants in the United States, Switzerland, Belgium, Spain, France, Germany, and Republic of Korea. Most patent applications were filed by Switzerland applicant and US applicants. CRISPR Therapeutics, AG was filed the patent applications for gene edited iPSCs or iPSC-derived cells used CRISPR/Cas system for treatment of neurodegenerative, metabolic, blood, immune, skin disorders, and pain. Sangamo Biosciences, Inc. was filed the patent applications for gene edited iPSC used ZFN system for treatment of immune, neurodegenerative, blood, digestive, ophthalmological disorders, cancer, and infection.

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F-4068

PLANT-DERIVED CELLULOSE HYDROGEL AS A MATRIX FOR 3D HUMAN STEM CELL PROLIFERATION AND DIFFERENTIATION

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In the pursuit of in vitro cell models that are biological relevant with improved functionality, new materials and methods for creating three-dimensional (3D) cell culture systems are a key requirement. GrowDex®, nanofibrillar cellulose (NFC) hydrogel which is derived from the Birch tree, has been shown to provide an effective support matrix for culturing various cell types in 3D. 1) hESC (WA07) and hiPSC (iPS(IMR90)-4): Cell colonies were embedded in 0.5% GrowDex (UPM) in mTeSR1 (STEMCELL Technologies) media and cultured on 96-well plate up to 26 days. hPSCs pluripotency was analysed with OCT4 and SSEA-4 marker expression, in vitro EB-mediated differentiation, and teratoma assay. 2) hESC derived neuronal cells: Pre-differentiated neuronal cells were embedded in 1.5 and 1.0% GrowDex. Formation of the neuronal networks was evaluated by immunocytochemical staining against neuronal markers MAP-2 and β-Tubulin III and confocal microscopy. 3) Adipose tissue derived hMSCs: Cells were embedded in 0.2% GrowDex in DMEM media and transferred on 24-well tissue culture inserts. Adiopogenic differentiation and osteogenic differentiation were induced with StemProTM differentiation kits (ThermoFisher) for 21 days, and analysed with Oil Red O and Alizarin Red S stainings. 1) hESC and hiPSC proliferated in GrowDex without

feeder cells, formed spheroids with 100-200 µm diameter, and the cells remained their pluripotency throughout the 26 day study. 2) GrowDex supported the 3D growth of hESC derived neuronal cells. The formation of neurospheres and neuronal networks by neurite outgrowth were observed. 3) Adipose tissue derived hMSCs were able to differentiate to both adipogenic, and osteogenic direction in GrowDex 3D culture. GrowDex offers a well-defined, tunable 3D culture matrix for various regenerative medicine applications. Authors would like to thank Yan-Ru Lou, Tiina Joki and Jonathan Sheard for performing the experiments.

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