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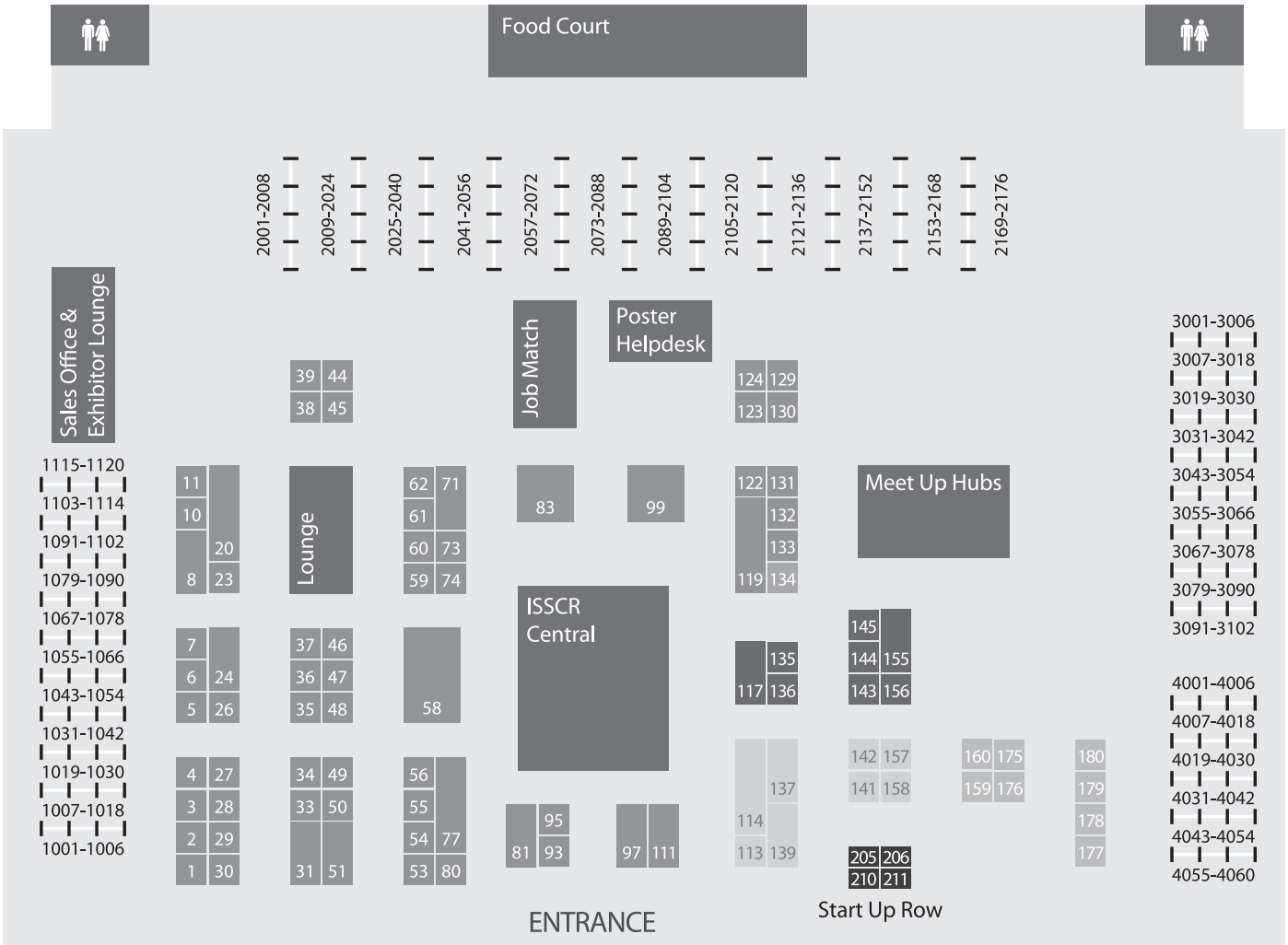

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WEDNESDAY, 20 JUNE, 2018

POSTER SESSION I-ODD
18:30 – 19:30

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-1001

HYPOXIA-ACTIVATED BICAUDAL D HOMOLOG 1 VIA AKT/MTOR/GSK-3β PATHWAY STIMULATES HIF-1α-MEDIATED GLYCOLYSIS AND ANTI-APOPTOSIS IN UMBILICAL CORD BLOOD-DRIVED HUMAN MESENCHYMAL STEM CELLS

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Adaptation to low oxygen tension is an essential process to maintain function and survival of stem cells under hypoxia. Hypoxia-inducible factor-1α (HIF-1α) is a major transcriptional regulator leading to hypoxia adaptation. However, it has been poorly understood that how hypoxia-induced HIF-1α translocates into the nucleus in the stem cells. Here, we investigated the role of a dynein adaptor protein BICD1, a bicaudal D homolog 1, in the HIF-1α-induced glycolysis and survival in the umbilical cord blood-derived human mesenchymal stem cells (UCB-hMSCs) under hypoxia. Our results firstly showed that hypoxia-induced nuclear translocation of HIF-1α is inhibited by pretreatments of nocodazole and ciliobrevin D, microtubule destabilizer and cytosolic dynein inhibitor, respectively. Silencing of BICD1 but not BICD2 blocked hypoxia-induced HIF-1α nuclear translocation and hypoxia response element (HRE) promoter activation. Furthermore, BICD1 overexpression enhanced hypoxia-induced nuclear translocation of HIF-1α. BICD1 silencing abolished hypoxia-induced mRNA expressions of glycolysis-related genes, HK1, LDHA1 and G6PD, hexokinase activity, lactate production, NHE1 mRNA expression and intracellular alkalization. Hypoxia stimulates the binding of HIF-1α to BICD1 and intermediate chain of dynein (Dynein IC). And, BICD1 silencing abolished the hypoxia-induced binding of HIF-1α to Dynein IC. Pretreatments of PI3K/AKT inhibitor wortmannin and mTOR inhibitor rapamycin inhibited the binding of HIF-1α to BICD1 and nuclear translocation of HIF-1α. Conversely, AKT activator SC-79 pretreatment and GSK-3β silencing enhanced hypoxia-induced nuclear translocation of HIF1α and HRE promoter activation. In conclusion, we demonstrated that hypoxia-activated AKT/mTOR/GSK-3β pathway stimulates nuclear translocation of HIF-1α through BICD1 activation, leads to glycolysis and anti-apoptosis in UCB-hMSCs.

W-1003

A POTENTIAL THERAPY FOR FETAL MYELOMENINGOCELE BY HUMAN AMNIOTIC FLUID STEM CELLS

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In spite of a poor prognosis of fetal myelomeningocele (fMMC), the options of prenatal treatments are still limited. The aim of this study is to investigate the therapeutic effects of concentrated human amniotic fluid stem cells (hAFSs) on fMMC. hAFS were isolated as CD117-positive amniotic fluid cells and these cells were proliferated in vitro. Sprague-Dawley rat dams were orally administrated retinoic acid to induce fMMC. On E 17, the model rats were inoculated with 1.0 × 105 cells/fetus of hAFSs suspended in PBS (group I) or PBS only as controls (group II). On E 21, the exposed area and the cross-section of the spinal cord were analyzed. To demonstrate the lesional change, the neuron / astrocyte ratio and the survival and characteristic of hAFS were investigated by immunohistochemistry. In addition, the expression of growth factors was analyzed by real-time PCR. While the hAFS inoculation significantly reduced the exposed area of the spinal cord (group I vs. group II; $p < 0.05$), the cross-sectional area of the spinal cord and the proportion of neurons were significantly increased in group I compared to group II ($p < 0.05$). Moreover, the decline of the neuron / astrocyte ratio ratio in group I compared to group II might show the ability of hAFS to reduce astrogliosis (scar formation) (group I vs. group II; $p < 0.05$). These results indicated that hAFS covered exposed spinal cord and supported neural regeneration. Human-derived cells survived on the surface of the spinal cord and some of these cells expressed cytokeratin, which implied that hAFS could differenciated into skin to cover the spinal cord and protect it from physical damage. Additionally, among growth factors analyzed in this study, hepatocyte growth factor (HGF) was highly expressed in the spinal cord of group I (vs. group II; $p < 0.05$). As HGF can suppress scar formation, taken all together, hAFS could cover and protect the exposed spinal cord and they also could secrete HGF to promote neural regeneration with less fibrotic scarring (i.e. glial

scarring) in the spinal cord. fMMC can be diagnosed during pregnancy and hAFS isolated from those patient can be proliferated in vitro. Therefore, the intraamniotic administration of concentrated hAFS could be a novel strategy to treat fetal MMC.

W-1005

ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELL FROM MACACA FASCICULARIS'S AMNIOTIC MEMBRANE: FIRST STEP TO APPLICATION ON DURAPLASTY PROCEDURE IN ANIMAL MODEL OF MACACA FASCICULARIS

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Dura mater has a vital function to protect all the anatomical and physiologic process of brain. Dura mater tears cause many fatal complication. Amniotic membrane have cellular content, physical and biochemical characteristic that could be use as a graft for duraplasty procedure. Amniotic membrane has two main stem cell i.e mesenchymal and epithelial stem cells. Because of dura mater develop from mesodermal lineage so that mesenchymal stem cell of amniotic membrane can predict has a role in dura mater's defect healing process. The objective of the study is to isolate and characterize of placenta-derived mesenchymal stem cell (MSCs) from amniotic-membrane of Macaca fascicularis. We have done the isolation and characterization of mesenchymal stem cell from amniotic membrane of placenta from Macaca fascicularis. This study was approved by the Institutional Animal Care and Use Committee (IACUC) Laboratory Animal Research, PT. Bio Farma (Persero), Bandung, Indonesia and by the Ethical Committee of Universitas Padjadjaran, Bandung, Indonesia. Five amniotic membrane from placenta that already free of infection of TBC, SIV, SV40, Polio and Foamy virus. All the cells already subculture for three times and we observe for the number of cells, immunophenotyping and cells differentiation. Cell caryotyping had performed to confirm the numbers of diploid chromosome of Macaca fascicularis. Cells culture and isolation show the adherent cells like fibroblastic-like cells. We obtained a mean of 147 x106 cells from the third passage of amniotic membrane. Karyotyping had perform to confirm the diploid chromosome integrity. FACS examination show that low expression of CD11b, CD19, CD34 and CD45; 98.97% of CD73-APC; 96,80% of CD90-FITC, 58.5% CD105-perCP from passage 2 and 3. Osteogenic, chondrogenic and adipogenic differentiation potential of placenta-derived mfMSC obtained from the isolation and culture process. Our study demonstrated that mechanical and enzymatic method can be used to obtain a sufficient number of mfMSCs amniotic-membrane. Furthermore, placenta-derived MSCs may be useful as a cell therapy application and has the potential in clinical application as in duraplasty procedure.

Funding Source: Research Grant from Ministry of Research, Technology and Higher Education of RI (PDD Scheme) No 1/E3/KPT/2018 for HB; (PUPT Scheme) No 30/E/KPT/2017 for AF. Internal Funding of PT.Biofarma (Persero) Indonesia for RN.

W-1007

CDX2 IS AN ESSENTIAL GATEKEEPER OF MOUSE TROPHOBLAST PLURIPOTENCY

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Cdx2 is crucial for the establishment of the murine trophoblast lineage, but the effect of Cdx2 loss on established trophoblast cells is unknown. We have generated ATACseq and RNAseq libraries from wild-type (WT) and Cdx2 knockdown (KD) trophoblast stem cells (TSCs) in vitro. Cdx2 KD triggers increased chromatin accessibility, which is reminiscent of what happens upon TSC differentiation. Consistent with this observation, the peaks with increased accessibility in KD cells are enriched for the Tfap2c consensus motif; a key player in TSC differentiation. Tfap2c is significantly upregulated in Cdx2 KD cells and likely contributes to the activation of newly accessible cis-regulatory elements given that many of these sites display Tfap2c occupancy. Furthermore, RNAseq analysis revealed significant transcriptional perturbation as a consequence of Cdx2 loss. By integrating published RNAseq differentiation data sets into analyses, it was established that those genes that are differentially expressed in Cdx2 KD compared with WT cells significantly overlap with changes observed as TSCs differentiate. Moreover, Cdx2 KD cells terminally differentiate into trophoblast giant cells, suggesting that Cdx2 prevents TSC differentiation and thus maintains pluripotency.

W-1009

COMBINED ANTI-TUMOR EFFECTS OF TRAIL INDUCED BY IL-1B IN HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS ON EMBELIN TREATED BREAST CANCER CELLS

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Human umbilical cord mesenchymal stem cells (HUCMSCs) have demonstrated high therapeutic value in cancer treatment owing to their ability of homing to cancer inflammation site. Our previous study has shown that pre-stimulated HUCMSCs with cytokines promotes tumor necrosis factor-related apoptosis inducing ligand (TRAIL) expression and enhances cancer suppressive activity. Furthermore, increasing evidence indicate that embelin, an active extraction of Embelia ribes, induces apoptosis in several cancer cell lines via upregulated TRAIL receptor 1 (DR4) and TRAIL receptor 2 (DR5). This study was conducted to examine the effect of embelin on apoptosis and DR4 and DR5 expression of the MDA-MB-231 and MCF-7 breast cancer cell lines and to investigate potential mechanisms involved in apoptosis pathways. Whether IL-1β induced TRAIL-expressing HUCMSCs, in combination with low-dose embelin, would further induce apoptosis in both breast cancer cell lines was also examined. In this study, MTT assay showed dose-dependent effect of embelin treatment ranging from 0 to 25 μM in 24 and 48 hr. Western blotting and immunofluorescence staining were conducted to determine DR4 and DR5 expression after treating with low-dose embelin. Additionally, embelin regulated caspase 8 or caspase 9 protein levels potentially leading to different apoptosis pathways were also examined. Moreover, flow cytometry confirmed that co-culture of IL-1β activated HUCMSCs and embelin treated MDA-MB-231 enhances the apoptosis rate of MDA-MB-231. In conclusion, we suggested that embelin stimulated the expression of DR4 and DR5 in breast cancer cell lines, and that IL-1β activated HUCMSCs in combination with embelin inhibited MDA-MB-231 cells proliferation through TRAIL-related pathways.

Funding Source: This work was supported by a research grant from Ministry of Science and Technology, Taiwan (MOST-104-2320-B-010-009-MY3).

W-1011

HUMAN AMNION EPITHELIAL CELL-DERIVED EXTRACELLULAR VESICLES RESCUE ACUTE AND CHRONIC PULMONARY DAMAGE IN MOUSE NEONATAL LUNG INJURY

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Despite advances in neonatal care, bronchopulmonary dysplasia (BPD) remains a significant contributor to infant mortality and morbidity. We have previously showed that human amnion epithelial cells (hAECs) can be a viable source of cell therapy for established experimental bronchopulmonary dysplasia (BPD), and extracellular vesicles (EVs) derived from pro-regenerative cells could be a potential remedy by transporting bioactive cargo. Here we characterized hAEC-derived EVs and assessed their therapeutic potential on a mouse model of BPD-like injury where a combination of antenatal inflammation and postnatal hyperoxia was used. The isolated hAEC-EVs had distinct cup shaped morphology with average size of 40-120nm. ALIX (96kDa), Grp94 (92kDa) and HLA-G (38kDa) were expressed in EVs and Pathway enrichment analysis showed that endothelin signaling pathway, Wnt signaling pathway, and inflammation mediated by chemokine and cytokine signaling pathway were enriched in hAEC-EVs. In mouse model of BPD-like lung injury, we observed that hAEC-EVs improved tissue-to-airspace ratio and septal crest density in a dose-dependent manner. hAEC-EV administration reduced the levels of inflammatory cytokines interleukin (IL)-1β, tumour necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF). The improvement of lung injury was associated with the increase of the percentage of type II alveolar cells. Surprisingly, neonatal hAEC-EV delivery reduced airway hyper-responsiveness and shifted pressure-volume loops. It also mitigated pulmonary hypertension and prevented right ventricle hypertrophy that associated with BPD-like lung injury, and this persisted until to 10 weeks of age. These findings suggest that hAEC-derived EVs can be a potential cell-free therapy for babies at risk of developing BPD.

ADIPOSE AND CONNECTIVE TISSUE

W-1013

TREATMENT RESISTANT STEM CELL SUBPOPULATION DEPLETION IN MORBID OBESITY AND DIABETES MELLITUS

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John Morton - *Stanford University, Stanford, CA, U.S.*
Geoffrey Gurtner - *Stanford University, Stanford, CA, U.S.*

Morbid obesity and diabetes mellitus are increasing globally but how they affect stem cell health and function remains poorly understood. We examine their impact on unique subpopulations of adipose-derived stem cells (ADSC) in humans and the extent to which depletion of these important subpopulations is rectifiable through conventional therapies such as diet, exercise and bariatric surgery. Adipose tissue samples were taken from over 100 patients during elective surgery (bariatric, post-bariatric and controls), with serial sampling when possible. In parallel, the effects of exercise and diet on recruitment of stem cells and subpopulations within adipose tissue were examined in human and murine studies. Blood samples were taken pre- and post-exercise in 12 patients and again following a 4-week exercise program in half the group with the remainder forming untrained controls. Diet effects were studied using a diet induced obesity model with crossover to normal chow across both short term (6 months) and long-term (18 months) periods with all experiments duplicated. The stromal vascular fraction of subcutaneous fat was isolated by fluorescence cytometry (CD45-CD31-CD34+). Single cell microfluidics was performed with hierarchical cluster-based analysis to examine for subpopulation changes between groups. Subpopulations were validated at a protein level by mass cytometry (CYTOF), including markers for stemness (Sox-2, Nanog, Oct 3/4) and relevant subpopulation surface markers (CD26, CD55). Subpopulations were further validated through cluster-based analyses including ViSNE, SPADE and CITRUS. Obesity and diabetes mellitus are both associated with reduction in a specific ADSC subpopulation that is potently pro-angiogenic. Subpopulation depletion is strongly associated with impaired wound healing ($p<0.05$, One-way ANOVA). Despite correction of diabetic status and massive weight loss, subpopulation depletion is not reversed in post-bariatric patients ($p<0.0001$), ANOVA). Exercise did not increase recruitment of these stem cell subpopulations and diet was unable to restore them to pre-morbid levels. These data offer a new paradigm for understanding stem cell health and function in obesity and diabetes mellitus, prompting the need for novel therapies to address this unmet clinical need.

Funding Source: This study was supported by: Fulbright Commission, National Health and Medical Research Council of Australasia, National Institutes of Health, Royal Australasian College of Surgeons, Australasian Foundation for Plastic Surgery.

W-1015

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

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

the range of 8-25 µm. hADSCs purified by the hybrid membrane migration method showed much better osteogenic differentiation ability than hADSCs isolated from the conventional culture method.

W-1017

TRANSCRIPTOMIC ANALYSIS OF ADIPOSE DERIVED STEM CELLS ISOLATED FROM PATIENTS WITH LIPIDEMA, A FAT DISORDER OF UNKNOWN ETIOLOGY

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Adipose derived stem cells (ADSC) are precursors for mature adipocyte development and fat deposition. Impairment in ADSC function can lead to disorders of adipogenesis. Lipedema is poorly understood fat disorder that presents as symmetrical deposition of fat in subcutaneous tissues of the lower limbs, with the feet often spared. Lipidema occurs predominantly in females, often at the onset puberty, suggesting an involvement of female hormone. Some reports suggested that lipedema has affected approximately 11% of all females worldwide. The etiology is unknown, although familial genetic factors are likely to be involved. Proper diagnosis and treatments available to patients with lipedema is lacking. To begin to understand the cause of this disease, we analysed tissue taken from patients diagnosed with lipidema by immunohistochemistry to assess the lymphatic vessels, adipocytes size, macrophages and ECM structure and found significant differences in tissue architecture compared to healthy control samples. To understand mechanisms involved in lipedema development, we have isolated ADSC from lipedema patients and compared them to those from healthy control fat tissue. Next-generation RNA sequencing of RNA from lipedema and control ADSCs revealed more than 924 (up>624 and down>246) significant (<0.05) differentially expressed genes. Pathway analysis showed changes to proliferation, cell cycle, cell adhesion, ECM and cytoskeleton remodelling signalling networks in cells from Lipidema patients. This study will facilitate our understanding of the cellular factors and mechanisms involved in lipedema development which may open new possibilities for therapeutic strategies to treat lipodema.

W-1019

THE REGULATORY EFFECT OF MICRORNAS IN THE CHONDROGENESIS OF CANINE ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Canine osteoarthritis (OA) is a common cause of chronic pain and lameness in dogs, and it is characterized by the destruction of articular cartilage, osteophyte formation, sclerosis of the subchondral bone, etc. In dogs with OA, it's generally believed that the cartilage homeostasis is changed, and the balance between biosynthesis and degradation of extracellular matrix (ECM) is disturbed. Some studies have shown that using stem cell therapy in OA patients might reverse the arthritic pathology and even regenerate cartilage. Therefore, canine adipose-derived mesenchymal stem cells (cADMSCs) have been used in OA canines by intra-articular injection, and they showed better and longer pain-relieving effect compared to the traditional analgesic therapy. MicroRNAs (miRNAs) are a class of small (18-22 nucleotides) non-coding single-stranded RNAs, which mediate post-translational gene silencing by binding to the 3'UTR region of the targeted messenger RNAs (mRNAs). Recent studies have demonstrated that miRNAs can regulate chondrogenesis by targeting Sox family (Sox9 and Sox5), for example, in human and murine mesenchymal stem cells (MSCs); however, there are no reports about canine mesenchymal stem cells. Therefore, the objective of this study is to find out the regulatory effects of miRNAs in the chondrogenesis of cADMSCs. We identify some miRNAs that showed different expression patterns at day0 (pre-induction), week1, and week2 of the chondrogenesis via RT-qPCR. This result provides the information that miRNAs might play a role in the chondrogenesis of cADMSCs just like in human and murine cells, and that they may be applied in conjunction with cADMSCs to elevate the efficacy of stem cell therapy in OA dogs.

Funding Source: National Taiwan University- Yonglin Humane Project

MUSCULOSKELETAL TISSUE

W-1021

RECAPITULATION OF EXTRACELLULAR LAMININ ENVIRONMENT MAINTAINS STEMNESS OF SATELLITE CELLS IN VITRO

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Satellite cells function as precursor cells in mature skeletal muscle homeostasis and regeneration. In healthy tissue, these cells are maintained in a state of quiescence by a microenvironment formed by myofibers and basement membrane in which laminins form a major component. Here we evaluated the satellite cell microenvironment *in vivo* and found that these cells are encapsulated by laminin α2-5. We sought to recapitulate this satellite cell niche *in vitro*by culturing satellite cells in the presence of recombinant laminin-E8 fragments. We show that treatment with laminin-E8 promotes proliferation of satellite cells in an undifferentiated state, through reduced phosphorylation of JNK and p38. On transplantation into injured muscle tissue, satellite cells cultured with laminin-E8 promoted the regeneration of skeletal muscle. These findings represent an efficient method of culturing satellite cells for use in transplantation through the recapitulation of the satellite cell niche using recombinant laminin-E8 fragments.

Funding Source: Supported by AMED and MEXT, Japan

W-1023

VOLTAGE-OPERATED CALCIUM CHANNELS AS MODULATORS OF CHONDROGENIC DIFFERENTIATION IN HUMAN MESENCHYMAL STEM CELLS

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Degradation of cartilage tissue caused by trauma or mechanical overload, leading to the chronic and progressive degenerative joint disease osteoarthritis has become a global problem for which no efficient therapy is available so far. Cell-based therapies using mesenchymal stem cells (MSCs) seem promising candidates for the treatment of cartilage lesions. During MSCs differentiation, calcium ions (Ca2+) play a crucial role in regulation of cellular functions, as the increase of intracellular Ca2+ was shown to improve cell differentiation potential. Although Ca2+ can enter cells through different pathways, voltage-operated calcium channels (VOCCs) have attracted an exclusive interest due to the significance of mechanical load and mechanotransduction for cartilage tissue maintenance. The aim of this study was to evaluate the effects of two different VOCCs regulators - antagonist Nifedipine and agonist BayK8644 on chondrogenic differentiation capacity of MSCs, isolated from two different sources - bone marrow (BMMSCs) and menstrual blood (MenSCs). BMMSCs and MenSCs were characterized to confirm classical stem cell properties, including typical cell surface markers and trilineage differentiation capacity. Intracellular calcium was measured using fluorescent dye Cal-520 (flow cytometry). Our results showed that in contrast to BMMSCs, MenSCs exhibited higher expression of CD10 and Notch1, and higher proliferative capacity, as determined by CCK-8 kit. Different patterns of intracellular Ca2+ oscillations in both cell types, being more frequent in MenSCs, were observed by fluorescence microscopy. Application of VOCCs regulators confirmed VOCC contribution to increased intracellular Ca2+ levels in MenSCs and BMMSCs. For the induction of chondrogenic differentiation, TGF-β3 was more efficient in BMMSCs, while activin A in MenSCs. Furthermore, chondrogenic differentiation of both cell lineages was differentially affected by Nifedipine or with Bay-K8644. In conclusion, this data demonstrates different regulation of chondrogenic differentiation of human MSCs from alternative sources. Moreover, chondrogenic differentiation is modulated through VOCCs, suggesting that they may become a perspective target for stimulation of cartilage regeneration.

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W-1025

THE EFFECTS OF RRM2B ON THE REGENERATION OF SKELETAL MUSCLE

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Wan-Jing Chen - *Taipei Medical University, Taipei, Taiwan*

Aging is the process that appears as a biological limit to lifespan. Previous reports indicated aging is a threat for skeletal muscle in mammals and is associated with a progressive loss of muscle mass, quality and strength. When people are getting older, resulting in severe muscle defects, this is termed sarcopenia. We hope to find a way that can efficiently measure the function of skeletal muscle, and improve the repair and regenerating capacity of muscle. Furthermore, we can come up with a therapeutic method that can cure sarcopenia and myopathy, even prevent skeletal muscle from aging. Rrm2b, the small subunit of ribonucleotide reductases (RNR), is not only induced by p53 with DNA damage, but also constitutively express in proliferating and non-proliferating cells. In the patients with mitochondrial depletion syndrome, RRM2B is direct correlated to severe muscle mitochondrial DNA depletion. Rrm2b deficiency in mice leads to systemically atrophy in skeletal muscle, while no clear mech-

anisms have been explored. We found Rrm2b is highly related to the differentiation of skeletal muscle, even to the regenerated capacity in damage or aged muscle. In this project, we will demonstrate that Rrm2b has a critical function on repair and regeneration of skeletal muscle and provide a chance for establishment of cell therapy in skeletal muscle disorders.

Funding Source: Ministry of Science and Technology (Taiwan) MOST 105-2320-B-038 -022 -MY3

W-1027

LINC-ROR PROMOTES OSTEOGENESIS DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELL THROUGH MIRNA SPONGING AND WNT/B-CATENIN SIGNALING ACTIVATION

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Long noncoding RNAs (lncRNAs) have been widely studied for their novel regulation mechanism. LncRNAs could regulate transcription activation, transcription interference and chromatic modification in nucleus as well as mRNA splicing, miRNA site masking and miRNA decoying in cytoplasm. LncRNA was revealed to be involved in various biological process as well as disease progression. Recent studies have shown that some lncRNAs could regulate osteogenesis differentiation in bone marrow derived mesenchymal stem cells (MSCs), which suggesting lncRNA as a potential therapeutic strategy for MSC-mediated bone regeneration. As a newly identified lncRNA, human linc-ROR was first identified to regulate the cell reprogramming of induced pluripotent stem cells and human embryonic stem cell stemness. However, the role which linc-ROR plays in the osteoblast differentiation of MSCs is still yet to be investigated. In this study, linc-ROR was found to be upregulated during osteogenesis of human bone marrow MSCs. Linc-ROR overexpression significantly accelerated MSC osteoblast differentiation in vitro and stimulated bone formation in vivo. Bioinformatic prediction and luciferase reporter assay results indicated that linc-ROR could decoy miR-138 and miR-145, both of which were negative regulators of MSC osteogenesis process as well as Wnt/β-catenin signaling activity. Further investigations revealed that linc-ROR antagonized the functions of these two miRNAs and led to ZEB2 upregulation, which further activated Wnt/β-catenin pathway and osteogenesis differentiation. In summary, linc-ROR modulates Wnt/β-catenin pathway by acting as a miRNA sponge to decoy miR-138 and miR-145, which may uncover unknown function of lncRNAs in coordinating MSC osteoblast differentiation and in vivo bone formation.

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CARDIAC TISSUE AND DISEASE

W-1029

PROFILING COMPOUNDS WITH KNOWN CARDIOTOXIC MECHANISMS OF ACTION USING PHYSIOLOGICALLY-RELEVANT, ANISOTROPIC, 384-WELL HIGH THROUGHPUT HIPSC-CARDIOMYOCYTE CULTURES

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Drug removal from the clinical market, as well as late-stage failures in clinical trials, are often linked to unforeseen cardiac toxicity. hiPSC-CMs are an integral component of a new paradigm, the Comprehensive in vitro Proarrhythmia Assay (CiPA) Initiative, through which panels of compounds with known mechanism of cardiotoxicity are being evaluated in hiPSC-CM platforms across independent test sites and through cutting-edge technologies. Key challenges under consideration for the hiPSC-CM system are sub-ideal cardiomyocyte geometry, sub-cellular structural organization, and electro-physiological maturity. Bioengineering approaches developed to enhance hiPSC-CM maturity have shown improvements in aspects of hiPSC-CM physiology, however those approaches have limited scalability and thus are not amenable to high throughput screening. hiPSC-CMs cultures plated on a high throughput platform which passively promote cardiomyocyte alignment have been shown to display physiologically-relevant features, including more physiological cellular geometry, coherent unidirectional contraction, cardiac cell junction re-modeling, and improved calcium handling. To evaluate whether the changes induced by this platform translated into differential responses to cardio-active compounds, high throughput calcium flux assays were performed on hiPSC-CMs cultured in standard high throughput screening cell cultureware or anisotropic 384-well plates and subsequently interrogated with the 28 compounds included in the CiPA initiative. Interestingly, differential responses were observed in nearly 60% of the compounds tested. Specifically, compounds in

the high risk category showed a dose-dependent progression in the severity of the pro-arrhythmic phenotypes in anisotropy. This was associated with a higher severity of early afterdepolarizations (EADs). Six out of eleven compounds in the intermediate risk category showed a more sensitive response in anisotropy. No EADs were observed in either control or anisotropic conditions treated with low risk compounds. Altogether, anisotropic high throughput hiPSC-CM cultures formatted in the platform employed in this study showed better resolution over the progression and severity of pro-arrhythmic events.

W-1031

TCF12/HEB CONTROLS CARDIOMYOCYTE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS BY REGULATING EXPRESSION OF GENES INVOLVED IN HEART DEVELOPMENT AND CARDIOMYOCYTE LINEAGE DEMARCATION

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The process of cardiomyocyte differentiation is regulated by a complex network of transcription factors dictating lineage-specific outcomes. This process involves a mesodermal branchpoint between the endothelial, hematopoietic, and cardiac lineages. We have previously shown that the basic helix-loop-helix (bHLH) transcription factor TCF12/HEB is required for hematopoietic specification, but not for endothelial development. However, the involvement of TCF12/HEB in cardiac specification and differentiation is poorly understood. We utilized a human embryonic stem cell line with a CRISPR/Cas9-mediated deletion of TCF12 to investigate the role of HEB in cardiomyocyte development. Analysis of RNA transcripts in TCF12-/- hESCs in cardiomyocyte differentiation conditions revealed a 2- to 20-fold greater expression of the cardiac transcription factors TBX5 and MEF2C, ventricular marker MYL2/MLC2V, and cardiac muscle proteins TNNT2 and TNNI3, suggesting that HEB may play a role in limiting this fate choice. HEB may play an important determining factor in deciding cardiomyocyte fate, likely by heterodimerization with other cell type-specific bHLH transcription factors.

W-1033

DGCR8 DELETION IN THE PRIMITIVE HEART UNCOVERED NOVEL MICRORNA REGULATING THE BALANCE OF CARDIAC-VASCULAR GENE PROGRAM

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Primitive mammalian hearts transform from a single tube to a four-chambered muscular organ during a short developmental window. We found that knocking out global microRNA by deleting Dgcr8 microprocessor in Mesp1 cardiovascular progenitor cells lead to the formation of extremely dilated and enlarged heart due to defective cardiomyocyte differentiation. Transcriptome analysis revealed unusual upregulation of vascular gene expression in Dgcr8 cKO hearts. Single cell RNA sequencing study further confirmed the increase of angiogenesis genes in single Dgcr8 cKO cardiomyocyte. We also performed global microRNA profiling of E9.5 heart for the first time, and identified that miR-541 was transiently highly expressed in E9.5 hearts. Interestingly, introducing miR-541 back into microRNA-free cardiomyocytes partially rescued their defects, downregulated angiogenesis genes, and significantly upregulated cardiac genes. Moreover, miR-541 can target Ctgf and inhibit endothelial function. Our results suggest that microRNAs are required to suppress abnormal angiogenesis gene program to maintain cardiomyocyte differentiation.

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W-1035

UNVEILING METABOLIC GENE EXPRESSION CHANGES ALONG THE HEART IN THE SETTING OF CHRONIC ISCHEMIC HEART FAILURE TO FIND NOVEL TARGETS FOR CARDIAC REGENERATION

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The heart is an organ capable to shift its metabolic substrate preferences and adapt energy production to meet the workload demands in diverse pathophysiological conditions. This capacity for energy metabolism remodeling is driven, in part, by a complex transcriptional regulatory network. Recent evidence indicates that this network is altered in failing hearts and a better understanding of these mechanisms could provide insights into novel targets for cardiac regeneration. In this study, we investigated the perturbations in the gene regulatory network that lead to metabolic dysregulation during the development of heart failure (HF). Specifically, we applied RNA sequencing to map and compare coding and non-coding signatures of different heart areas in the setting of chronic ischemic HF with reduced ejection fraction in Göttingen mini pigs. Myocardial infarction (MI) was induced by 120 min balloon occlusion in the LAD artery. Three months post-MI, echocardiography and invasive left ventricular (LV) hemodynamic measurements confirmed significantly reduced global and regional heart function (~15-20% reduction in LV ejection fraction) and histological assessment showed an infarct size of 15% of LV. The hearts were excised and specimens taken from the right ventricle (RV), the infarcted LV, the MI border zone and remote from the MI were used for comprehensive analysis of spatial metabolic gene expression changes. Bioinformatics analyses were applied to identify differentially expressed genes and pathways, and reveal clusters of co-regulated genes. The results from these analyses identified substantial changes in the metabolic transcriptome between the infarcted LV, remote LV and RV areas. Several pathways involved in the regulation of energy production were shown to be activated in non-infarcted regions, suggesting that these zones may present a central role in compensatory protective energy metabolism remodeling mechanisms. The insights generated herein unveiled novel therapeutic targets to directly address the metabolic derangements occurring in the failing heart. These targets are being tested in hiPSC derived cardiomyocytes and the knowledge gained on metabolic remodeling during HF progression, is being applied to generate more reliable and predictive in vitro stem cell based disease models.

W-1037

UNDERSTAND THE FATE OF CARDIAC PDGFRA+ CELLS IN HEALTHY AND INJURED MOUSE HEARTS USING SINGLE CELL RNA-SEQ

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Myocardial infarction (MI) and subsequent heart failure are one of the leading causes of mortality worldwide. Different strategies are being explored to promote injury resolution and heart regeneration, including targeting cardiac resident progenitor cells. Previously, we have demonstrated that SCA1+PDGFRα+ (S+P+) cardiac stromal cells contain a colony-forming fraction sharing properties with mesenchymal stem cells described in bone marrow. We hypothesise that cardiac PDGFRα+ cells incompass progenitor cells involved in cardiac homeostasis and repair after injury. To unravel the differentiation potential of S+P+ cells, we permanently tagged PDGFRα+ cells using a Cre-based lineage tracing approach. Labelled cells isolated from ventricles of sham-operated and MI mice 7 days post-surgery were then analysed using single-cell RNA-seq. The transcriptome of more than 14000 cells have been explored allowing us to identify 9 and 11 clusters in Sham and MI, respectively. As expected, the main population in both conditions was fibroblasts (FB) divided into several subclusters, showing the heterogeneity of these cells. Moreover, the MI hearts contained myofibroblasts that were absent in Sham hearts. The second main population highlighted in our dataset was immune cells. It was mainly constituted with cells from the myeloid lineage in both conditions and expanded after MI, reflecting the complexity of the immune response after cardiac injury. Furthermore, while differentiation of FB into endothelial cells (ECs) is controversial, we have found a significant EC population in Sham hearts. Notably, this population was subdivided into two distinct clusters after MI. We also observed a small population characterized as mural cells in MI hearts, suggesting that PDGFRα-derived

cells participated to neo-vascularization post-injury. Finally, a distinct cycling cell cluster including cells expressing myofibroblasts, endothelial and immune cell markers, demonstrated the proliferative nature of these tagged cells. In depth study of lineage traced single cell gene expression profiles should provide us with a comprehensive analysis of the fate of PDGFRα+ cells after injury demonstrating their plasticity. Moreover, this approach may lead to the identification of new therapeutic strategies to improve cardiac repair.

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W-1039

SINGLE-CELL EXPRESSION PROFILING REVEALS DISTINCT SUB-POPULATIONS IN THE CARDIAC STROMA THROUGH HOMEOSTASIS AND INJURY RESPONSE

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Heart failure following myocardial infarction (MI) is in epidemic proportions and is set to increase. Congestive cardiac failure after MI is caused by the low level of cardiomyocyte (CM) regeneration after injury. We hypothesise that Sca1+/PDGFRα+/CD31- cells(S+P+) which reside in cardiac interstitium, are dedicated to cardiac homeostasis and repair, acting as a specialised mesenchymal stem cell source for smooth muscle cells, adipocytes, and possibly endothelial cells (Chong et al., 2011). They may also act as stress sensors to support CM and vascular tissue through dedicated paracrine functions. To investigate activation of these cells during initial stages of inflammation after MI, single-cell RNA-seq (scRNA-Seq) was performed using GFP+/CD31- and total interstitial cell isolated from a mouse with a H2B-eGFP fusion gene knocked-in to the PDGFRα locus. The gene expression (GE) profiles of more than 30,000 cells were analysed 3 and 7 days following induction of MI by coronary artery ligation. We will describe our initial findings, including the stepwise induction of known and novel stromal cell states, and the presence of minority populations suggestive of progenitor cell activity. Two major differentiation pathways direct GFP+ cells towards myofibroblast or smooth muscle cell fates with major involvement of Wnt signalling. scRNA-Seq analysis of freshly isolated S+P+ cells stimulated in vitro with PDGF and bFGF have myofibroblast GE signatures resembling those cells found in vivo following MI. High content, single cell GE analysis provides extraordinary resolution of the cellular repair process. Our findings highlight the role of resident S+P+ as cardiac progenitor cells in healthy and diseased heart. S+P+ cells are activated following MI, providing a source of differentiated cell types and secretory functions required for cardiac repair. These molecular findings may identify new therapeutic approaches for preserving myocardial contractility following MI.

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W-1041

THE MEVALONATE PATHWAY CONTROLS REGENERATION IN HUMAN CARDIAC ORGANOIDS

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Induction of cardiomyocyte proliferation is emerging as a promising strategy for heart regeneration. Using a recently developed human cardiac organoid (hCO) system, we have recently discovered a small molecule (Compound 6.28) that inhibits both GSK3 and MST1 and drives robust proliferation of hIPS-cardiomyocytes (Mills et al., PNAS, 2017). However, chronic stimulation with this compound also causes a decline in contractile function, which would prevent its use as a therapeutic. We have now performed quantitative proteomics of ~50 individual hCOs under different conditions to de-convolute the effects of GSK3 and MST1 on the pro-proliferative response. GSK3 inhibition was found to be responsible for a cell cycle network whereas MST1 inhibition activated the mevalonate pathway; the detrimental functional effects were specifically associated with inhibition of GSK3. An additional screen of 105 compounds was performed to identify new compounds that could activate proliferation in hCOs without detrimental effects on functionality. We identified a compound targeting p38, which activated a similar cell cycle network to GSK3, and another compound targeting TGFBR/ACVR/BMPR, which activated the mevalonate pathway similar to MST1 inhibition. A strong synergistic pro-proliferative response was observed when both of these compounds were combined, without any detrimental effects on cardiac function. The pro-proliferative effect could be blocked by inhibition of β-catenin transcriptional networks using ICRT14 or inhibition of the mevalonate pathway using a statin. In addition, RNA-seq data shows large scale changes in the mevalonate pathway during heart maturation in vivo and blocking the mevalonate pathway in immature cardiomyocytes reduces proliferation. By re-supplementing different metabolites in the mevalonate pathway, a specific function for the prenylation branch of this metabolic pathway for proliferation was identified and direct inhibition of a downstream kinase activated by Rho prenylation, ROCK, also lead to

a reduction in proliferation. Our results reveal a novel role for the mevalonate pathway in cardiomyocyte proliferation and suggest that inhibition of this pathway occurs during postnatal heart maturation.

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W-1043

MICROPHTHALMIA-ASSOCIATED TRANSCRIPTION FACTOR PLAYS A VITAL ROLE IN SUBSTANCE P-MEDIATED CARDIAC STEM CELL ACTIVATION

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There have been no studies exploring the relationship between microphthalmia-associated transcription factor (MITF) and substance P (SP) in the local activation of c-Kit+cardiac stem cells (CSCs) in cardiac repair. Herein, we investigate the mechanism of SP underyling cardiac repair after I/R. Male Sprague-Dawley rats (SD, 8weeks) were subjected to ischemia/reperfusion-injury (I/R) and 5 nmole/kg SP injection (SP+I/R). At the end of 7 days, the left ventricle (LV) and infarcted area (IA) were sampled from the heart. We found that compared to LV 7 days I/R, there were higher levels of c-Kit+ CSC in LV 7 days SP+I/R, which also expressed higher levels of major CSC markers and MITF. In agreement with these observations, elevated expansion of c-Kit+CSCs in the explant-derived cells (EDCs) from IA 7 days SP+I/R was detected than in EDCs IA 7 days I/R using the primary explant technique. c-Kit and MITF expression increased in the SP-treated IA c-Kit+CSCs, which increased the phosphorylation of c-Kit and MITF at 1 h. Interestingly, FTY720, known to efficiently down-regulation MITF, suppressed the IA c-Kit+CSC proliferation and migration stimulated by SP-induced expression of MITF. Overall, our results demonstrate for the first time that the expression of MITF may interact with SP-induced local activation of c-Kit+CSC in cardiac repair.

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ENDOTHELIAL CELLS AND HEMANGIOBLASTS

W-1045

NEWLY OPTIMIZED MEDIUM FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS

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Human endothelial cells (ECs) are widely used in the field of vascularization study such as drug discovery, safety/toxicological test, tumor growth or tissue engineering including organ-on-a-chip technology. Although primary ECs like HUVECs are generally used for these purposes, there are some drawbacks in utilizing primary cells because of the donor difference due to a wide variety of genetic background. To overcome this, human pluripotent stem cell-derived ECs (hPS-ECs) are expected to be supplied as stable cell source with similar properties and without the donor difference among manufacturing batches. We have already developed the differentiation method from pluripotent stem cells into ECs population having more than 95% of CD31 and CD144 positive cells, which are identified as endothelial cell. Our hPS-ECs also express CD34 in more than 95% of cells, suggesting immature phenotype. This is considered to be one of the appropriate features for tissue engineering study because ECs may be needed to be educated to fit in each tissue. However, it was difficult to obtain a large number of hPS-ECs by using commercially available medium for primary ECs due to poor proliferation capacity, even though many cells are required for tissue engineering. Thus, we have also developed the culture medium for hPS-ECs. As a result, it was observed that hPS-ECs show about 10-fold expansion in one week by newly optimized medium. The expanded hPS-ECs maintained ECs surface marker (CD31 and CD144) expression similar to the cells in pre-expansion. Furthermore, tube-like structure formation on matrigel was also observed after expansion, indicating an angiogenic capacity of hPS-ECs. Overall we successfully developed hPS-ECs culture system using newly optimized medium. The cells and medium can overcome the lot variation problems of primary endothelial cells, making them suitable for industrialization with mass cell production. Further, we are currently developing xeno-free medium for hPS-ECs to apply to the manufacture of regenerative medicine.

W-1047

HIGHLY EFFICIENT HEMATOPOIETIC AND MACROPHAGE DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS USING A SIMPLE MONOLAYER CULTURE SYSTEM

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Generation of hematopoietic stem cells from human pluripotent stem cells (hPSCs) hold great promise for the treatment of hematological diseases and providing sufficient cells for immune therapy. Here, we established a monolayer, chemically defined culture system to induce hematopoietic differentiation from hPSCs in 8 days. During stage one, hPSCs were induced to leave pluripotency promptly and preferably enter the vascular lineage. Hemogenic endothelial progenitor cells were then induced for 2 days. At the third stage, inhibition of TGFβ signaling with small molecule lead to rapid conversion of hemogenic endothelium to generate large numbers of CD34+CD43+ hematopoietic stem/progenitor cells (HSPCs). Global transcriptome profiling revealed that HSPC differentiated using our protocol was similar to EB derived HSPC. HSPCs obtained from our differentiation system formed robust erythroid, granulocyte, monocyte/macrophage colonies in CFU assay, and can be induced to generate macrophages resembling tissue residential macrophages. Moreover, these hPSC derived macrophages displayed strong phagocytotic activity towards pathogenic bacteria and released cytokine in response to LPS stimulate and Zika virus infection. Sum together, our monolayer differentiation system can offer a cost effective means to generate large quantities of cells of desired blood lineages for regenerative medicine.

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HEMATOPOIESIS/IMMUNOLOGY

W-1049

COLOR BARCODING AND MOSAIC MUTAGENESIS OF HEMATOPOIETIC STEM CELLS REVEAL CLONAL HEMATOPOIESIS IN GATA2 DEFICIENCY

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Myeloid malignancies, such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), arise from abnormal hematopoietic stem and progenitor cells (HSPCs) with acquired somatic mutations or cytogenetic abnormalities. Germline GATA2 haploinsufficiency underlies an inherited syndrome of predisposition to myeloid malignancies, with disease onset often occuring in childhood and young adulthood. We generated gata2b heterozygous zebrafish to study the hematopoietic stem cell clonal architecture and dynamics over time at steady-state and in the presence of somatic mutations found in GATA2-associated MDS and AML. We used a lineage tracing system of zebrafish Brainbow, Zebrabow, to track the hematopoietic output of individually colored HSPC clones, with the goal to detect abnormal expansion of single color clones indicating clonal hematopoiesis. Color barcoding was induced at the time of HSPC emergence from dorsal aorta during development when about 20 stem cell clones exist in the embryo. Analysis of adult hematopoiesis at 3 months showed myelocytopenia in gata2b+/- fish. Surprisingly, gata2b+/- fish displayed frequent color dominance at baseline, with a single color clone contributing to over 30% of granulocytes, while the myeloid output in wild-type clutchmates was polyclonal. To study effect of MDS/AML associated mutations, we induced mosaic mutagenesis in wild-type or gata2b+/- embryos by injecting with Cas9 mRNA and guide RNAs targeting zebrafish orthologs of ASXL1 and STAG2. In the presence of secondary mutations, gata2b+/- fish showed in an increase of precursor cells and a more pronounced myelocytopenia in the kidney marrow at 3 months, compared to wild-type controls, without changes in the lymphoid compartment. Single color expansion was observed in over 60% of gata2b+/- fish, and correlated with stag2a mutations. In summary, gata2b deficiency results in oligoclonal hematopoiesis and myelocytopenia, reminiscent of monocytopenia in GATA2 haploinsufficiency in patients. Mutations in stag2 highly correlated with clonal dominance in gata2b+/- but not wild-type fish. Our studies demonstrate that stem cell clonal expansion and selection is dependent on the dose of GATA2, and provide insight into the mechanism of preleukemic clonal expansion.

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W-1051

ASSOCIATION OF THE PROTECTIVE FOXO3 LONGEVITY VARIANT WITH TELOMERE DYNAMICS DURING AGING

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Telomere attrition in proliferative tissues, including adult stem cells, is a hallmark feature of human aging. To date, identification of genetic influence on the rate of telomere attrition is poorly understood. The two genes with the most robust effect on human longevity are FOXO3 and APOE. Notably, we discovered a genetic variant of the FOXO3 gene that is strongly associated with human longevity. Importantly, this observation has

been now reproduced in independent studies of over a dozen different populations around the world. In the present study, we sought to assess the effect of the longevity associated variant of FOXO3 (rs2802292 - G allele) as well as the variants of APOE on telomerase activity and the rate of telomere attrition during aging in peripheral blood leucocytes. The preliminary results from a cohort of Okinawan Japanese (N=122) ranging in age from 25 - 90 years, indicates no substantial effect of the variants for either FOXO3 or APOE on telomerase levels in peripheral blood leukocyte (PBL) samples. Analysis of the rate of telomere attrition during aging as a function of the different variants of APOE also revealed no significant effect. In contrast, carriers of 1 or 2 copies of the rare longevity-associated G allele of FOXO3 showed markedly reduced rates of telomere loss in PBL during aging, as compared to carriers of the more common variant of FOXO3 (TT - common genotype). Interestingly, no loss of telomere length was observed as a function of age for G allele carriers. These results mark the first report for a protective effect of a gene variant on the rate of telomere attrition in humans, and imply a mechanism for enhancing long term renewal capacity for hematopoietic stem cells during aging.

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W-1053

DEVELOPMENT OF FUNCTIONAL, MATURE CYTOTOXIC T-CELLS FROM CORD HEMATOPOIETIC STEM CELLS, VIA A MOLECULARLY-DEFINED, SCALABLE AND CLINICALLY TRANSLATABLE CULTURE SYSTEM

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Chimeric Antigen Receptor (CAR-) T cell immunotherapy is revolutionising cancer treatment, but most are autologous. Inevitably these will struggle to reach mass adoption given the complexity and cost (~US$400,000 per treatment). Logically, a precisely defined, consistent, 'off-the-shelf' CAR-T product with broad histocompatibility is the future of this technology. In vitro directed differentiation of T-cells from stem cells sources, could provide a platform to generate a 'limitless' supply of CAR-T cells. One logical, readily available stem cell source is cord HSC. Previous studies have successfully generated T-cells from HSC but these were reliant on mouse stromal cells negating their clinical utility. We have now established a molecularly defined, xeno-free, stroma-free, serum-free human T cell differentiation culture system suitable for upscale manufacture. For the first time we demonstrate the generation of mature CD8αβ+CD4-TCRαβ T-cells from cord blood HSCs without an animal stromal cell component. When activated via CD3/CD28 co-stimulation, the in vitro generated T-cells have strong dose-dependent cytotoxic function against human cancer cell lines. Using this system we can create ~100x mature cytotoxic T-cells per cord HSC, over 47 days of differentiation. With the prospect of post T-cell expansion, using our system 1 cord blood sample can yield up to 2.5 x 1011 T-cells. We have, however, observed donor cord variability, appearing to affect the end-state of T-cell maturity. This manufacture system serves as a stand-alone technique to enable immune reconstitution for a variety of diseases.

W-1055

RUNX1B OVEREXPRESSION TOGETHOR WITH ENHANCED TGF-β SIGNALING UPREGULATE P21 GENE WHICH LEAD TO CELL CYCLE G1 ARREST AND HEMATOPOIESIS BLOCKAGE

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RUNX1 play a key role in regulatory function on 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 AGMS-3 co-culture system and PiggyBac inducible system, which could be partially rescued by RepSox, the TGF-β signaling inhibitior against ALK5. D4 co-cultures were detected by cell cycle assay kit and 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 by RUNX1b, the cell cycle-related genes were detected by RT-qPCR to screen the differentiated expression genes between DOX-induced and untreated

D4 co-culture of RUNX1b/hESC. Among them p21WAF1 was up-regulated after DOX induction and reverted to original expression level when RepSox was added. The expression level of p21WAF1 at D4 was highly consistent with RUNX1b when the RUNX1b/hESC co-culture was induced by different concentration of DOX. Above results indicated that up-regulation of p21WAF1 depend on both overexpression of RUNX1 and enhanced TGF-β signaling. The p21WAF1 inducible hESC based on PiggyBac inducible system was also established and the overexpression of p21WAF1 could obviously block the hematopoiesis regardless which days DOX induction started. Though the blockage of p21WAF1 to hematopoiesis was not development stage-specific (just as RUNX1b did) the up-regulation of p21WAF1 lead to cell cycle G1 arrest, which ought to be a key step to cause the blockage of RUNX1b to hematopoiesis. TGF-β1 could up-regulate TGF-β signaling but couldn't block hematopoiesis when RUNX1b was not induced; on the contrary, RUNX1b could not completely block the generation of CD34+ population when TGF-β signaling was inhibited by RepSox, which indicated that RUNX1b itself or other down-stream pathway controlled by RUNX1b could regulate the expression of p21WAF1 with the indispensable help of enhanced TGF-β signaling. The severe blockage to hematopoiesis depend 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-1057

FUNCTIONAL DOMINANCE OF CHIP-MUTATED HEMATOPOIETIC STEM CELLS IN PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATIONS

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Normal hematopoiesis is maintained by a polyclonal pool of hematopoietic stem cells (HSCs). Single somatic mutations in recurrent genes cause the emergence of expanded clones in elderly persons, a phenomenon called clonal hematopoiesis of indeterminate potential (CHIP). While the multilineage differentiation of these clones suggests an involvement of HSCs in CHIP, direct evidence of the fitness of CHIP-mutated human HSCs in blood reconstitution is lacking. Interestingly, previous studies showed a constant clone size for decades under steady-state conditions. Myeloablative treatments and stem cell transplantation put enforced stress on the ability of HSCs to reconstitute the blood system, and the fitness of HSCs is maximally challenged. To assess whether human CHIP-mutated HSCs outcompete their wildtype counterparts in stress hematopoiesis, we took advantage of a well-characterized cohort of currently 74 patients undergoing high-dose chemotherapy conditioning and autologous stem cell transplantation (autoPBSCT) for the treatment of solid tumors or lymphoid diseases in our center. Using deep next generation sequencing of 54 myeloid cancer genes, we found that 19 patients (26%) are affected by CHIP (variant allele frequency >2%) in their blood at a time 6-102 months after autoPBSCT, with a mean variant allele burden of 13%. DNMT3A was affected most often, followed by TET2, ASXL1, TP53, KRAS, MYD88, KIT, and RUNX1. Most patients carry only one mutation while few have 2-3 mutations. To explore whether the mutations had been present before the high-dose chemotherapy, and whether the CHIP-mutated HSCs expanded after autoPBSCT, we sequenced frozen samples of the transplanted graft from all patients. Importantly, the mutations can be detected in the majority of patients already in the transplanted cells, suggesting that they are not induced by high dose chemotherapy; however, the allele burden in the graft is significantly lower, often below 0.5%, than in the blood months to years after autoPBSCT suggesting a selective advantage of mutated HSCs. Here we show that human HSCs harboring CHIP mutations outcompete normal HSCs upon transplantation and severely contribute to the increase in CHIP clone size during stress hematopoiesis.

W-1059

INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS ACTIVATE QUIESCENT T CELLS AND ELEVATE REGULATORY T CELL RESPONSE VIA NF-κB IN ALLERGIC RHINITIS PATIENTS

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The unique immunomodulatory properties of mesenchymal stem cells (MSCs) make them an invaluable cell type for the treatment of chronic inflammation or autoimmune disorders. We have successfully derived MSCs induced pluripotent stem cell (iPSC), which exhibited lower immunogenicity, and superior survival and engraftment following transplantation in a mouse ischemia model compared to that of traditional tissue-derived MSCs. The induced iPSCs-MSCs were demonstrated have immunosuppressive effects on activated T cells. However, the effects of iPSC-MSCs on quiescent T cells are unknown. This study was to identify the immunomodulatory role of iPSC-MSCs on resting peripheral blood mononuclear cells (PBMCs) from allergic rhinitis (AR) patients. The PBMCs from AR patients were co-cultured with iPSC-MSCs without any stimulation, following which lymphocyte proliferation, activation of T cells, T_H1/T_H2 and regulatory T (Treg) cell differentiation and Treg cell function were analyzed. The roles of stem cell-derived soluble factors and cell-cell contact were examined to investigate the mechanisms involved in iPSC-MSC mediated immunomodulatory effects. By using transduced shRNA of IKKβ in iPSC-MSCs, the NF-κB signalling pathway involvement was investigated. Results revealed that iPSC-MSCs promoted, but did not decrease, the proliferation of resting lymphocytes. Flow cytometry analysis found that iPSC-MSCs activated $CD4^+$ and $CD8^+$ T cells. Besides, iPSC-MSCs upregulated and activated Treg cells without any additional stimulation, further co-culture tests found that the upregulated Treg cells showed good inhibitory function on the stimulated PBMCs. In addition, iPSC-MSCs balanced biased T_H1/T_H2 cytokine levels as examined by ELISA assays. Cell-cell contact was confirmed to be a possible mechanism involved by transwell test. NF-κB was identified to play an important role in the immunomodulatory effects of iPSC-MSCs on quiescent T cells by IKKβ knockdown. Our findings demonstrated that iPSC-MSCs activate quiescent T cells and elevate regulatory T cell response in AR patients, suggesting different immunomodulatory functions of iPSC-MSCs according to the phases of diseases. Therefore, iPSC-MSCs are a potential therapeutic candidate for treating allergic airway inflammation.

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W-1061

DIFFERENTIATION OF HUMAN ES-CELLS INTO MORE DEFINITIVE HEMATOPOIETIC PRECURSOR CELLS

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In vitro production of human hematopoietic stem/precursor cells (HSPCs) represents an important model/therapy for hemoglobin disorders. Traditional HSPC generation methods from human embryonic stem cells (hESCs) result in a predominance of ε- and g-globin production among erythroid progeny, limiting their utility. Recently, we developed hESC-derived erythroid cell generation via serum-free ES-sacs allowing definitive ß-globin protein production. ES-sacs are derived from hESCs cultured on C3H cells using Knockout Serum Replacement (KSR) and VEGF for 15 days and have hemangioblast-like structures including an external layer of endothelial cells, and internal spherical hematopoietic-like cells. We generated ES-sacs and characterized the different populations among the spherical cells, including hematopoietic progenitor cells (HPCs CD34+CD45+) and erythroid precursors including more definitive (dEP CD235a-CD34+) and more primitive (pEP CD235a+CD34-). After erythroid differentiation, we quantitated globin gene expression by qPCR and protein levels by HPLC. Extension of the generation phase of our serum-free protocol (from 15 days to 18, 19 and 20 days) allows for more mature ES-sacs. Higher amounts of HPCs (10.934.1, 11.332.2, 11.031.3 vs 0.930.1%, $p<0.01$) and dEPs (22.034.6, 21.131.2, 16.533.0 vs 14.030.8%, $p<0.05$) were produced during the extended generation phase, compared to 15 days. Additionally, ε-globin expression was lower (1432, 1436, 1231 vs 3235%, $p<0.05$) and ß-globin expression higher (632, 932, 733 vs 131%, $p<0.01$) in the extended generation phase. This trend was also obtained at the protein level. To further increase definitive hematopoiesis, we examined increasing concentrations of KSR (20, 25, 30 and 35%) during the ES-sac generation. Increased KSR resulted in higher percentages of dEPs (6.630.6, 6.830.4, 7.730.5 vs 5.330%, $p<0.01$) and lower of pEPs (1.430.1, 1.530.2, 1.430.2 vs 2.030%, $p<0.0$) among the spherical cells, compared to 20% KSR. More HPCs (1.130.4 vs 0.0430%, $p<0.01$) were obtained in 35% compared to 20% KSR. Lower RNA expression of ε-globin (1230 vs

24311%, p<0.05) was detected in 35% compared to 20% KSR, similar to the protein level. Extension of the ES-sac generation phase or increase of KSR concentration leads to the production of more definitive HSPCs from hESCs.

W-1063

TRACKING HEMATOPOIETIC PRECUSOR DIVISION EX VIVO IN REAL TIME

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Deciphering molecular mechanisms underlying division of hematopoietic stem cells (HSCs) and malignant precursors would improve our understanding of basis of stem cell-fate decisions and oncogenic transformation. Using a novel reporter of hematopoietic precursor Evi1-GFP, we track the division of hematopoietic precursors in culture in real time. First, we confirmed that Evi1-GFP is a faithful reporter of HSC activity and identified three dividing patterns of HSCs: symmetric renewal, symmetric differentiation, and asymmetric division. Moreover, we found that the cytokines and growth factors combination (STIF) promotes symmetric renewal, whereas OP9 stromal cells balance symmetric renewal and differentiation of HSCs ex vivo. Interestingly, we found that Tet2 knockout HSCs underwent more symmetric differentiation in culture compared to wild type control. Intriguingly, OP9 stromal cells reverse the phenotype of Tet2 knockout HSCs ex vivo. Furthermore, we demonstrated that Tet2-/- ; FLT3ITD acute myeloid leukemia (AML) precursors primarily underwent symmetric renewal divisions in culture. Our study establishes a new system to explore the molecular mechanisms of the regulation of benign and malignant hematopoietic precursor division ex vivo. The knowledge learned from these studies will provide new insights into the molecular mechanisms of HSC fate decision and leukemogenesis.

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PANCREAS, LIVER, KIDNEY

W-1065

MODULATION OF SIGNALING PATHWAYS AND 2D/3D CULTURE TO DIRECT PANCREATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO INSULIN-PRODUCING CELLS IN FULLY DEFINED CONDITIONS

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Stem cell-derived endodermal progenitor cells have potential applications in regenerative medicine and basic research, particularly in autoimmune diseases, such as Type 1 diabetes. Here, we describe a novel protocol for pancreatic differentiation of human pluripotent stem cells through sequential stages, in which combinatorial activation and inhibition of the BMP and TGFb signaling pathways are used to direct the cells specifically to the posterior foregut. Although we have based our strategy on findings from previous in vivo development and in vitro differentiation studies, we have combined and systematically tested them in a novel manner using defined conditions, in order to optimize the definitive endoderm (DE) and posterior foregut stages in terms of the concentration and temporal modulation of the many signaling pathways involved. We have also optimized the 2D/3D culture environment throughout the differentiation process. We have fully characterized each stage of differentiation in detail, including transcriptomic profiling of the differentiating cells in the context of data from a prior pancreatic differentiation study, as well as fetal and adult human pancreas samples, to determine the fidelity of the in vitro differentiation process. We observed that the early stages of endodermal differentiation of human pluripotent stem cells follow similar transcriptional routes, even using different protocols and stem cell lines. However, final stages of pancreatic maturation appear to be more strongly influenced by external factors, such as in vitro culture conditions or transplantation for in vivo maturation. This work provides a deeper understanding of the transcriptional dynamics of cells during in vitro differentiation of hPSCs to the pancreatic lineage, which can be applied to development of stem cell based therapies for developmental and degenerative disorders of the pancreas.

Funding Source: UCSD Department of Reproductive Medicine

W-1067

TREATMENT OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (MSCS) CAN SIGNIFICANTLY ATTENUATE THE KIDNEY DAMAGES IN TYPE 2 DIABETIC NEPHROPATHY

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Diabetic nephropathy (DN) is a major complication of diabetes and represents the leading cause of end-stage renal disease worldwide. Now, there is no effective therapy for DN. Therefore, a new treatment strategy aimed to delay or revert DN is highly urgent. To investigate the therapeutic role of human bone marrow-derived MSCs in type 2 DN, SD rats were fed with high-glucose-high-fat diet, then a small dose of streptozotocin were injected into the peritoneal cavity of the uninephrectomy rats, so to construct type 2 DN models. When significant proteinuria appeared in the rats indicating that type 2 DN models has been successfully established. The model rats received injection of human MSCs via tail vein at 5 x 106 every 4 weeks for five times. The results showed that reduction of proteinuria was not observed in diabetic model rats untill 24 weeks after three doses of MSCs. However, since 28 weeks, urinary protein excretion was significantly suppressed, and persisted up to 32 weeks after streptozotocin. Hypoalbuminemia and hyperlipidemia were also improved in MSCs-treated group. Renal pathological analysis showed that MSCs significantly attenuated glomerular hypertrophy and renal tubular interstitial injury. Western blot revealed that MSCs up-regulated the expression of the Glomerular markers WT-1 and snaptopodin decreased in the DN. To observe the migration and localization of MSCs in tissues and organs, fluorescent dyes CFSE- and Hoechst-labeled MSCs were injected into diabetic rats via tail vein. The results found that MSCs were detected in lung and spleen, and kidney peritubular regions, but rarely in glomeruli and pancreas within 48 hours after injection. The MSCs did not improve hyperglycemia and pancreatic damages. These findings indicate that repeated intravenous MSCs can alleviate diabetic kidney damage in rats even at the progressive stage.

W-1069

ALDH1B1 EXPRESSION DEFINES AN ADULT PANCREAS STEM CELL POPULATION AND IS NECESSARY FOR PANCREATIC CANCER IN MICE

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The existence of bona fide pancreatic adult stem cells remains contentious. Centroacinar cells have been proposed to be adult pancreas progenitor cells, but this has been difficult to address due to the lack of a unique molecular marker. We show that centroacinar cells are characterized by the exclusive expression of Aldh1b1. Aldh1b1-expressing cells are necessary and sufficient to form self-renewing adult pancreatic organoids and Aldh1b1 function is required for their formation. The Aldh1b1^{+} cells are largely quiescent, self-renew and contribute to all three pancreatic lineages in the adult organ under homeostatic conditions. Single cell RNA sequencing identified the molecular signature of Aldh1b1^{+} stem cells and demonstrated distinct differentiation pathways to early progenitors. Kras is specifically expressed in this stem cell population suggesting that Aldh1b1 expressing cells may be the origin of pancreatic cancer. Consistent with that, loss of Aldh1b1 function in a Kras^{G12D} - mediated model of pancreatic cancer completely abrogated tumor initiation and progression.

W-1071

LONG-TERM CORRECTION OF DIABETES IN MICE BY IN VIVO REPROGRAMMING OF PANCREATIC DUCTS

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Direct lineage reprogramming can convert readily available cells in the body into desired cell types for cell replacement therapy. This is usually achieved through forced activation or repression of lineage defining factors or pathways. In particular, reprogramming towards the pancreatic β-cell fate has been of great interest in the search for new diabetes therapies. It has been suggested that cells from various endodermal lineages can be converted to β-like cells. However, it is

unclear how closely induced cells resemble endogenous pancreatic β-cells and whether different cell types have the same reprogramming potential. Here, we report in vivo reprogramming of pancreatic ductal cells through intra-ductal delivery of an adenoviral vector expressing the transcription factors Pdx1, Neurog3 and Mafa. Induced β-like cells are mono-hormonal, express genes essential for β-cell function and correct hyperglycemia in both chemically and genetically induced diabetes models. Compared to intrahepatic ducts and hepatocytes treated with the same vector, pancreatic ducts demonstrated more rapid activation of β-cell transcripts and repression of donor cells markers. This approach could be readily adapted to humans through a commonly performed procedure, endoscopic retrograde cholangio-pancreatography (ERCP) and provides potential for cell replacement therapy in type 1 diabetes patients.

W-1073

GLOBAL TRENDS OF CLINICAL TRIALS OF CELL THERAPY FOR LIVER CIRRHOSIS

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Cell therapy medicine for liver cirrhosis would be a potential alternative to liver transplantation which is the only radical treatment currently available but the number of donors is very limited. In this study, we analyzed the clinical research trends related to cell therapy for cirrhosis based on data obtained from the ClinicalTrial.gov website. Although this website does not provide comprehensive results of clinical trials, it offers information on prospective clinical trials, including work in progress, and thus allows chronological analysis of the data. We selected 48 studies related to the field of cell therapy for mainly hepatic cirrhosis patients from ClinicalTrial.gov. The results showed a shift in the clinical translational trend in cells used in such research from the oligopolistic situation with bone marrow derived cells to a variety of cells derived from bone marrow, umbilical cord, adipose tissue and so on as adipose tissue entered into this field in 2012. The similar trend was observed on the origin of cell source, which is that autologous stem cells were used in most studies in the earlier years, then allogeneic cells have been increasingly used since 2010. Looking at the number of the clinical trials by country, 22 trials in China stood out dominantly followed by 5 in Iran, 4 in Korea and 3 in Japan and Turkey each and 2 in each of India and Brazil, whereas merely 3 countries of UK, Belgium and Italy conducted each of one trial in Europe and none in the North America. We found in the Clinical Trials.gov website that the clinical researches on cell therapy for cirrhosis have been conducted more actively in Asia and the Middle East comparing to the Western countries up until now. The use of ClinicalTrials.gov as the sole data source can yield a perspective view of the global clinical translational trends.

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W-1075

DIFFERENTIATION OF BI-POTENT HEPATOBLASTS INTO HEPATOCYTES AND CHOLANGIOCYTES IN THE HEPATOBLAST-DEPLETED FETAL LIVER OF AN AFP-HSVTK TRANSGENIC MOUSE

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The liver consists of hepatocytes and various non-parenchymal cells such as cholangiocytes. Hepatocytes are an indispensable tool in drug discovery as they play an important role in drug metabolism. Numerous groups have reported the establishment of humanized-liver mice, in which the liver parenchyma has been repopulated with human hepatocytes, as an in vivo model of human liver physiology. It is well known that in the developmental process, both hepatocytes and cholangiocytes are derived from the bi-potent progenitor, hepatoblasts. Therefore, we speculate that the transplantation of hepatoblasts into the fetal liver of a hepatoblast-depletion mouse makes it possible to reconstitute not only the liver parenchyma but also the bile duct structure. To prove this concept, we newly established an AFP-HSVtk mouse as a drug-inducible hepatoblast-depletion model. AFP-HSVtk mice express the mutant herpes simplex virus thymidine kinase (HSVtk) under the control of the mouse alpha-fetoprotein (AFP) promoter. The administration of gancyclovir to a pregnant AFP-HSVtk mouse caused fetal liver aplasia by hepatoblast depletion. We performed an ex utero transplantation of the hepatoblasts, which were isolated from PgkEGFP mouse, into the fetal liver of AFP-HSVtk and wild-type mice. The colonies comprising of >100 EGFP-expressing cells were observed in the AFP-HSVtk mice liver, whereas few EGFP-expressing cells were observed in the wild-type mice liver. Moreover, EGFP-expressing hepatocytes were observed in the fetal liver of AFP-HSVtk mice at the embryonic day E19.5. Since the prenatal bile duct structure is immature, we plan to confirm the contribution of the transplanted cells in

neonatal and adult AFP-HSVtk mice. It is expected that the ex utero transplantation of human hepatoblasts into the fetal liver of AFP-HSVtk mice will reconstitute both human liver parenchyma and bile duct structure.

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W-1077

INTEGRATED PHOSPHOPROTEOMIC AND TRANSCRIPTOMIC PROFILING REVEALS CRITICAL ROLES OF PIM2 IN HEPATIC TRANSDIFFERENTIATION

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Transcription factor induced cell fate conversion holds great promise in disease modeling and clinical therapy, yet the mechanisms behind successful lineage-specific reprogramming remain elusive. To investigate regulatory pathways that involved in hepatic conversion process we used iTRAQ-based quantitative phosphoproteome and RNA-seq strategies. Based on the phosphoproteomic analysis, we reconstructed the kinome in early stages of human hepatic transdifferentiation. The activities of 15 kinases were predicted to be significantly upregulated. After performing RNAi screen of the predicted kinases, we identified PIM2 as an important regulator for hepatic transdifferentiation. Overexpression of PIM2 significantly promoted hepatic transdifferentiation by phosphorylation of TSC1/2. However, suppression of PIM2 inhibited this process with increased cell death. This study provided comprehensive profiling of the phosphoproteome and transcriptome of cells undergoing hepatic conversion and identified PIM2 as a novel regulator of hepatic transdifferentiation.

W-1079

CHEMICAL REPROGRAMMING OF HUMAN HEPATOCYTES INTO HEPATIC PROGENITOR CELLS

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Cell-based regenerative medicine is a breakthrough technology, which holds promises for gene and/or stem cell therapy and given the shortage of donor organs, may provide a valuable option for patients with end stage of liver disease. Despite much progress in isolation and long-term expansion of bipotent progenitor cells with regenerative capacity from terminally differentiated mouse hepatocytes, expansion of the adult human hepatocytes remains a major challenge. We report a successful generation of the patient-specific hepatic progenitor cells from human hepatocytes from healthy and diseased liver using two small molecules and growth factor. Three days of treatment small molecule in the presence of growth factor, a key driver of hepatic progenitor cell activity, triggered expansion of small polygonal cells, which co-expressed known hepatic progenitor cells and lineage specific marker genes. These chemically derived human hepatic progenitor cells (hCdHs) could self-renew for at least 10 passages while retaining phenotype, normal karyotype and potential to differentiate into functional hepatocytes and biliary epithelial cells in vitro. A next-generation sequencing confirmed a high degree of molecular similarity between hCdHs and human hepatoblasts Upon intrasplenic transplantation into immunocompromised mice with a diseased liver, hCdHs effectively repopulated and restored. 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.

EPITHELIAL TISSUES

W-1081

THE ROLE OF TRANSLATION IN HUMAN KERATINOCYTE CELL FATE DETERMINATION

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Keratinocyte cultures are an excellent model for investigating the molecular basis of cell fate. Colonies of cultured human keratinocytes can be classified as 'balanced' or 'expanding' based on the ratio of outcomes after division. On average, 'balanced' colonies give rise to equal numbers of proliferative and differentiating cells, whereas 'expanding' colonies grow in an exponential manner, with the majority of divisions resulting in the generation of two proliferative daughter cells. Our previous work has demonstrated that cells in the middle third of a growing 'expanding' colony spontaneously switch their behaviour towards the 'balanced' state. Cells are also able to switch back from 'balanced' to 'expanding' after a scratch injury. These results suggest the existence of a tuneable mechanism for determining

the fate of proliferative keratinocytes, but the molecular identity of this switch remains unknown. Here we present that global translation levels may define the division outcomes of cultured human keratinocytes. Using a combination of cell imaging and state of the art RNA sequencing technologies, we show that the two modes of proliferation produce distinct transcriptional and translational profiles. We are able to distinguish between the 'balanced' and 'expanding' colonies at a very early stage during colony growth using specific markers for translation and a global transcription marker suggesting a colony's fate is set early on. Utilising RNAi technology allowed us to partially inhibit translation in cultured keratinocytes, leading to an apparent switch in the colony's proliferative potential. This suggests that global translation levels may actually determine the cell fate of human keratinocytes. Overall, these findings bring us closer to understanding the molecular basis of keratinocyte cell fate determination, and may help us explain how through simple cell dynamics an entire epithelial sheet can be generated from a single cell, which is one of the great questions in the epithelial stem cell research.

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W-1083

ESSENTIAL ROLES FOR THE RHO-KINASES, ROCK1 AND ROCK2, IN INTESTINAL STEM CELL VIABILITY AND MAINTENANCE OF ORGAN HOMEOSTASIS

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The Rho-kinases, Rock1 and Rock2, regulate cell shape/cytoskeletal rearrangement downstream of the RhoA small GTPase. These processes are critical to many cellular functions including contraction, polarity, proliferation, motility, adhesion and viability. While genetic data support distinct and essential roles for each kinase during development, how Rock1 and Rock2 contribute to homeostatic functions in adult tissues is unknown. Although small molecule inhibitors are widely used to investigate Rho-kinase function in acute settings, their pharmacological and selectivity profiles preclude their application in addressing this question. Here, we used inducible gene targeting to ablate Rock1 and Rock2 individually or together in adult mice, and found an obligate requirement for these enzymes in maintaining stem cell viability and proliferative capacity. $Rock1^{flox/flox}$, $Rock2^{flox/flox}$, or $Rock1^{flox/flox}$: $Rock2^{flox/flox}$ mice were crossed to the Rosa26-Cre ERT2 line to globally delete the floxed alleles with tamoxifen. Loss of either allele had no impact on survival, suggesting functional redundancy between the two kinases for sustaining homeostatic processes. In contrast, deletion of both genes caused mortality within 5 days of tamoxifen injection. Histological evaluation revealed a disturbance of tissue homeostasis in organs with rapid cell turnover and renewal, such as the alimentary tract and lymphoid tissues, with lesions resembling radiation induced injury. Specifically, the epithelial barrier of the small intestine was severely compromised, impeding nutrient absorption, promoting systemic inflammation, and ultimately causing death of the animals. Akin to $RhoA^{-/-}$ mice, deletion of both Rock1 and Rock2 led to mitotic arrest of stem cells due to defective cytokinesis, signaling apoptotic cell death. Biochemical studies demonstrated Rho-kinase activity in epithelial stem cells stimulated with Wnt ligands, factors critical to intestinal stem cell survival and differentiation. Moreover, this phenotype was not exclusive to the small intestine, with doubly deficient mice displaying severe cytopenia because of a failure in hematopoietic stem cell division. Our data reveal a fundamental role for this pathway in stem cell renewal and the maintenance of tissue homeostasis.

Funding Source: ALL AUTHORS ARE EMPLOYEES OF GENENTECH

W-1085

REST (RE1-SILENCING TRANSCRIPTION FACTOR) AFFECTS MOUSE NEURAL CREST CELL-DERIVED MELANOCYTE STEM CELLS AND THEIR HOMEOSTASIS

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Rest is a transcriptional repressor of neural genes and is expressed during embryonic development to prevent neural gene expression in non-neural cells. The role of Rest in control of neural crest cells (NCCs) fate has little been examined. NCCs have a multipotential cell fate to differentiate into neuronal and non-neuronal lineages including melanocytes. Analyses of Rest functions in vivo have been hampered by early embryonic lethality of Rest null mice. To evaluate the role of Rest in NCCs we developed a conditional Rest knockout (CKO) system and observed the established NCC-specific homozygous Rest CKO mice cause neonatal death. The heterozygous NCC-specific Rest CKO mice are viable and some of them showed the white spotting phenotype. A reduction in the number of melanoblasts is observed in NCC-specific Rest CKO embryonic skin. However, melanocyte lineage-specific Rest CKO mice and their niche keratinocyte-specific Rest CKO mice did not show the white spotting phenotype. Therefore, the expression of REST during the early neural crest specification stage was necessary for the normal development of melanoblasts to cover all of the skin. Interestingly, it is recently reported that Rest induction suppresses apoptotic cell death and the aged-related stress or toxic effect. By using an irradiation induced hair graying model, we also investigated the Rest function in melanocyte stem cell system in adult

hair follicles. Among conditional Rest loss- and gain-of-function mutant mice, a conditional loss-of-function mutation of Rest using a doxycycline inducible Cre to ablate floxed Rest facilitates the radiation-induced hair graying. Thus, Rest is also important for the protection from the stress in follicular melanocytes stem cells of adult skin.

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W-1087

DIRECTED DIFFERENTIATION OF RHESUS MACAQUE IPSC-DERIVED ENDOTHELIA CELLS

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Endothelial cells form a monolayer covering the luminal surface of all vessels, and play a significant role in angiogenesis, modulation of smooth muscle cell function, and regulation of vascular responses to hemodynamic forces. Endothelial cell dysfunction contributes to cardiovascular diseases, including coronary heart disease, myocardial infarction, and other ischemic processes. Embryonic stem cells (ESCs) derived or induced-pluripotent stem cells (iPSCs) derived endothelial cells are a promising regenerative medicine tool to study cardiovascular disease phenotypes. In contrast to rodent models, the nonhuman primate Rhesus monkey is an excellent preclinical transplantation model for modeling human disease. We developed a protocol for the generation of endothelial cells with high efficiency from Rhesus monkey induced pluripotent stem cells (RiPSCs). These RiPSC-derived endothelial cells (RiECs) display phenotypic endothelial cell markers and gene expression profile, possess a similar capacity for tube-like structure formation, and uptake acetylated low density lipoprotein (acLDL), as compared to primary endothelial cells. In a xenograft assay, co-injection of RiECs and tumor cells increased tumor burden, suggesting that the RiECs promote tumor angiogenesis. Histological analysis of xenograft tumors showed the existence of RiECs around the vessel structure, indicating their potential angiogenesis role during tumor growth. Taken together, we have successfully developed a method to generate RiECs from RiPSCs that are readily expandable, possess characteristic endothelial cell identities, and display similar in vitro and in vivo functional capabilities compared to primary endothelium. Future studies will assess the ability of RiECs to promote RiPSC-derived other tissue specific cells proliferation and maintenance to generate functional organoid in vitro and in vivo.

W-1089

BIOLOGICALLY RELEVANT LAMININ MATRIX FOR CULTURING HUMAN EPIDERMAL KERATINOCYTES

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For decades, the expansion of human epidermal keratinocytes relies on the use of murine 3T3 fibroblasts as feeder cells and animal-derived component in the culture media. Despite its robust clinical efficacy on severe burns, the presence of undefined xenogeneic components in the 3T3 culture system still poses as a safety concern in today's regulatory requirements for cell therapy. In vivo organized cell types such as epithelial cells are tightly anchored to a basement membrane (BM). Among many of the BM components, laminins are the only components that are highly cell type specific and contribute to the BM structure and behavior of the associated cells. Therefore, we hypothesize that the use of biologically relevant laminin matrices could be used to replace feeder and support the growth of human epidermal keratinocytes in vitro. In this work, we found two laminin isoforms could best support adult human keratinocytes growth in a completely xeno-free and chemically defined method. We validated keratinocytes cultured on these two laminin systems vs. the standard 3T3 co-culture method by assessing their growth potential, expression of basal and differentiation markers by qPCR, immunostaining, FACS, as well as organotypic culture functional assay. When compared to 3T3 co-culture control, we found that keratinocytes grown on laminin system showed similar expression levels of basal and differentiation markers. Furthermore, through in-vivo flap model, we demonstrated that keratinocytes cultured in our laminin systems were able to form fully stratified epidermis. As our culture system is xeno-free and fully defined, this method will not only provide safer products for the patient, but also open up greater applicability in epithelial stem cell therapy (e.g. management of less severe burns and chronic wounds).

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W-1091

TRANSCRIPTIONAL PROFILE OF HUMAN EPIDERMAL STEM CELLS AND STEM-DERIVED TRANSIENT PROGENITORS

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The epidermis is 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. In the last thirty years, a method for cultivating and expanding human keratinocytes has been consolidated and widely used in disease modelling, drug discovery and regenerative medicine. Autologous cultures of human keratinocytes generate epithelial grafts used for treatment of massive full-thickness skin burns, chemical burn-dependent ocular burns with limbal stem-cell deficiency, or ex-vivo gene therapy approaches for severe genetic skin diseases, such as Epidermolysis Bullosa. In all these cases, a correct amount of KSC is required in order to permanently regenerate a functional epithelium. Clonogenic keratinocytes can be analysed at clonal level. KSCs give rise to Holoclones (H), whilst TACs give rise to Meroclones (M) and Paraclones (P). We characterized the molecular profile of H vs M to unveil molecular pathways sustaining KSCs. We isolated RNA from H, M and P from different strains and microarray analyses was performed. We identified differentially expressed genes and delineated a molecular signature of each class of clones. We demonstrated that H have a characteristic profile: genes involved in DNA repair, checkpoint control, mitosis and homologous recombination are upregulated, and genes linked to apoptosis, cell movement and DNA damage are downregulated. In particular, we focused on the transcription factor FOXM1, which is involved in G1/S transition, S progression and in G2/M transition, cell migration and kinetochore assembly and known to regulate hematopoietic stem cells. Western blot, real time PCR and immunofluorescence analysis confirmed that FOXM1 is upregulated in H vs M. To investigate the role of FOXM1 in cultured primary human keratinocytes we modulated its expression with loss and gain of function experiments. These experiments reveal that FOXM1 maintain the correct amount of KSC and stemness markers, such as p63 and Survivin, during mass culture serial cultivation. Moreover, we found that FOXM1 protein levels depend on the transcriptional activity of YAP, a known regulator of KSCs, confirming the role of FOXM1 in stemness maintenance.

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EYE AND RETINA

W-1093

HUMAN CULTURED AUTOLOGOUS ORAL MUCOSAL EPITHELIAL CELL SHEET (CAOMECS) AND PROGENITOR STEM CELL MAINTENACE

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Limbal stem cell deficiency (LSCD) is currently treated with donor corneal graft tissue. Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) have been successfully used as a non-limbal cell source to treat unilateral and bilateral LSCD. However, despite intensive investigation, it remains obscure why the reconstructed epithelium derived from CAOMECS has most of the self-renewing progenitor stem cells observed in normal corneal epithelium. The present study characterized the human carrier free produced CAOMECS, compared to human cultured corneal epithelial cells (hCEC). CAOMECS was produced using donated small biopsies obtained following informed consent procedures. Histological analysis demonstrated that CAOMECS basal cells and the supra-basal daughter cells expressed the renewing progenitor stem cell deltaNp63 as well as the proliferating cell nuclear antigen (PCNA) and Ki-67. Semi-quantitative analysis showed that PAX6, Bmi1, and Vimentin were also expressed in CAOMECS. Our results also show that cytokeratins K4 and K13 were only expressed in the apical cells in CAOMECS, which suggested that these basal cells

might promote differentiation to apical cells during perpendicular divisions. The underlying mechanism of this differentiation is proposed to be regulated by the activity of proteasomes. Indeed, we found a significantly elevated proteasome chymotrypsin-like activity and a significantly high expression of the proteasome catalytic core, 20S, in CAOMECS, as compared to hCEC. Proteasomes were then semi-purified from CAOMECS and hCEC using non-denaturing zonal centrifugations. Proteomic analysis showed that CAOMECS have more than one proteasome population, while hCEC have only one proteasome subtype. We previously reported a change in proteasome population in rabbit with experimentally induced LSCD. It is possible that CAOMECS exhibits a high proteasome activity to maintain and regulate the levels of transcriptional factors and epithelial stem cell markers (DeltaNp63, PAX6, or BMi1) in basal and supra-basal cells. ***Funding Source:*** Supported by Emmaus Life Sciences, Inc.

W-1095

CHARACTERISING THE ENGRAFTMENT AND ALLOGENEIC REJECTION OF MOUSE CORNEAL STEM CELL TRANSPLANTS

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Destruction or dysfunction of corneal stem cells, most commonly through chemical injury, leads to the development of limbal stem cell deficiency (LSCD), which causes painful inflammation and progressive vision loss. Current treatment for LSCD patients requires the ex vivo culture and transplantation of corneal stem cells. Whilst the outcomes of autologous transplants are good these are only possible in unilateral LSCD. Outcomes following allogeneic cell transplants are poor, most likely due to immune mediated rejection; the precise mechanisms of which are not yet fully understood. In order to investigate this process we have established a murine model of LSCD and allogeneic limbal epithelial stem cell (LESC) transplantation. The characteristic features of neovascularisation, corneal opacity, and epithelial defects develop within a few days of the initial chemical injury, and are accompanied by a significant infiltration of inflammatory monocytes, neutrophils, NK, and T-cells into the cornea. Using a combination of fluorescently labelled LESCs and serial in vivo imaging we have characterised the engraftment kinetics and survival of transplanted allogeneic corneal stem cells. We have established a similar model in immune deficient NOD/SCID gamma (NSG) mice to investigate the mechanisms of allorecognition and graft rejection by adoptively transferring purified immune cell types, permitting a detailed analysis of their role in allograft rejection.

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W-1097

INDUCTION OF IN VIVO RETINAL STEM CELL PROLIFERATION, EXPANSION AND ENHANCED GROWTH FACTOR STIMULATION THROUGH 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 culture, RSCs have the capacity to self-renew and differentiate into all the cell types of the retina, and readily proliferate to form clonal, free-floating spheres in 7 days. However, in vivo, RSCs do not proliferate or generate new retinal cells in adult mammals. Previously, we identified BMP and sFRP2 proteins as mediators of adult RSC quiescence with in vitro experiments. Here, we investigated whether BMP and sFRP2 inhibition could induce RSC proliferation in vivo in 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 inhibition, each single factor induced a ~5-fold increase in proliferating (EdU+) CE cells (Pax6+). Combined Noggin and anti-sFRP2 inhibition did not have an increased effect. In contrast, Noggin and anti-sFRP2 inhibition combined with the growth factors FGF2 and Insulin (GFs) did induce an additive increase in EdU+/Pax6+ cells (~12 fold), whereas GFs alone showed a ~5-fold increase in EdU+/Pax6+ cells. At 31 days after inhibition, the EdU+/Pax6+ population persisted but were Ki67-, indicating these cells survive, but do not continuously divide. Some of those EdU+/Pax6+ populations were in discrete clusters within the CE, suggesting there may have been clonal growth, and a neural retinal progenitor or CE identity for the cells. At both 7 and 31 days after Noggin or anti-sFRP2 inhibition, clonal sphere assays revealed an over two-fold increase in the number of sphere-forming RSCs present in the eye. Together, these results demonstrate that blocking BMP and sFRP2 in the adult mouse eye can bring RSCs out of quiescence, induce them to proliferate and expand, and enhance their response to growth factors. A recent study established that the CE contributes neurons to the retina during embryonic development, using an Msx1-CreERT2 inducible lineage tracing model. We have acquired this model and have demonstrated that the clonal RSC spheres derived from the adult CE are labeled by this lineage marker. Therefore, we are

currently using this paradigm to investigate if RSCs activated in the adult eye will migrate and differentiate into retinal neurons, recommencing the developmental phenomenon.

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W-1099

HUMAN EMBRYONIC STEM CELL-DERIVED CELLS AND EYE ORGANOIDS ARE RELEVANT IN VITRO SYSTEMS TO MODEL OCULAR DISEASES INVOLVING THE MACULA

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The macula is the region of the retina responsible for bright-light high-resolution and color vision. In maculopathies, such as Age-related macular degeneration (AMD), the photoreceptors and underlying Retinal Pigmented Epithelium (RPE) degenerate, leading to progressive vision loss. Mice do not have a macula and limited relevant *in vitro* systems have been developed to understand maculopathies. DRAM2 (DNA damage-regulated autophagy modulator 2) mutations cause a monogenic early onset of maculopathy by an unknown cellular mechanism. Our goal was to compare different human *in vitro* systems to determine if they could help understand the functional consequences of DRAM2 loss. Relevant systems might then be used for the study of more complex ocular diseases such as AMD. First, we compared primary human fetal RPE (hfRPE) cells to human embryonic stem cells-derived RPE (hESC-RPE) cells. We found that both primary and stem cell-derived cells had similar differentiation potential, with pigmentation, expression of tight junctions and phagocytosis of photoreceptor outer segment capacity. We then used lentivirus shRNA to knock-down DRAM2 in hfRPE, and CRISPR/Cas to knock-out DRAM2 in hESC followed by RPE differentiation. We found that hESC-RPE DRAM2 ko/ko cells had delayed maturation and resisted less upon passaging compare to the DRAM2 wt/wt cells. Furthermore, we found that DRAM2 loss increased cell death of both hfRPE and hESC-RPE cells when challenged by toxic exposure to sodium iodate or the lipofuscin component A2E. Second, we investigated the consequence of DRAM2 loss on photoreceptors, using hESC-derived eye organoids. After directed differentiation, we confirmed the presence of both cones and rods photoreceptors in the eye organoids using single cell RNA sequencing. Our preliminary results show that loss of DRAM2 causes a decrease in the number of mature photoreceptors in the hESC-derived eye organoids. In conclusion, we found that human embryonic stem cells can be differentiate into mature RPE cells and photoreceptors, providing relevant *in vitro* systems to model and study ocular diseases. Using these systems, we found that DRAM2 loss accelerates cellular degeneration and increases stress-induced cell death, potentially mimicking the pathophysiology of the DRAM2 maculopathy in human patients.

STEM CELL NICHES

W-1101

THE ROLE OF CTGF IN THE BONE MARROW NICHE IN THE PROGRESSION OF MYELOPROLIFERATIVE NEOPLASMS

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Myelofibrosis (MF) describes a myeloproliferative neoplasm (MPN) in which an aberrantly self-renewing stem cell population results in scarring of the bone marrow, reducing its capacity to produce myeloid cells. Connective tissue growth factor (CTGF) is a mitoattractant that has been implicated in a range of fibrotic diseases, and has more recently been proposed as a potential biomarker for MF. However, the role of CTGF within the bone marrow niche in the malignant progression of MPNs has yet to be elucidated. DNA sequencing of CD34+ cells from saliva (germ line control) and corresponding peripheral blood (somatic cells) of 54 patients for inherited or acquired mutations identified VWA1 component of the CTGF protein as the most highly mutated gene in all MPNs. RNA sequencing analysed the differential gene expression of 96 samples of bone marrow and peripheral blood from a variety of human MPNs sorted for stem (CD34+CD38-), progenitor (CD34+CD38+) and stromal (CD34-) cells. This revealed a significantly higher expression of CTGF in stromal cells of the bone marrow in patients with myelofibrosis compared to stem and progenitor populations in both the peripheral blood and bone marrow. Upon transduction of a CML cell line using a CTGF-expressing vector, an increased release of BCL2L, BCLXL and MCL1L correlated with a significantly increased percentage of viable cells following Dasatinib treatment at increasing concentrations, compared to controls. The use of shCTGF and CTGF anti-sense oligonucleotide (ASO) to knockdown the expression of CTGF in both CML cells overexpressing CTGF and stromal cells resulted in alterations to cell cycle progression, detected using qPCR and flow cytometry, and self-renewal. Our data demonstrates that reducing the expression of CTGF,

involved in the interaction of myeloid and stromal cells, in the bone marrow niche may be important in the therapeutic targeting of myeloid cells that currently resist chemotherapy.

W-1103

RAT CRANIAL BONE-DERIVED MESENCHYMAL STEM CELLS TRANSPLANTATION PROMOTES FUNCTIONAL RECOVERY IN ISCHEMIC STROKE MODEL RATS

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The functional disorders caused by central nervous system (CNS) disease such as ischemic stroke are clinically incurable and current treatments have limited effects. Previous studies have suggested that cell-based therapy using mesenchymal stem cells (MSCs) have therapeutic effects for ischemic stroke. In addition, the characteristics of MSCs might depend on their sources. Among various derived tissues of MSCs, we focused on the cranial bone originated from neural crest. We previously demonstrated that human cranial bone-derived MSCs (cMSCs) have higher neurogenic potential than human iliac bone-derived MSCs. Therefore, we presumed that the cMSCs have higher therapeutic potential for central nervous system disease such as ischemic stroke. However, detailed therapeutic effects of cMSCs remain unclear. In the present study, we aimed to demonstrate the therapeutic effects of rat cranial bone-derived MSCs (rcMSCs) transplantation for ischemic stroke model rats. The rcMSCs showed significantly higher mRNAs expression of brain-derived neurotrophic factor and nerve growth factor than those of rat bone marrow-derived MSCs (rbMSCs). The rcMSCs transplantation group resulted in remarkable functional recovery in the ischemic stroke model rats compared to no transplantation and rbMSCs transplantation groups. Furthermore, in vitro study, conditioned medium of the rcMSCs significantly suppressed cell death of neuroblastoma x glioma hybrid cells (NG108-15) exposed to oxidative and inflammatory stresses. These results suggest that the cMSCs might be a valuable candidate for cell-based therapy to CNS diseases.

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W-1105

CALCINEURIN REGUALTES PROPOTIONAL GROWTH OF ZEBRAFISH FINS BY REGULATING KCNK5B

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Zebrafish regenerates organs and appendages to the exact same dimensions as the original lost structures. This control on proportional growth is a fundamental yet poorly understood phenomenon that involves the coordinated scaling of all the stem and progenitor cells of each tissue within the organ. We previously found that the phosphatase calcineurin regulates scaling of zebrafish appendages, but the mechanism through which calcineurin regulated the proportional growth remained unknown. We now show that calcineurin controls proportional growth by regulating Kcnk5b, a potassium channel that regulates membrane potential. Electrophysiology measurements of cells overexpressing the zebrafish Kcnk5b show that increasing calcineurin activity inhibits channel-mediated conductance at the plasma membrane; whereas, inhibiting the endogenous calcineurin activity of the cells increases Kcnk5b activity. Removal of a predicted consensus calcineurin binding site in the C-terminal cytoplasmic tail of KcnK5b increased channel activity even in cells overexpressing activated calcineurin. As a phosphatase, calcineurin should regulate the channel by dephosphorylation. We found that a phosphorylation mimic at serine 345 (Kcnk5bS345E) not only increased conductance but also made the activity of the channel resistant to calcineurin inhibitory effects, while the converse dephosphorylation mimic of the channel Kcnk5bS345A decreased channel conductance. Mimics at other serines did not have these effects and were still regulated by changes in calcineurin activity, suggesting that calcineurin regulates Kcnk5b by dephosphorylating through a specific serine. Current results of transgenic overexpression of the mutant serine channels in vivo indicate that serine345 regulates the

scaling of the fish appendages. Thus, our results provide a mechanism through which calcineurin mediates post-translational regulation of cell membrane potential to scale tissue.

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W-1107

UNCOVERING NOVEL ASPECTS OF HUMAN NEURAL CREST BIOLOGY USING MULTIOMIC APPROACHES

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Abnormalities in neural crest cell (NCC) development lead to neurocristopathies such as Hirschsprung's disease, craniofacial and congenital heart defects. Infants born with Down syndrome (DS) have an increased risk of developing these, suggesting that trisomy 21 causes NC defects. We developed a protocol to generate SOX10+ NCCs from human pluripotent stem cells (hPSCs). Temporal analysis of gene expression demonstrated that hPSCs exit from pluripotency, and sequentially progress through a neural plate border stage, before upregulating definitive premigratory NC and next cranial migratory NC genes. To obtain pure populations of SOX10+ NCCs, we employed CRISPR/Cas9 genome editing technology for site-specific insertion of a reporter cassette containing coding sequences for mMaple and Puromycin-N-acetyltransferase into the 3'UTR region of the SOX10 locus. We show that reporter line derived mMaple+ cells uniformly express SOX10 protein and are resistant to Puromycin, permitting their facile purification. Pure SOX10:mMaple+ cells could be expanded 35 fold in our differentiation medium without antibiotic selection and without significant loss of SOX10 expression or NC differentiation capacity. We next performed multiomic analyses to better define the molecular signatures of SOX10:mMaple positive and negative cells. Bulk RNA-seq revealed that SOX10+ cells are akin to cranial migratory NCCs, while SOX10- cells resemble specified premigratory NCCs. We further demonstrate that SOX10+ NCCs exhibit morphological and motility profiles consistent with an early migratory cranial NCC identity and are patternable with retinoic acid. Single cell RNA-seq of these NCCs revealed intra- and inter- population heterogeneity and potential causes thereof. Discovery proteomics further identified known and novel regulators of NC EMT, migration and morphology, and a cell surface marker specific to SOX10+ cranial NCCs. This marker next permitted the isolation of pure SOX10+ cells from non-tagged wild type and DS-affected hPSCs, and identification of defects in migration and proliferation kinetics of DS-NCCs that could be overcome by the addition of SAG. Collectively, our datasets contribute to a better understanding of NC developmental biology and exemplify a strategy for investigating human neurocristopathies.

Funding Source: BioPlatforms Australia; Stem Cells Australia; Australian Institute of Bioengineering and Nanotechnology

W-1109

DEMYSTIFYING PARALOGOUS STEM-CELL NICHES TO UNFOLD PROGENITOR CELL IDENTITIES

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Elucidation of stem-cell niches both in vivo and in vitro is critical for understanding of cellular lineage commitment and differentiation. It would also provide significant insights into the resolutions of many existing controversies in the stem-cell field. We have recently proposed a paralogous stem-cell niche (PSN) concept that clarifies the coupling and uncoupling mechanisms between a specific stem-cell niche with its dynamic regeneration sites at different developmental stages. Developmentally, PSNs are gradually transformed analogous niches within an individual species during its life span. After the first epithelial-mesenchymal transition (EMT) in the primitive streak, progressive variations of cell identity give rise to a cluster of adjacent stem-cell niches (i.e., PSNs) for fostering specific types of progenitors and adult stem cells. The PSN model was further supported by analysis of transcriptional signatures

of mouse *Nes*-GFP and leptin receptors, which are two representative marker genes that are frequently used to monitor skeletal stem-cell (SSC) lineage development in murine bone marrow. Notably, we used large human genomic data-informatics to facilitate genetic analyses of mouse models and to resolve existing controversies (e.g., the debated "mesenchymal stem cells" or "MSCs") in bone marrow stem-cell biology. Unraveling of complicated mouse genetic data and their translational relevance to human biology would pave the way to achieving precision use of desired stem-cell resources for regenerative medicine and drug discovery.

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W-1111

CANINE SKIN-DERIVED NEURAL PRECURSORS ENGRAFT IN THE AGED RODENT HIPPOCAMPUS AND RESCUE MEMORY FUNCTION

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The isolation and culture of neural precursors show promise as an autologous stem cell therapy for the regeneration of depleted or dysfunctional neuronal circuits in patients with neurodegenerative disorders. The rapid clinical translation of this research has however been limited by the frequently reported propensity for glial differentiation in vitro and in vivo, or due to a technical reliance on transgenic modifications. We utilize a two-step culture system to generate high yields of P75-Nestin-CD133-positive neural precursor cells from adult canine skin without the use of genetic manipulation. These skin derived neural precursors are highly homogenous in culture, rate-limited by virtue of low number of maximal cell doublings, and differentiate almost exclusively into neurons - demonstrating high expression levels of neuron specific markers such as βIII-tubulin (96%) and MAP2 (74%). Following transplantation into the hippocampi of aged rats, these cells survive, migrate extensively, and mature structurally and functionally into electrophysiologically active neurons capable of synaptic integration with host hippocampal circuitry. Moreover, in aged rats, selective hippocampal-dependent age-related memory deficits are reversed, restoring memory function back to levels equivalent with young rats. Adult skin-derived neural precursors are from an easily accessible, readily available source, homogenous in culture, and bias to a neuronal fate in vivo. Our success in reversing age-related memory impairment in rodents has paved the way for our current autologous cell therapy trial treating a naturally occurring dementia syndrome in older pet dogs.

W-1113

ISOLATION AND CHARACTERISATION OF TASMANIAN DEVIL (SARCOPHILUS HARRISII) DENTAL PULP STEM CELLS - A 'ONE HEALTH' APPROACH TO A PERIPHERAL NERVE SHEATH TUMOUR MODEL: PRELIMINARY RESULTS

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Studies investigating Tasmanian devil dental pulp stem cells (tdDPSC) have not previously been reported. Wild populations of the devil are currently threatened by an aggressive and transmissible cancer of Schwann cell origin: Devil Facial Tumour (DFT). To better understand DFT a pure devil Schwann cell population is required. Schwann cells are a well-established target for DPSC differentiation. Taking a One Health approach to this disease by utilising human DPSC techniques will generate both a Schwann cell model for DFT, and also a peripheral nerve sheath tumour (PNST) model for human research. Before a tdDPSC derived Schwann cell population can be produced, tdDPSC must first be isolated and characterised. Utilising a protocol developed for human DPSC, this study aims to isolate Tasmanian devil dental pulp cells (tdDPC) and characterise them as tdDPSC. Canine teeth were removed from 3 devils free of DFT - euthanised on grounds unrelated to this study. The dental pulp from each tooth (n=12) was extracted and digested in a solution of collagenase/dispase. The tdDPC were then suspended in DPSC medium and seeded into T25 culture flasks. Phenotypic/morphological analysis via light microscopy was regularly observed and cells were passaged at 80% confluence. Cell growth was measured

using a WST-1 cell proliferation assay. Multipotency was assessed by culturing tdDPC in osteogenic and adipogenic media for 4wks. Following tdDPC isolation, growth of the adherent cultures was observed after a 5 day period. Preliminary results show successful isolation and propagation of tdDPC from 11 out of the 12 canine teeth. WST-1 results show that proliferation of tdDPC has remained stable. There are no statistically significant differences in cell proliferation amongst tdDPC populations ($p > 0.05$). tdDPC have shown promising potential for multilineage differentiation. At 2-3wks of culture morphological changes are visible in tdDPC cultured in osteogenic media. Differentiation potential will be confirmed via cell staining for mineral and fat deposits. In conclusion, tdDPC were extracted and isolated successfully. Preliminary results indicate tdDPC demonstrate stem-like qualities; this is an important step towards further characterisation of tdDPSC and ultimately Schwann cell differentiation as a model for DFT and other PNSTs.

Funding Source: Sir Mark Mitchell Foundation Research Grant

NEURAL DEVELOPMENT AND REGENERATION

W-2001

MATURE SCHWANN CELLS BUT NOT DEVELOPING SCHWANN CELLS SUPPORT AXON REGENERATION AFTER PERIPHERAL NERVE INJURY

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Despite of the fact that peripheral nerve can regenerate, clinical outcomes after peripheral nerve injuries (PNI) are still unsatisfactory, especially in severe and proximal injury cases. Accumulated evidences show that Schwann cell (SC) graft is one of potential approaches for regenerative therapy after PNI. While the graft of glial precursors supports axon regeneration in adult central nervous system, it remains to be elucidated that SCs at developmental stages promote regeneration of adult axons after PNI. The purpose of the current study is to elucidate the axon promoting effects of developing SCs after PNI. Total of 4 types of SCs were tested, including 1) SC precursors (SCPs), 2) immature SCs (ISCs), and 2 types of mature SCs, which were 3) repair SCs (RSCs) and 4) non-RSCs. All cells were prepared from RFP transgenic Lewis rats. SCPs, ISC, and non-RSCs were harvested from intact sciatic nerves at embryonic day 14 (E14), E18, and postnatal 10-12 weeks. RSCs were isolated from transected adult sciatic nerves at 1 week after injury. One million cells were grafted into 25 mm long cell-free area between crush injuries in a sciatic nerve of syngenic Lewis rat. Cell-free area was achieved by repeated freeze and thaw procedures with liquid nitrogen. Crush alone (no decellularization) and no cell graft groups were used as positive and negative controls. Two weeks after injury and grafts, RSCs group showed the greatest axon regeneration among all cell graft groups, although its extent was still significantly reduced compared to a positive control. Non-RSCs was the next effective cell type. Surprisingly, SCPs and ISCs failed to support axon regeneration at all, even though they maintained proliferative ability after grafting. Further, in vitro culture of dorsal root ganglion (DRG) neurons at adult and embryonic (E14) stages in combination with RSCs and SCPs demonstrated that RSCs but not SCPs promoted neurite outgrowth of adult DRG neurons and that neither of RSCs and SCPs stimulated neurite outgrowth of E14 DRG neurons. These findings indicate that, unlike CNS, SCs at developmental stages don't support regeneration of adult axons after PNI and that mature SCs, especially RSCs, are good candidates as a graft cell type for regeneration therapy after PNI.

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W-2003

THE KYNURENINE PATHWAY OF TRYPTOPHAN METABOLISM MODULATES NEURAL STEM CELL PROLIFERATION

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The search for molecules which critically regulate neural stem cell (NSC) proliferation is ongoing, underpinning future production of cell lineages for therapy, while helping understand why innate repair in neurodegenerative diseases fails. Our ongoing research has investigated a role of the kynurenine pathway (KP) in healthy metabolism and neurodegenerative diseases. The KP critically regulates bioavailability of the essential amino acid tryptophan. In MS the KP is dysregulated, producing high levels of metabolites like neurotoxic Quinolinic acid. We investigated if modulating the KP altered NSC proliferation. In particular, if interferons (IFNs) activate KP and drive changes in the proliferation of NSCs. Developing mouse NSCs from E14 neurospheres were cultured. Agonists, antagonists or siRNAs to KP enzymes were used to dissect the pathways. IFN-gamma (IFN-g) activates indoleamine-2,3-dioxygenase (IDO-1) expression, the initial rate-limiting enzyme metabolising Tryptophan, and indeed significantly induced IDO-1 in NSCs. NSCs express all KP enzymes, and IFN-g lead to impaired proliferation and an alteration of metabolic state of NSCs including their NAD+/NADH ratio (cell energy levels) via Trp depletion (required for protein biosynthesis), rather than through effects of KP metabolites. IFN-b negligibly affected IDO-1 levels, but induced IDO-2, and significantly decreased proliferation and downstream enzyme kynurenine-3-monooxygenase. 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 e.g. IFN-g and IFN-β. Selective KP inhibition could minimize cell death during inflammatory episodes and optimize NSC proliferation and differentiation with direct therapeutic applications.

W-2005

SYNAPTIC INTEGRATION OF INTRASTRIATAL VERSUS INTRANIGRAL GRAFTS OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS IN THE 6-OHDA-LESIONED ADULT RAT BRAIN

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Human embryonic stem cell (hESC)-derived neurons survive long-term, release dopamine, and extend axons to fill functionally-appropriate host structures after transplantation into the adult rat brain. Using a monosynaptic rabies virus-based tracing technique, we have recently shown that hESC-derived neurons integrate into host circuitry, establishing both host-to-graft and graft-to-host synaptic connections. Here, we use the same methodology to further investigate the connectivity of midbrain- and forebrain-patterned hESCs-derived neurons transplanted either to the striatum or substantia nigra of 6-OHDA-lesioned rats. To assess for host-to-graft synaptic connectivity, animals were injected with modified rabies virus 23 weeks after transplantation, and were perfused one week later. Analysis 24 weeks post-grafting revealed that both local and distant host neurons made extensive synaptic contacts onto both intrastriatal and intranigral grafts. The gross anatomical location of host cells labelled with rabies depended more on the location of transplantation than on the phenotype of the cells grafted. Further, we have identified host neurons making monosynaptic contacts onto graft-derived neurons which express molecular markers of cells that participate in canonical circuits of the basal ganglia. We are now beginning to assess the kinetics of host-graft integration. In summary, we show that intrastriatal and intranigral grafts of hESC-derived neurons can integrate into host circuitry, and that the pattern of host connectivity is primarily dependent on location of the transplant.

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W-2007

SREBF1, A NOVEL REGULATOR OF MIDBRAIN DOPAMINERGIC NEUROGENESIS

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Liver X Receptors (LXRs, NR1H2 and 3) and their ligands (oxysterols and 24,25-epoxycholesterol) are potent regulators of midbrain dopaminergic (mDA) neurogenesis and differentiation. However, the molecular mechanisms by which LXRs control these functions remain unknown. To elucidate the mechanisms by which LXR regulates mDA neurogenesis, we performed a combined transcriptome and ChIP-seq analysis of mouse midbrain cells after LXR activation. Gene set enrichment and causal network analysis of the data allowed us to identify a novel transcriptional network controlling mDA neurogenesis. Our results show the basic helix-loop-helix transcription factor, sterol regulatory element binding protein 1 (SREBP1), as part of a cluster of proneural transcription factors in radial glia that controls a transcriptional network formed by factors such as Foxa2 and Ferd3l. Loss and gain of function experiments in vitro and in vivo demonstrate that Srebf1 is both required and sufficient for mouse embryonic mDA neurogenesis. .Moreover, activation of this pathway in human embryonic or neuroepithelial stem cells enhanced the generation of mDAs. In sum, our data identifies Srebf1 as a novel central player in mDA neurogenesis.

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W-2009

METABOLIC GLYCAN LABELING-ASSISTED DISCOVERY OF CELL-SURFACE MARKERS FOR PRIMARY NEURAL STEM AND PROGENITOR CELLS

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Cell surface proteins play important roles in biological processes such as cell-cell adhesion, signal transduction, immune response, and cellular homeostasis. Most cell surface proteins are modified by glycans, which regulate protein function and cellular complexity in diverse tissues including the central nervous system. Cell surface glycoproteins that are expressed specifically on stem cells can also serve as markers for identifying stem cells and their location in vivo, and for generating a pure population of stem cells for genetic analyses and therapeutic use. Neural stem cells are capable of self-renewing and differentiating into multiple neural lineages, either directly or indirectly through intermediate progenitor cells. However, due to their rarity in tissue and lack of suitable methodology, the surface glycoproteome of neural stem cells remains to be identified. Here we developed a highly efficient and sensitive approach to selectively enrich surface proteome by metabolically labeling sialylated proteins with azide followed by click chemistry and mass-spectrometry analysis. Using endothelial cell coculture to enrich primary neural stem cells, we labeled and identified surface proteins in neural stem cells and differentiating cells. We have revealed differential expression levels of sialylated surface proteins in neural stem cells compared to that in differentiating cells, including known neural stem cell markers such as EGFR, Glast and VCAM1. In addition, we propose that Igsf8 as a novel candidate marker for intermediate neural progenitor cells in the embryonic cerebral cortex. This work offers a new strategy to specifically label and identify surface proteins of stem cells through metabolic glycan labeling and can be applied to other systems for cell surface marker exploration.

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W-2011

PROTEOMIC EVALUATION OF KEY NEURAL PROTEINS DURING NEURAL DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS

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The incidence of neurological disease in the population is increasing, however the mechanisms underlying the etiology of a host of neurological diseases remain ambiguous. Current evidence implicates disturbances of signalling pathways during neurodevelopment as a causative factor, highlighting the necessity of simple and robust models for studying neurodevelopment. This study aimed to utilize the neural differentiation of mesenchymal stem cells as a model for neurodevelopment, and subsequently analyse the expression and function of the proteins Reelin and GM1 gangliosidase, the malfunction of which are correlated with an increased risk of schizophrenia, Alzheimer's Disease, and Parkinson's Disease. Cell samples and secretions were collected at various time points during neural differentiation of human adipose-derived stem cells, and underwent proteomic analysis via shotgun LC-MS/MS, BN-PAGE, Western blotting, and Bioplex multiplex immunoassay. Reelin and proteins pertinent to Reelin signalling and neuronal migration were detected, whilst GM1 gangliosidase and proteins relating to ganglioside catabolism were also observed. Furthermore, a potential interaction between Reelin and catechol-O-methyltransferase, a proposed etiological factor in schizophrenia, was

detected. Finally, the upregulation of neuroprotective cytokines and limited expression of pro-inflammatory cytokines was consistent with literature indicating their role in signalling pathways during neurodevelopment. Together, this data shows potential Reelin and GM1 gangliosidase signalling during neurodevelopment, and validates neuronal differentiation of adipose-derived stem cell as a neurodevelopmental model.

W-2013

NEURAL REGENERATION OF RAT BRAIN INJURY MODELS VIA CELL THERAPY

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Neurogenesis by activation of endogenous neural progenitor cells is considered as a potential treatment strategy for brain injuries. Although several researches support the benefit of erythropoietin (EPO) for neuroprotection and neuronal survival, yet there are still some technical problems needed to be overcome, such as the delivery system. In our studies, cDNAs of mouse EPO were transfected into cell lines of 3T3 fibroblasts. The expression and bioactivity for EPO were analyzed by immunocytochemistry, Western blot, Enzyme-Linked Immunosorbent Assay (ELISA), and functional assays. In this study, we produced ischemic stroke in adult rats by the approach of middle cerebral artery occlusion (MCAO) and examined the infarct zone with MRI. The EPO-overexpressing NIH/3T3 (EPO-3T3-EGFP) cells were directly injected to the infarct zone. The brain function was assessed via modified Neurological Severity Score (mNSS). On day 14 after stroke induction, the infarct volume was measured again by MRI and the animal was euthanized for the study of angiogenesis and neurogenesis. The result of neurological assessment suggested that both 3T3-EGFP-treated and EPO-3T3-EGFP-treated groups showed significantly improvement of functional ability in both acute and chronic phase of ischemic stroke. Our data suggests that EPO-overexpressing NIH/3T3 cells treatments could provide stable release of EPO in the brain injury area, facilitate neurogenesis and neuroprotection ability that may contribute to the functional recovery of brain.

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W-2015

ARYL HYDROCARBON RECEPTOR PATHWAY DEFINES TIME FRAME FOR RESTORATIVE NEUROGENESIS

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Zebrafish has high capacity to replace lost neurons after brain injury. New neurons involved in repair are generated by the specific set of glial cells, ependymoglia. Therefore, we analysed changes in the transcriptome of ependymoglia cells and their progeny after injury to infer molecular pathways governing restorative neurogenesis. We identified Aryl hydrocarbon receptor (AhR) to regulate ependymoglia differentiation towards post-mitotic neurons. In vivo imaging showed that high AhR signalling promotes direct conversion of specific subset of ependymoglia into post-mitotic neurons, while low AhR signalling promotes ependymoglial proliferation. Interestingly, we observed inactivation of the AhR signalling shortly after the injury and return to the basal levels 7 days post injury. Interference with the timely AhR regulation after injury leads to the aberrant restorative neurogenesis. Taken together, we identified AhR signalling as a crucial regulator of neurogenic fate of ependymoglia and of timing restorative neurogenesis in zebrafish brain.

W-2017

STRIATAL PROJECTION NEURONS ARE NOT REPLACED AFTER ISCHEMIC DAMAGE

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Neurogenesis from the subventricular zone (SVZ) is increased after striatal ischemia in both adult and neonatal rats. Under normal conditions the adult SVZ generates interneurons that migrate to the olfactory bulb, while the neonatal SVZ generates both interneurons and medium spiny neurons (MSN), the main neuronal subtype affected after striatal ischemia. In addition, it was reported that the adult brain is capable of redirecting SVZ progenitors to the injury site and reprogram their fate to become MSN. These results suggested an attempt of the brain to repair itself and stimulated research into successful 'endogenous cell therapies'. However, few years later conflicting results reported that the adult brain was in fact not capable of generating MSN after ischemia, thus reconsidering the real potential of endogenous cell therapies. In this study, we aimed at comparing the adult and neonatal brain after striatal ischemia, by investigating proliferation, neurogenesis levels and progenitors' phenotypes. The results showed that while the neonatal brain was producing MSN, the production rate was not increased by the injury. In addition, the results showed that the adult brain only generates interneurons in response to injury. This suggests that while endogenous cell replacement therapies are attractive, many factors including neurogenesis rate and neuronal fate, need to be manipulated in order to have a successful treatment for brain injury such as stroke.

W-2019

A STUDY ON THE IMPORTANCE OF REGENERATION OF GABAERGIC NEURONS IN RESTORING COGNITIVE FUNCTION BY HIPS DERIVED NEURAL CELL TRANSPLANTATION TO DEMENTIA MODEL MICE

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Cell replacement is one of the radical treatments on regenerative medicine. We have already reported that transplantation of neural stem/progenitor cells derived from human iPS (hiPS) cells improved cognitive dysfunction of human APP transgenic mouse (PDAPP) at the ISSCR. This year, we would like to report on the association between improvement cognitive dysfunction and regeneration of GABAergic neurons. The hiPS cell lines, 253G1 (RIKEN, Tsukuba, Japan), were used in this study. We first developed embryoid bodies (EB). Then EB were cultured on fibronectin (FN)-coated dishes and we added retinoic acid (RA), noggin-Fc (NOG) and sonic hedgehog (SHH). We transplanted the neuronal precursors into the PDAPP mice at day 8. Neural stem/progenitor cells derived from hiPS cells (2 x 105 cells; n=30) and PBS (n=19) were stereotaxically transplanted into hippocampus. MWM test was conducted 14 days before and 15 days after the transplantation to assess the spatial memory function of PDAPP mice. First, we conducted histological analysis of serial brain sections. We found that neural fiber increased in the transplanted brains. Some of neural fibers extended from transplanted cells. In addition, synapsin positive neural fibers were also observed. From these results, neurons derived from transplanted cells seem to form axons with transplanted neurons and host neurons. Some of these transplanted neurons were differentiated into VGAT positive GABAergic neurons in hippocampus. Furthermore, when GABA inhibitor was administrated to mice with restoration of cognitive function by transplantation, cognitive function of mice decreased again. In summary, after the transplantation, platform escape latency of the transplanted PDAPP mice was shorter compared with that of vehicle injected PDAPP mice and transplanted neurons were differentiated into VGAT positive GABAergic neurons in hippocampus. Since administration of GABA inhibitor reduced cognitive function restored by neural transplantation, regeneration of GABAergic neural system seems to play some important role in restoring cognitive function.

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W-2021

TRANSDIFFERENTIATION OF HADSCs INTO MOTONEURON-LIKE CELLS FOR CELL REPLACEMENT THERAPY OF SPINAL CORD INJURY

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Human adipose-derived stem cells (hADSCs) are increasingly presumed to be a prospective stem cell source for cell-replacement therapy in various degenerative and/or traumatic diseases. The potential of trans-differentiating hADSCs into motor neuron cells indisputably provides an alternative way for Spinal Cord Injury (SCI) treatment. In the present study, a stepwise and efficient hADSC trans-differentiation protocol with Retinoic Acid (RA), sonic hedgehog (SHH) and neurotrophic factors were developed. With this protocol

hADSCs could be converted into electrophysiologically active motoneuron-like cells (hADSC-MNs) which expressed both a cohort of pan neuronal markers and various motor neuron specific markers. Moreover, after being primed for neuronal differentiation with RA/SHH, hADSCs were transplanted into SCI mouse model and they survived, migrated and integrated into injured site and led to partial functional recovery of SCI mice. When ablating the transplanted cells with HSV-TK-mCherry-GCV overexpression system, functional relapse was detected by electrophysiological and behavioral assays, implying that transplanted hADSC-MNLCs participated in rebuilding the neural circuits, which was further confirmed by cis-neuronal tracing system (WGA). GFP-labelled hADSC-MNs were subjected to whole-cell patch-clamp recording in acute spinal cord slice preparation and both action potentials and synaptic activities were recorded which further confirmed that those pre-conditioned hADSCs indeed became functionally active neurons in vivo. As well, transplanted hADSC-MNs largely prevented the formation of injury-induced cavities and exerted obvious immune-suppression effect as revealed by preventing astrocyte reactivation and favoring the secretion of a spectrum of anti-inflammatory cytokines and chemokines. Our work suggests that hADSCs can be readily transformed into MNs in vitro and stay viable in spinal cord of the SCI mouse and exert therapeutic effect by rebuilding the broken circuitry and optimizing the microenvironment through immunosuppression.

W-2023

DISTINCT AND SYNERGISTIC ROLES OF NFIA, NFIB AND NFIX IN POSTNATAL MOUSE NEURAL STEM CELL SELF-RENEWAL AND DIFFERENTIATION

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Understanding postnatal neural stem/progenitor cell (pNSPC) self-renewal and lineage specification is key to future 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*. Germline loss of *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. 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) of such mice. pNSPCs of P10-20 mice were cultured in the presence of EGF and bFGF (proliferation (prolif.) conditions) then placed into medium lacking these growth factors (differentiation (differen.) conditions). Transcript levels of markers of self-renewal and differentiation were assessed by qPCR from RNA of cells cultured without (WT) or with 4HT (NFI-deleted) during both prolif. and differen. conditions. Treatment with 4HT during prolif. efficiently deleted all floxed alleles with >99% loss of transcripts within 3 days. Deletion of *Nfix* resulted in no changes in prolif. or neuronal or astrocytic differen., but a bias towards the oligodendrocyte lineage, consistent with our previous studies on germline loss of *Nfix*. Deletion of *Nfib* also resulted in no obvious changes in self-renewal or neuronal differentiation but reduced the expression of astrocyte markers, consistent with our previous studies on loss of *Nfib in vivo*. Surprisingly, simultaneous deletion of *Nfia* & *Nfib* resulted in a major reduction in self-renewal as seen by reduced PCNA and Nestin expression and the loss of colony forming ability. Upon differentiation there were increases in the neuroblast marker DCX and reduced expression of astrocyte and oligodendrocyte markers. This loss of self renewal appears specific for the combined loss of Nfia & Nfib as the combined loss of Nfib & Nfix does not result in this phenotype. 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.

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W-2025

THE ROLE OF PROGRANULIN ON HUAMN DOPAMINERGIC NEURONAL DIFFERENTIATION IN VITRO

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The method to enrich dopaminergic neurons in vitro is essential for Parkinson's transplantation. Our hypothesis is that maintenance of progranulin (PGRN) expression in neural precursors may be essential to ensure neuronal differentiation. The reported results from our previous studies indicate that the dopaminergic neuronal differentiation occurs only among the nestin^{+}/CD133^{+} human fetal brain cells rather than the cells with same markers isolated from adult human brain tissue. We also notice that the level of PGRN expression in fetal neural precursors is higher than that in adult neural precursor cells. To explore the molecular mechanism of PGRN to regulate dopaminergic neuronal differentiation, both PGRN antisense deoxynucleotides (PGRN -ADON) and

human recombinant PGRN protein were used for this study. Prior incubation of the nestin^{+}/CD133^{+} human fetal brain cells in dopaminergic differentiation medium, cells were cultured with the PGRN-ADON medium. After PGRN antisense treatment, the level of PGRN expression in the nestin^{+}/CD133^{+} human fetal brain cells was reduced. The decreased PGRN expression was confirmed using RT-PCR, Northern blotting and protein assays. No elevated apoptotic signal was detected after the cultures treated with PGRN-ADON. Interestingly, inhibition of PGRN expression declines the potential of dopaminergic differentiation among nestin^{+}/CD133^{+} human fetal brain cells. Meanwhile, it is likely reducing of PGRN expression increases GFAP expression and astrocytic differentiation. In contrast, using exogenous recombinated-PGRN protein to pretreat the nestin^{+}/CD133^{+} human fetal brain cells is able to supress the PGRN-ADON induced glio-differentiation. The results from this study indicate that high level of PGRN promotes neuronal differentiation. Although the mechanism of dopaminergic neuronal differentiation may be not limited to the intracellular PGRN expression level, effect of PGRN on DND deserves further investigation.

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NEURAL DISEASE AND DEGENERATION

W-2027

UNDERSTANDING AND CHALLENGING THE CONTRIBUTION OF HSC TO BRAIN MYELOID CELL TURNOVER

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We have shown that hematopoietic stem and progenitor cells (HSPCs) can contribute to brain myeloid cell turnover upon transplantation in recipients pretreated with proper conditioning. The transplanted HSPCs home to the brain, engraft locally and give rise to a mature progeny that shares transcriptional, morphologic and functional features with central nervous system (CNS) microglia (Capotondo et al. 2012). We thus postulated the existence within the HSPCs pool of functional hematopoietic equivalents of microglia progenitors (HE μP) that can replace CNS resident μP in defined experimental conditions. We demonstrated that i) HE μP are comprised within long-term (LT) hematopoietic stem cells (HSC)(Capotondo, Milazzo et al., 2017) and ii) GFP+ HE μP retain clonogenic potential when plated for the colony forming cell (CFC) assay after LT engraftment in the brain of myeloablated mice. We then transplanted hematopoietic cells isolated from the brain of primary GFP+ HSPCs recipient mice into secondary myeloablated recipients to assess whether CNS-engrafted HE μP also retain hematopoietic reconstitution potential. Interestingly, donor cells were able to engraft in hematopoietic tissues and brain of secondary recipients, showing multi-lineage differentiation capability. These results indicate that HE μP retain HSC functional features upon brain engraftment. An integration site analysis on the transplanted GFP-HSPCs and on the tissues isolated from primary donors and secondary recipients is ongoing to strengthen these results with clonal tracking data. Based on these findings, we hypothesized that also CNS-resident μP may be endowed with HSC features. We are thus experimentally addressing this hypothesis exploiting the Fgd5-Zs green mouse, a reporter animal model suitable for HSC identification (Gazi et al. 2015), since we already showed that Fgd5 expressing HSC are enriched in functional HE μP. Overall, the characterization of these cell populations will provide an ideal target for advanced, brain-directed myeloablative conditioning regimens and will get new insights for the identification of the ideal population for HSPCs transplantation protocols aimed at microglia reconstitution.

W-2029

MODELLING INTESTINAL GANGLIONEUROMATOSIS IN MEN2B DISEASE USING HUMAN EMBRYONIC STEM CELLS

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The ENS is an enormous ganglionated neural network in the wall of the gastrointestinal tract which develops from a small number of vagal neural crest cells (VNCC) that progress to enteric NCC (ENCC) then differentiate as enteric neurons (EN) and glia. Uncontrolled proliferation of ENS cells results in intestinal ganglioneuromatosis

(iGN). The first and prominent presentation in MEN2B patients is diffuse iGN and it is characterised by a disseminated, intramural or transmural proliferation of neural elements involving the enteric plexuses. With the advent of programmable engineered nucleases (ZFN, TALEN and CRISPR/Cas9), it is possible to generate specific mutations in pluripotent stem cells to study the effect of these mutations in the downstream differentiated tissue and cell types. Here, we generated the MEN2B mutation in RET, M918T, in human embryonic stem cells and differentiated them to ENCC, EN and enteric glia. Human Embryonic Stem Cells with the 918T mutation were generated by Homology Directed Repair (HDR), in three different lines, H9, HES3 and MEL2 using the CRISPR/Cas9 system and karyotyped. These cells were analysed for the expression of RET and other NC-related genes. The generated hESC mutants were differentiated to VNC-like cells and further differentiated to ENC-like cells, EN and glia by modifying previously published protocols. We further immunostained the differentiated ENCC, EN and enteric glia with the appropriate marker antibodies. In the pluripotent state, the MEN2B mutants showed an increased proliferation rate by EdU staining with a slight increase in RET mRNA expression. After differentiation, exposure of the M918T mutants to GDNF led to an increase in size of the neurospheres and in a monolayer culture, the mutants differentiated into bigger ganglion-like structures with abundant coarse and bundled neurites whereas control cells assembled into smaller groups with smooth and fine projections. This phenotype was reproducible in all of the 3 cell lines. Intestinal ganglioneuromatosis is the first and most prominent feature presented in MEN2B. The MEN2B mutant lines differentiated to ENCC, EN and glia mimics the diffuse iGN phenotype observed in patients. Modelling the disease in vitro will provide a platform to study this disease and other iGN cases, and for drug discovery.

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W-2031

USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO INVESTIGATE THE CONTRIBUTION OF RISK VARIANTS AND AGING TO THE ONSET AND PROGRESSION OF ALZHEIMER'S DISEASE

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Developing therapies for the treatment of Alzheimer's disease (AD) requires an understanding of the mechanisms that cause the disease. Animal models of AD have provided important insights but do not display important AD-related pathologies and have not been useful in modeling the complex genetics associated with "sporadic" AD. Although the majority of AD patients are sporadic, multiple genetic risk variants have been identified, the most powerful and prevalent of which is the E4 variant of Apolipoprotein E (APOE) gene. Compared to individuals with an APOE 3/3 genotype, heterozygosity for the E4 allele increases AD risk by 3 fold, and homozygosity for the E4 allele increases risk up to 12 fold. Amyloid-dependent and -independent mechanisms have been postulated to explain the APOE4 effect, but currently how APOE4 modulates AD disease risk, especially during aging, remains unclear. To that end, we are generating a diverse set of human induced pluripotent stem cell (hiPSC) lines from AD and non-demented control (NDC) patients with no (i.e. APOE 3/3) and two (i.e. APOE 4/4) copies of the E4 allele. We are using these hiPSCs to elucidate the potential genetic, molecular, and cellular mechanisms by which the APOE 4 allele contributes to AD onset and age-related disease progression. By using a novel 3D cortical neuronal culture model and genome-wide expression analysis (RNA-seq), we are identifying unique gene expression profiles that are independently defined by APOE genotype, disease status, and age. Future bioinformatic analysis will reveal candidate genetic, biochemical, and signaling pathways that will provide more definitive relationships between APOE genotype and AD onset and age-related progression. In the future, we will investigate how modulation of these candidate target genes and pathways regulates the manifestation of AD-related phenotypes. Such future investigations will have significant impact on the design of molecularly targeted therapeutics to treat AD.

W-2033

LARGE-SCALE GENERATION AND TESTING OF PATIENT DERIVED MOTOR NEURONS TO IDENTIFY CANDIDATE THERAPEUTICS FOR MOTOR NEURONS DISEASE

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Despite decades of intense research into Motor Neurons Disease (MND), people diagnosed with the disease have few therapeutic options capable of modifying the disease progression. The lack of effective therapeutics is driven largely by an inability to model sporadic forms of disease, which represent a majority of cases. However, the development of induced pluripotent stem (iPS) cells is now revolutionising MND research. The approach provides a powerful tool capable of modelling the disease process in sporadic patient motor neurons for the first time, and holds great promise for the development and testing of candidate therapeutics to treat MND. Here we report the implementation of a large scale, high throughput drug screening program aimed at accelerating the identification and validation of potential treatments for Motor Neuron Disease using patient derived iPS cells. We have generated iPS lines from patients with sporadic and familial MND, and healthy controls. To address the increasing recognition that disease heterogeneity plays an important role in MND, iPS lines from 100 sporadic and 20 familial patients were generated across a range of clinical phenotypes. The iPS lines were differentiated into spinal cord motor neurons using established directed differentiation protocols, and the cellular phenotype characterised to identify a subset of representative lines for drug screening. Drug screening will be conducted using an automated liquid handling robot to identify candidate drugs capable of increasing motor neuronal survival. This program represents an important advancement in the search for disease modifying therapeutics for the treatment of MND, and the iPS lines generated will provide a valuable resource for future research into MND in Australia.

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W-2035

HIGH GLUCOSE INCREASES Aβ PRODUCTION THROUGH ENDOSOMAL TRAFFIC JAMS: INVOLVEMENT OF AP2A1/PICALM-AND AMPK/MTOR-PATHWAY

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Growing evidence supports that diabetes mellitus (DM) is the important risk factors for Alzheimer's disease (AD). Many researchers have demonstrated that the increment of residence time of Amyloid precursor protein (APP) at endosome is critical for Aβ production. However, detail mechanism how DM regulates Aβ processing is still unclear. Therefore, this study investigated the effect of high glucose on the Aβ producing endosome. Our results using STZ-induced diabetic mouse, showed that DM upregulated Aβ secretion and activation of endosome. In vitro results with mouse hippocampal neuron and SK-N-MC demonstrated that high glucose increased Aβ production and enlarged endosomes which were reversed by treatment of Rab5a siRNA. High glucose increased APP endocytosis which is crucial for endosomal enlargement proved by pretreatment of dynasore(endocytosis inhibitor) that reversing the effect of high glucose on endosome. In addition, high glucose increased endocytosis related proteins, such as AP-2 complex subunit alpha-1 (AP2A1) and Phosphatidylinositol binding clathrin assembly protein (PICALM). And, silencing of each protein inhibited APP endocytosis, respectively. In addition, high glucose decreased endosomal clearance through inhibition of fusion with autophagosome by AMPK/mTORC1 activation. And, high glucose induced enlarged endosome and increased Aβ secretion were reversed by pretreatment of trehalose and rapamycin, autophagy inducer and mTOR inhibitor, respectively. In conclusion, high glucose induces endosomal traffic jams through AP2A1/PICALM-induced endocytosis and AMPK/mTOR-inhibited endosomal clearance, upregulating Aβ production.

W-2037

ALTERATIONS IN PROTEIN DEGRADATION IN DIRECTLY INDUCED DOPAMINERGIC NEURONS DERIVED FROM SPORADIC PARKINSON'S DISEASE PATIENTS

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Directly reprogrammed neurons (induced neurons; iNs) hold great promise for disease modeling of neurodegenerative disorders associated with aging as they maintain some of the signature associated with age from the parental cells. As such, they could also express the intra-cellular disease associated features occurring in idiopathic forms of Parkinson's disease (PD). Here, we have developed a reprogramming approach that successfully reprograms PD patients skin fibroblasts directly to dopaminergic neurons (iDA). This method leads to 70% of the fibroblast converting to neurons, of which approximately 20% express the dopaminergic marker tyrosine hydroxylase. We next investigate autophagy as well as ubiquitin-proteasome system alterations in iDAs derived from PD patient's skin fibroblasts. More specifically, we show that iNs from 42% of PD patients depict p62 accumulation, a marker of protein degradation, upon starvation-induced autophagy. Moreover, late stage suppression of the autophagic flux using chloroquine leads to a LC3II+ autophagosome depletion in 42% of PD lines.

These results suggest autophagy alterations, a feature that is believed to participate to the pathophysiology of PD, in PD-iNs from a subset of patients. As such, we are currently investigating factors that could influence those alterations with the hope to be able to segregate and categorize different type of patients based on the disease-associated features expressed specifically in their iNs.

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W-2039

MODELLING CHILDHOOD NEURAL PATHOLOGIES IN 3-DIMENSIONS

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Studying development and normal or abnormal function of the human central nervous system (CNS) is hampered by the very limited possibility of experimentally manipulating it *in vivo*. While monolayer cultures of human neural stem cells (NSCs), neurons and glia have conventionally provided means to study the human CNS, there is mounting evidence of the effect of dimensionality on cellular phenotype and behaviour. Hence, we have developed 3D (3-dimensional) cultures and established NSC lines, either from embryonic CNS or iPSCs (induced pluripotent stem cells), for modelling human damage in normal CNS and in CNS affected by congenital diseases. Focus has been on comparing response to damage in 2D and 3D cultures following disruption of calcium homeostasis (e.g. using thapsigargin) and oxygen-glucose deprivation (OGD), that can affect the developing CNS in uterus or perinatally with devastating consequences on brain function in the affected children. In addition, we are testing the hypothesis that children with certain congenital diseases, such as Down syndrome and Duchenne muscle dystrophy, have increased susceptibility to neural damage. Significantly, we have found that NSCs and neurons cultured in 3D hydrogels display a reduced susceptibility to Ca^{2+} and OGD-induced cell death compared to 2D culture. We show that the higher thapsigargin doses required for Ca^{2+}-induced cell death in 3D cultures is not due to limited drug permeability in the hydrogel, as dead cells were found to be distributed throughout the gel. Together, these results indicate that 3D cultures provide a more favourable environment for the cells, allowing one to analyse cell behaviour and pharmacological responses in a more "tissue-like" situation. Work suggesting differences in cellular responses between the normal and diseased iPSC-derived neural cells we have generated will also be discussed.

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W-2041

USING A NOVEL CRISPR/CAS9 SCREENING PLATFORM TO ELUCIDATE THE ROLE OF APOE RISK ALLELES IN ALZHEIMER'S DISEASE

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Late-Onset Alzheimer's disease (LOAD) is a common form of dementia that affects over 40 million people worldwide and is the 6th leading cause of death in the United States. The gene APOE presents in 3 isoforms (E2, E3, & E4), and consistently emerges as the most significant genetic risk factor for LOAD. 60% of homozygous carriers for the E4 allele develop LOAD, as compared to only 10% of homozygotes who carry E3, while the E2 variant is suspected to have neuroprotective properties. However, this disease allele is not fully penetrant, and other genes involved in the development of this multigenic neurodegenerative disease remain undetermined. To further discover the other risk factors involved in the APOE interactome, we propose using the novel CRISPR-Cas9 system as a tool to conduct a functional genomic-wide screen. The CRISPR-Cas9 system allows for precise and efficient editing, activation, or knockout of specific target sequences, making it an ideal candidate for the systemic perturbation and identification of genes that interact with the different APOE isoforms. Additionally, we aim to develop a co-culture system of stem cell derived astrocytes and neurons to study this disease in conditions that more closely resemble the natural tissues of the human brain. Using this co-culture system in tandem with CRISPR-Cas9, we deliver a library of guide RNAs (sgRNA) via lentivirus to the co-cultures, grow them for several days, then perform deep sequencing on surviving neurons to identify lethal guides. Using the gene editing capabilities

of CRISPR-Cas9, we have generated several isogenic lines of human embryonic stem cells and are in the process of using them for the differentiation and maturation of the co-culture tissue system. Our preliminary data show that resulting neurons and astrocytes express anticipated genes of the corresponding mature cell type and are responsive to stimuli such as inflammatory cytokines. We expect to elucidate genes and corresponding cell signaling networks that interact with APOE, illuminating potential genetic risk factors that contribute to Alzheimer's disease.

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W-2043

ASSESSING THE ROLE OF TAU-DEPENDENT MICROTUBULE STABILITY AND DYNAMICS IN TRAUMA-INDUCED NEURODEGENERATION USING MAPT-KNOCKOUT HIPSC-DERIVED NEURONS

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Traumatic brain injury (TBI) is a high risk factor for cognitive decline and dementia. The mechanisms leading to neurodegeneration following injury remain widely unknown. Disruptions in the neuronal microtubule cytoskeleton are hypothesized to lead to axonal morphological changes observed in animal models of TBI and in post-mortem brains of TBI patients. These axonal morphological changes, including wavy and swollen axons, may be exacerbated by the activity of Tau, a microtubule associated protein whose reduction and elimination has been shown to alleviate TBI-induced microtubule pathology in animal models. To elucidate Tau's role in injury induced cytoskeletal disruptions in human neurons, we first developed a microfluidic biomedical device to produce a sub lethal mechanical strain on human induced pluripotent stem cell (hiPSC)-derived neurons seeded on a flexible substrate, simulating the biomechanical loading neurons experience during TBI. We compared axon morphology in unstretched cells to those subjected to 50 stretch-release cycles at 24% strain. We found that mechanical loading results in immediate and transient morphological changes, including waves along the length of axons that mimic those found in post-mortem brains of individuals previously exposed to TBI. Axons aligned in the direction of stretch were most likely to display structural changes. Also, monitoring microtubule dynamics in these neurons demonstrated a transient cytoskeletal reorganization, in which axonal microtubule levels decreased and then returned towards pre-injury levels within 24 hours. To evaluate Tau dependence in injury-induced axonal pathology, we engineered a MAPT knockout hiPSC line by introducing a premature stop codon within exon 2 of the MAPT gene using CRISPR/Cas9 genome editing technology. Here, we compare axonal morphological changes and microtubule dynamics after injuring neurons derived from wild type and MAPT knockout hiPSC-derived neurons. Examining Tau's role in the cytoskeletal disturbances following injury might provide mechanistic insight into TBI induced neurodegeneration and may lay the groundwork in understanding how brain injury could ultimately lead to increased risk for Alzheimer's disease.

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W-2047

REPEATED ADMINISTRATIONS OF HUMAN MESENCHYMAL STEM CELLS INTO THE HIPPOCAMPI OF NORMAL BEAGLE DOGS

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According to the Phase I clinical study which we've conducted in the past, we administered human mesenchymal stem cells (MSCs) into the brain parenchyma of Alzheimer's disease patients. Improvement of cognitive symptoms was observed but was not long-lasting. Based on this clinical trial, we came to a conclusion that repeated administrations of mesenchymal stem cells are necessary in order to enhance the therapeutic efficacy of the administered MSCs. Therefore, we invented a device that can be implanted into the brain parenchyma of patients. Not only is repeated administrations of MSCs possible through this device but additional surgical procedures are also not required after the initial implantation. To test the function of the device, this device was implanted into the hippocampi of normal beagle dogs. Two weeks after implantation, ferumoxytol-labeled MSCs (F-MSCs) were repeatedly administrated for a total of 3 times (4

week intervals) into the hippocampi of beagle dogs. Additional surgeries were not performed for each of the injections. Based on 3T-MRI scans, we confirmed the transplantation of F-MSCs into the hippocampi of beagle dogs. Four months after the implantation of the device, the dogs were sacrificed and the brain was harvested to perform histological analysis. Based on the analysis, signs of inflammation or other side effects were not observed near and at the site the device was implanted. According to the results of this study, we believe that we can prolong the therapeutic efficacy of MSCs by performing repeated administrations through this device. This device can be widely applied to various diseases that require the repeated injections of stem cells or drugs.

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CANCERS

W-2049

AN INTRINSIC FAST CELL CYCLE QUALIFIES THE CELL-OF-ORIGIN FOR MLL-AF9 MEDIATED TRANSFORMATION

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Active proliferation and impaired differentiation are two key features of cancer. However, whether these features are acquired consequent to oncogene activity, or inherited from the cancer cell-of-origin remains elusive. Here, we report that an intrinsically fast-proliferating progenitor population initiates transformation by preserving its progenitor-like state in MLL-AF9 mediated leukemic transformation. Using a novel doxycycline (Dox) inducible MLL-AF9 knock-in mouse model yielding acute myeloid leukemia (AML), we uncoupled the normal intrinsic cell cycle kinetics from the proliferative response to prolonged oncogene expression. Combined with a single cell assay relating transformation efficiency to the cell cycle kinetics, we determined that the probability of a cell undergoing transformation is dictated by their intrinsic cell cycle kinetics. Overall, the faster a cell divides, the more likely it transforms. The fastest cycling subset of myeloid progenitors, those that divide 3 times or more within 24 hours, significantly enrich for transformed colony formation in vitro and induce lethal AML earlier in vivo. Importantly, transient cell cycle deceleration by a CDK4/6 inhibitor, Palbociclib, at the onset of oncogene induction but not afterwards, significantly reduces transformation, both in vitro and in vivo. These data indicate that cells with an intrinsically fast cell cycle provide the oncogene with a permissive cellular context to exert its function, qualifying them as the cell-of-origin for MLL-AF9 mediated transformation. We investigated the molecular mechanism of this phenomenon and discovered that MLL-AF9 functions to sustain the pre-existing gene expression program, rather than to dramatically alter it, to initiate malignancy. Thus, the malignant cell fate induced by MLL-AF9 reflects the preservation of a molecular and cellular state normally occupied by the fast cycling myeloid progenitors. The quiescent/slow cycling hematopoietic stem cells are unfit to be the direct cell-of-origin, but rather are the likely source for sustaining the rapidly cycling progenitor compartment.

W-2051

FASCIN REGULATES INTEGRINS TO MAINTAIN BREAST CANCER STEM CELLS

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Recent years have witnessed a significant increase in the development of new drugs that can target cancer cells more effectively. Nonetheless, cancer remained one of the leading causes of death worldwide mainly due to tumor relapse and metastasis, which are widely believed to be regulated by a small subpopulation of cancer cells that possess stem cell-like features, and thus called "Cancer Stem cells". We have shown significant association between worse clinical outcome in breast cancer patients, including metastasis and shorter survival, and their expression of fascin, an actin-bundling protein. Moreover, we have also reported that fascin is a critical mediator of breast CSCs and chemoresistance via the activation of Focal Adhesion Kinase (FAK), which is known to directly bind members of the integrin adhesion molecules and was recently reproted to interact with fascin. Here we have used f*ascin* loss and gain of function approaches to examine if fascin influences integrin expression to regulate breast CSC function. Our results have demonstrated that fascin expression in breast cancer cells is directly associated with increased expression of selected integrins including: ITGA1, ITGA3, ITGA6, ITGB1 and ITGB3. Fascin-mediated integrin expression on breast cancer cells enhances their adhesion, chemoresistance and tumorsphere formation ability. This study supports

a role for fascin in the maintenance of breast CSCs via the regulation of integrin expression. The outcome of this study is expected to provide another evidence that fascin targeting may present a new approach for optimal treatment of breast cancer from the root.

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W-2053

STEM/PROGENITOR CELLS OF THE HUMAN TUBAL EPITHELIUM AND THEIR ROLE IN OVARIAN CARCINOMA

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Ovarian cancer is the most lethal gynecologic malignancy and is the 5th leading cause of cancer-related deaths among women in the USA. About 50% of high-grade serous carcinomas (HGSCs), the most common and aggressive type of ovarian cancer, are likely to develop from the distal region of the uterine (aka Fallopian) tube. Many cancers arise from stem cell niches. However, stem/progenitor cells of the distal tubal epithelium (TE) and their role in the ovarian carcinoma pathogenesis remain insufficiently defined. We isolated putative adult TE-SCs from the normal distal regions of human uterine tube based on their high aldehyde dehydrogenase (ALDH) enzymatic activity. ALDHhi epithelial cells had a distinct gene expression profile and were marked by stemness-related markers KRT5 and LEF1. Furthermore, unlike ALDHlow/- cells, they formed TE organoids, which faithfully recapitulated the mucosal fold architecture in serial dissociation/clonal regeneration assays. Both KRT5+ and LEF1+ cells were located in the vicinity of tubal-peritoneal junctions, consistent with stem cell niche locations in other epithelial transition/junction areas. However, compared to KRT5+ cells, LEF1+ cells were expanded towards the proximal region of the uterine tube. Supporting different functional properties of KRT5+ and LEF1+ cells only few cells co-expressed both markers in TE organoids. In primary HGSCs and HGSC cell line SKOV-3 both KRT5+ and LEF1+ cancer cell populations had higher proliferative index, as compared to negative cells. Consistent with this observation, presence of either KRT5+ or LEF1+ cancer cells correlated with the worst prognosis of HGSC patients. Yet, only few cancer cells co-expressed both markers. Moreover, all precursor lesions of HGSCs, serous tubal intraepithelial carcinomas (STICs), expressed LEF1 but not KRT5. In sum, our findings suggest that TE contains distinct populations of KRT5+ and LEF1+ stem/progenitor cells, which may have different roles in HGSC pathogenesis.

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W-2055

ISOLATION OF COLON CANCER CELLS AND CANCER STEM CELLS FROM PRIMARY HUMAN COLON CANCER TISSUE FOR ESTABLISHING PATIENT-SPECIFIC CANCER CELL LINES

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Tumors contain a small subpopulation of cells, i.e., cancer-initiating cells or cancer stem cells (CSCs), which exhibit stem cell properties, possess a self-renewing capacity and are responsible for tumor generation and metastasis. Cancer stem cells persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. Since patient specific colon cancer cell lines is in great advantages of developing patient specific therapy in clinical application, it is expected to establish primary colon cancer cell lines from patient's tumor tissue in vitro. In this study, we tried to develop a method of establishing cancer cell lines from primary human colon cancer tissue. Human colon cancer tissue is digested by collagenase to generate colon cancer cell solution. Subsequently, primary colon cancer cell lines were established by (a) the culture method on specific cell sorting materials, and (b) the membrane migration method through Nylon mesh filter. We determine optimal isolation method for capturing primary human colorectal cancer cells. We also investigate which factors are more important for establishing primary cancer cell lines from minimum amount of colon cancer tissue. Moreover, cell sorting dishes in the culture method are designed from two combined concepts: physical cues and biological cues.

Different extracellular matrix (ECM) and cell binding domain oligopeptides (biological cues) with different elasticity (physical cues) are immobilized on culture dishes for capturing CSCs. Optimal ECM/ECM-derived oligopeptide and elasticity of culture dishes for (a) establishment of patient-specific cancer cell lines and (b) isolation or depletion of CSCs have been discussed in this study. In addition, CSCs identification is quantified by colony forming assay and tumor generation in serial xenotransplantation model. Establishing patient specific cancer cell line from human colon tumor will be in great advantage for improving future cancer treatment.

W-2057

THE BHLH PROTEIN E47 REGULATES CANCER STEM CELL MARKERS IN PANCREATIC CANCER

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In human pancreatic ductal adenocarcinoma (PDA), as in many other cancers, stem cells are responsible for tumor formation. This population of stem cells self-renew and sustain the cancer, making conventional cancer treatment inefficient. Tumorigenic human PDA cells express high levels of the pro-stem markers c-MYC and CD44. Concurrently, the stem cell population loses expression of acinar cell fate markers including the bHLH factors PTF1a and MIST1. Our lab showed that PDA cells also exhibit increased expression of ID3, an inhibitor of bHLH transcription factors. In particular ID3 binds to and sequesters the subclass of bHLH factors that include E47 and E12 expressed from the E2A gene, rendering cells deficient in E2A activity. We found that restoring E47 expression in patient derived PDA lines down-regulated stem cell markers c-MYC and CD44, inhibiting pancreatic cell growth. Concurrently, E47 promoted the acinar cell differentiation program, which included strong expression of acinar digestive enzyme genes and MIST1. Furthermore, we demonstrate E47 is sufficient to inhibit PDA tumorigenesis in vitro. Cells expressing E47 sustained growth arrest and acinar gene expression after transplantation. To examine the role of E47 in PDA development in vivo, here we have generated mice with pancreas specific expression of mutant Kras and mutant p53 on a WT E2A background (KPC mice) or on an E2A null background (KPCE mice). As previously reported by others, KPC animals exhibit CD44 in PanIn lesions but not in morphologically normal tissue. Lesions in KPCE mice similarly expressed high levels of CD44. Interestingly however, we find that silencing E47 in KPC animals also leads to aberrant CD44 expression in morphologically normal acinar tissue. The data suggest that loss of E47 accelerates acquisition of stem cell traits, which may predispose to PanIn lesions and frank carcinoma. Future experiments include evaluation of myc and additional stem cell markers in KPC versus KPCE mice and determining whether loss of E2A alters the trajectory of disease pathogenesis.

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W-2059

USING PATIENT-DERIVED ORGANOIDS TO PREDICT TREATMENT OUTCOMES IN RECTAL CANCER

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Colorectal cancer (CRC) is the third most commonly diagnosed cancer, and the fourth leading cause of cancer death worldwide. The incidence of rectal cancer accounts for approximately one-third of CRC cases, but more than half of the CRC-related deaths. The standard of care for locally advanced rectal adenocarinoma involves neoadjuvant chemoradiotherapy (CRT) followed by radical surgery. A significant proportion of patients (15-25%) who receive neoadjuvant therapy achieve a pathologic complete response (CR), with histological examination reporting no residual tumour. In the absence of residual tumour, these patients could be spared from the perioperative and long-term morbidity of rectal resection. Several studies evaluating the safety and feasibility of a "watch and wait" strategy for clinical CR patients report similar disease-free survival and overall survival in non-surgical management versus surgical resection, however physicians remain reluctant to adopt this practice. A personalised cancer therapy approach for rectal cancer would transform the treatment and management of disease. Organoids recapitulate the features of the tissue from which they are derived, and therefore serve as an innovative tool for cancer research. As cultures can be established quickly, organoid technology holds great promise for optimising patient outcomes by allowing therapies to be trialled before they reach the patient. We have undertaken preliminary studies for the radiation treatment of rectal tumour organoids and patient-matched metastatic tumour organoids. The responses observed in these organoid lines will be correlated with clinical outcomes to determine if this is reflective of a patients' sensitivity or resistance to radiation treatment. Our aim is to develop these models for use as a predictive tool that will guide patients and clinicians to make informed choices on appropriate treatment regimens. The ability to predict

which patients are likely to achieve pCR would enable physicians to utilise the "watch and wait" approach with confidence. This would spare patients from unnecessary surgery, preserve organ function and quality of life, whilst also saving the community from unnecessary cost.

W-2061

VAPB OVEREXPRESSION ENHANCES TUMORSPHERE GENERATION CAPACITY OF HUMAN MEDULLOBLASTOMA CELLS

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VAPB (vesicle-associated membrane protein-associated protein B) is an ER protein that regulates multiple biological functions. It was previously reported that it has decreased levels in ALS motor neurons, leading to neuronal degeneration. Recently, VAPB expression have been correlated with human breast cancer cell proliferation in vitro and in vivo. Medulloblastoma is the most common type of malignant embryonic brain tumor in children up to four years of age, accounting for about 18% of all pediatric brain tumors. It originates from undifferentiated primitive cells during neural development, involving signaling pathways relevant to the development of the nervous system. Cancer cells with neural stem cell properties are present in medulloblastoma and are capable of generating new tumors. For these reasons, medulloblastoma is an interesting model to investigate a possible relationship between VAPB levels and development of tumor stem cells in the Central Nervous System (CNS). In order to address this question, we overexpressed VAPB in different cell lines derived from medulloblastoma patients. Distinct clonal tumor cell lines were generated and characterized regarding VAPB expression level and tumorsphere generation capacity. The tumorsphere formation assay showed that clones overexpressing VAPB had increased numbers of cells after seven days under sphere culture condition, in a dose dependent manner. In summary, this functional analysis suggests a prooncogenic role of VAPB in human Medulloblastoma.

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W-2063

HYALURONIC ACID CONJUGATED NANOPARTICLE DELIVERY OF SIRNA AGAINST TWIST REDUCES TUMOR BURDEN AND ENHANCES SENSITIVITY TO CISPLATIN IN OVARIAN CANCER STEM CELLS

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TWIST1 and TWIST2 are two basic helix-loop-helix transcription factors critical to organismal development. These proteins are often aberrantly activated in many cancers. TWIST proteins regulate epithelial to mesenchymal transition (EMT), the process underlying metastatic spread. The majority of epithelial ovarian cancer (EOC) patients with advanced metastatic disease respond well to first line chemotherapy consisting of a platinum drug and/or paclitaxel. Unfortunately, most of these patients relapse with disease that is both metastatic and drug resistant, leading to a five-year survival rate under 20%. We are investigating the role of TWIST family proteins in mediating these relapses. We have identified several genes and pathways that are differentially expressed in cells expressing TWIST proteins compared to those where it is silenced. However, **t**ranscription factors are difficult to target with small molecule drugs due to their nuclear localization. To circumvent this issue, we employ siRNA to target *TWIST* mRNA to reduce metastasis and re-sensitize cancer cells to conventional chemotherapeutic agents. We have designed and validated two therapeutic siRNAs against *TWIST*, and have created a polyethylenimine (PEI) coated, mesoporous silica nanoparticle (MSN) tagged with HA to be used as a nanoparticle-based delivery system for the siRNA. Using fluorescent microscopy, we demonstrated that all tested cell lines efficiently take up MSNs, delivering their siRNA cargo to the cytoplasm. Furthermore, MSN-HA delivered anti-TWIST siRNAs were able to knockdown *TWIST,* and sensitized cells to chemotherapeutics compared to cells treated with non-targeting control siRNA. These studies reveal TWIST family proteins as promising targets to address the compound problems of metastasis and acquired drug resistance in EOC, and provide evidence for MSN as a possible therapeutic. We utilized cell lines and patient derived xenografts to test TWIST knockdown as a therapeutic approach in various forms of ovarian cancer,

as a combination therapy with standard chemo. We also evaluated the effects of TWIST knockdown using MSNs in animal models on a metastatic phenotype. We hope that MSNs will be a platform for therapies to prevent both metastatic spread and acquired resistance in ovarian and other cancers.

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CHROMATIN AND EPIGENETICS

W-2065

ACCUMULATION OF N6-METHYLADENOSINE ON THE DNA: RNA HYBRIDS IN HUMAN PLURIPOTENT STEM CELLS

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R-loops are three-stranded nucleic acid structures composed of a DNA: RNA hybrid and a displaced single-stranded DNA. DNA: RNA hybrids have been implicated in a number of conserved biological processes ranging from transcriptional regulation to genome instability in mammals. Importantly, the presence of non-canonical bases on the RNA component of the R-loops has not been reported. Here we show that N6-methyladenosine (m6A), a modification that has previously been implicated into the regulation of mRNAs stability and translation is present on majority of the DNA: RNA hybrids in human pluripotent stem cells (hPSCs). We demonstrate that RNAse H-mediated depletion of DNA: RNA hybrids abolishes chromatin associated m6A signal in hPSC nuclei in immunostaining experiments. Immunoprecipitation of m6A modified DNA: RNA hybrids followed by deep sequencing (m6A-DRIP-Seq) reveal the enrichment of m6A-containing R-loops in the 300 kb up and downstream of coding sequences, promoter regions, introns, intergenic regions and in LINE I elements. Moreover, our ongoing analysis shows that siRNA-mediated depletion of m6A methyltransferases METTL3/14 decreases the levels of DNA: RNA hybrids immunostaining in hPSCs. Taken together, our results suggest the potential involvement of m6A in regulating the stability of DNA: RNA hybrids and provide a new perspective on this modification as an integral component of R-loops contributing to their functions in hPSCs.

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W-2067

DISEASE MODELLING OF HUMAN CARDIAC LAMINOPATHY REVEALS SELECTIVE DISRUPTION OF CHROMATIN ARCHITECTURE

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Mutations in nuclear lamina genes such as LMNA (encoding for Lamin A/C) cause laminopathies characterized by prominent defects in skeletal and cardiac muscles. Besides providing mechanical support to the nucleus, the lamina regulates chromatin topology by anchoring Lamina-Associated Domains (LADs), largely silent regions correlated with inactive (B) compartments and found at the nuclear periphery. Thus, the pathophysiology of laminopathies may involve functional disruption LADs, a process that could be exacerbated in mechanically active cells. To test this, we generated cardiomyocytes from human induced pluripotent stem cells (hiPSC-CM) carrying a heterozygous nonsense LMNA mutation (R225X), and from two isogenic control hiPSC lines obtained by CRISPR/Cas9 scarless gene editing. Patients with such mutation suffer from familial dilated cardiomyopathy with severe conduction disease and arrhythmia. Mutant hiPSC-CM show disease-associated phenotypes such as electrophysiological abnormalities (irregular beat rate and prolonged field potential duration), stronger and prolonged calcium fluxes, and dysregulated contractility (diastolic dysfunction and systolic hyperfunction). RNA sequencing analyses revealed that laminopathic hiPSC-CM inefficiently silence certain cardiac progenitor and alternative lineage genes, upregulate genes involved in MAPK and TGFbeta signaling, and show impaired expression of selected cardiac regulators. Further, chromosome conformation capture analysis using

in situ DNase Hi-C indicated that approximately 9% of the genome changes its active/inactive (A/B) compartmentalization status. Most notably multiple regions that normally transition from A to B during hiPSC-CM differentiation are found in the A compartment in laminopathic cells. These include chr5 q31.3, chr19 p13.13, and chr19 q13.33-q13.41, corresponding to ectopic activation of several genes such as the neuronal P/Q-type calcium channel CACNA1A. Interestingly, however, A/B compartment changes do not largely correlate with the most robustly differentially expressed genes. Collectively, our findings indicate that LMNA mutation in mechanically active cells induce pathogenic gene expression alterations that are both dependent and independent from changes in 3D chromatin organization.

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W-2069

SPECIFIC FUNCTIONS OF TET1 AND TET2 IN REGULATING MESENCHYMAL STEM CELL LINEAGE DETERMINATION

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Mesenchymal Stem cell lineage determination is under the control of epigenetic regulation. We have previously reported that the histone lysine 27 methyltransferase, Ezh2 and the demethylase counterpart, KDM6A regulate mesenchymal stem/stromal cell (BMSC) differentiation. As DNA methylation is linked with H3K27me3 we examined the function of all three Tet DNA dioxygenases, responsible for hydroxylating methylated DNA and discovered that Tet1 is a repressor of both osteogenesis and adipogenesis as it repressed genes involved in their respective lineage differentiation. Conversely, Tet1 was found to promote expression of pluripotency genes and promote proliferation of BMSC. Tet2 was discovered to promote both osteogenesis and adipogenesis as well as proliferation of BMSC. 5hmc was found to be present on osteogenic, adipogenic and pluripotency genes suggesting that 5hmc is needed for active gene expression. Tet2 is mainly responsible for 5hmc on lineage associated genes, whereas Tet1 is responsible for 5hmc on Stemness genes. Finally, in conditions where MSC renewal and differentiation is deregulated such as osteoporosis, the levels of Tet1, Tet2 and 5hmc are downregulated emphasizing their importance in BMSC and bone health.

W-2071

O-GLCNAC PROFILING IN MOUSE EMBRYONIC STEM CELLS BY QUANTITATIVE CHEMOPROTEOMICS

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O-GlcNAcylation is the attachment of β-N-acetylglucosamine (O-GlcNAc) onto serine or threonine residue of intracellular proteins. This dynamic modification is catalyzed by a sole known enzyme O-GlcNAc transferase (OGT) and removed by the O-GlcNAcase (OGA). To date, more than four thousand O-GlcNAc proteins have been reported. The O-GlcNAc modification plays significant roles in a broad range of cellular processes and diseases. Emerging evidence indicates that O-GlcNAc participates in ESC transcription regulation and facilitates pluripotency maintenance. However, mESC O-GlcNAc proteome is still poorly understood. Here, we identified totally 1,408 O-GlcNAcylated proteins including 342 high-confidence modification sites in mESCs with the chemoenzymatic labeling strategy. Interestingly, these proteins were significantly enriched in transcription, including the pluripotency transcription network (PTN), general transcription factors, chromatin remodelers and mRNA stability regulators. Especially, about 70% of the PTN components and one fifth of mESC identity candidates were identified. These findings will provide comprehensive insights into mESC O-GlcNAcylation at the proteomic level and expand our understanding of the pluripotency maintenance at the molecular level.

ORGANOIDS

W-2073

THREE-DIMENSIONAL CO-CULTURE OF SALIVARY GLAND STEM CELLS WITH PERIVASCULAR STEM CELLS: A NEW MODEL FOR STUDYING RADIATION-INDUCED SALIVARY HYPOFUNCTION

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Salivary organoids provide a potential opportunity to study the mechanisms of salivary diseases as well as develop a new therapeutic approach. Multipotent salivary gland stem cells (SGSCs) have a potential to self-organize into salivary cell-containing organoids under three-dimensional culture conditions, however, little is elucidated regarding the functions of surrounding stromal cells for generation of salivary organoids. We first established a salivary organoid using human SGSCs and perivascular stem cells (PVSCs) which enables to study the interactions with mesenchymal cells. To develop ways to exploit salivary organoids for studying salivary hypofunction, we then tested the application of it through organoid-derived modeling of radiation-induced salivary hypofunction. Human parotid SGSCs are cocultured with human umbilical cord-derived PVSCs in the growth factor-reduced Matrigel. To assess the genetic signature of salivary organoids, we performed microarray and found that the transcripts of salivary organoids were different from respective SGSCs or PVSCs and similar to salivary epithelial cells or tissues. Progressive differentiation was noted after transplantation of salivary organoids in vivo. In addition, when we cultured SGSCs with conditioned medium of PVSCs to assess the niche function of PVSCs, the structural marker levels as well as acinar and ductal functional indexes were declined, suggesting the importance of cell-to-cell contact. Finally, we could assemble a radiation-inflicted salivary hypofunction-mimicking disease model based on our salivary organoids, in which radiation-induced structural and functional deterioration caused by depletion of salivary cells were recapitulated. In conclusion, coculture of SGSCs with PVSCs promotes generation of salivary organoids by supporting cell-to-cell contact and our novel salivary organoid can be used as a disease model of radiation induced salivary hypofunction.

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W-2075

CORTICAL ORGANOIDS FOR PSYCHIATRIC DISEASE MODELING

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The current models for investigating psychiatric or neurodegenerative diseases rely on animal and/or neuronal cell culture based platforms. While these formats have been integral for elucidating many underlying disease mechanisms, this often leads to a failure to translate into effective therapeutics. Many of these failures can be attributed to the biological differences between human disease and animal models, or the lack of proper neuronal development/maturation in vitro (e.g. spine formation). Our lab has pioneered a rapid and robust procedure for differentiating human stem cells into functional excitatory cortical neurons, providing a species-specific model, which can be CRISPR edited to mimic human disease. We have since applied this method to three dimensional cultures (brain organoids), to allow neurons to develop in an environment more closely related to the human brain. This allows basic biological investigation into complex psychiatric diseases, where information is lacking. One such psychiatric disease is that caused by mutations in Synagap1, which causes a spectrum of intellectual disability, autism, epilepsy and has been linked to schizophrenia. Syngap1 is a post-synaptic density protein, where it influences spine plasticity and structure, as well as NMDA-mediated embedding of AMPA receptors into the post-synaptic membrane. Syngap1 interacts with many different proteins in the postsynaptic density; such as SHANK3, PSD-93, PSD95 and AMPA/ NMDA subunits, which have all been implicated in psychiatric disease. It has been shown that mutations in the aforementioned Syngap1 interacting proteins affect individuals in a similar way, leading to the hypothesis that many of these post-synaptic density proteins may converge on a common disease pathway. However, it is unknown how mutations in Syngap1 affect human neuronal differentiation, morphology (dendritic and synaptic structure), excitability or downstream effects on interacting proteins. We investigated the changes in development of Syngap1 heterozygous KO human organoids (achieved by CRISPR editing) to better understand how these mutations contribute to disease, as well as applying tissue clearing techniques with 3D confocal microscopy to image changes in morphology.

W-2077

GENERATION OF FUNCTIONAL SALIVARY GLAND ORGANOID FROM MOUSE EMBRYONIC STEM CELLS

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The salivary glands arise as a thickening of the primitive oral epithelium, invaginate downwards into mesenchyme, and continually develop by branching morphogenesis. However, little is known about the exact molecular mechanism during the earliest stages of salivary gland formation. Here, we generated gene expression profiles of embryonic salivary gland rudiment and identified a specific combination of transcription factors responsible for the differentiation of mouse embryonic stem cell-derived oral ectoderm into the salivary gland rudiment in an organoid culture system. The induced salivary gland rudiment from mouse embryonic stem cells consisted of aquaporin 5-positive acinar cells, cytokeratin 18-positive ductal cells, and alpha smooth muscle actin-positive myoepithelial cells, and morphologically mimicked the embryonic salivary glands. We compared the gene expression profiles of the induced salivary gland with those of the embryonic salivary gland in each developmental stage via RNA-seq. Through hierarchical clustering analysis, the induced salivary gland gene expression profiles were found to be relatively similar to those observed at E15.5 and E18.5. Moreover the induced salivary glands developed in vivo and had physiological functions, following orthotopic transplantation into mice whose salivary glands have been removed. Engrafted induced salivary gland developed into the correct gland structures with successful connections to surrounding tissues including excretory ducts and could produce the saliva by muscarinic receptor-mediated stimulation but also gustatory stimulation of citrate. The transplanted induced salivary gland included nerve fibres derived from recipient mice. This study provides novel evidence of the successful replacement of a functional organ through orthotopic transplantation of the salivary gland organoid from pluripotent stem cells.

W-2079

APPROACHES FOR THE GENERATION OF HUMAN KIDNEY ORGANOIDS FOR MODELLING NEPHROPATHIES

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Induced pluripotent stem cell (iPSC)-derived kidney cells enable studies of nephropathies in patient-derived cells. Several approaches for the differentiation of kidney cells from iPSCs have been published, including the generation of kidney organoids. However, cell line characteristics and culture conditions may affect the end result. In addition, current organoid protocols cannot be used directly for high-throughput analyses. In this study, the reproducibility of the generation of iPSC-derived kidney organoids was tested using different cell lines and culture conditions. In addition, four different approaches with different culture systems, multiwell plate products, and amounts of cells were tested to set up more efficient and consistent organoid protocol for the needs of extensive functional analyses and drug screenings. This study was based on pre-established healthy controls and affected iPS cell lines representing GRACILE syndrome (OMIM:603358). The survival and the amount of intrinsic structures positive for well-established markers for kidney cells of differentiating organoids were analysed. Kidney organoids were managed to be generated from three out of five iPS cell lines tested so far. However, the amount of intrinsic structures was variable. Initial cell confluence had different effects on the outcome between separate cell lines. In general, the amount of nephrin-positive glomeruli was cell line-dependent, although in case of GRACILE kidney organoids, this phenomenon may be a sign of a potential disease-associated phenotype. All tested modified approaches also allowed formation of structures positive for kidney cell markers but the total outcome of them all was not comparable to the organoids generated using the original protocol. However, some of the tested approaches were found to be highly promising, and may enable processing of a plateful of 24 - 96 kidney organoids simultaneously. These results highlight the importance of carefully controlled cell culture conditions and isogenic controls. In the future, kidney organoids generated from iPSCs of patients with nephropathy, their healthy relatives, and isogenic control cell lines using the here established, more efficient differentiation method will be utilised for the functional analyses of diabetic and other nephropathies.

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W-2081

INTERACTIONS BETWEEN ENDOTHELIAL CELLS AND CARDIOMYOCYTES REGULATE HUMAN CARDIAC ORGANOID FUNCTIONALITY

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Bioengineered cardiac organoids (hCO) provide a platform to study heart development, disease and regeneration in human tissue. hCO possess many in vivo-like properties and represent a physiologically relevant model that is amenable to a range of manipulation that cannot be achieved in vivo. Most engineering approaches focus on the importance of cardiomyocytes for force production and stromal cells for tissue integrity. However, recent studies have revealed that endothelial cells are the most populous non-myocyte population within the heart, yet, there are limited studies on the impact of endothelial cells on hCO function, phenotype and proliferative capacity. The aim of this study is to characterise the effect of incorporating two distinct endothelial populations - lymphatic and blood vascular endothelial cells into micro-hCO. Directed differentiation protocols were used to generate distinct populations of cardiomyocytes, fibroblasts and endothelial cells from human pluripotent stem cells, which were then used to fabricate hCO microtissues. Vessel-like structures spontaneously organised within the organoids with the addition of lymphatic and blood vascular endothelial cells, and the addition of endothelial cells had a vast improvement on contractile function and cardiomyocyte proliferation. Ongoing investigation is underway to further understand the signalling between cardiomyocytes and endothelial cells in vitro to understand the mechanisms driving the increased function and proliferation.

W-2083

INTERELATIONSHIP BETWEEN HUMAN PLURIPOTENT STEM CELL MAINTENANCE CONDITIONS AND CEREBRAL ORGANOID DIFFERENTIATION

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The human brain, particularly the cerebral cortex, has many features that are distinct to humans and not accurately recapitulated in lower species such as mice. However, experimentation with human brain tissue, particularly at the fetal stages, is inherently challenging particularly with respect to long-term gene manipulation studies and environmental perturbations. Cerebral organoids generated from human pluripotent stem cells (hPSC) are thus emerging as a promising alternative system for studying human neocortical development and disease. While progress in organoid technology is rapidly advancing, many challenges remain including rampant batch-to-batch and line-to-line variability and irreproducibility as well as unwanted differentiation into different classes of neural cells and other tissue types. In our previous work, we established reproducible and efficient methods for cortical organoid differentiation that faithfully recapitulates in vivo neocortical development. However, we have found that optimal results are only achieved when hPSCs are grown under particular feeder-supported conditions. Here, we define differences in the transcriptional state of hPSC maintained under different conditions and demonstrate how this in turn relates to success or failure in cerebral organoid development. Utilizing this information, we further identify specific growth factor supplements that can significantly enhance organoid formation. Together, our findings illustrate how different hPSC culture methods impact their developmental potential and provide guidelines for achieving reproducibility and consistency in cerebral organoid production.

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W-2085

NEURONAL NETWORK FUNCTION AND PLASTICITY IN BIOENGINEERED NEURONAL ORGANOIDS (BENOS)

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3D modelling of human brain in combination with reprogramming and genome editing technologies represent powerful tools for understanding and treating human neurological diseases. Despite the advances in these technologies there is still little known about neuronal network function and plasticity in brain organoids. 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 (iPSC) embedded in a collagen matrix was directed under defined serum-free conditions to derive BENOs of an approximate diameter of 2 mm. RNA-sequencing at different time points of BENO-development (n=3-6/time-point) showed that similar to human brain development, in our model neurogenesis (d30-40) precedes gliogenesis (d50-60). Whole mount immunofluorescence revealed the presence of dopaminergic (TH), glutaminergic (VGLUT) and gabaergic (GABA) neurons. Calcium imaging revealed spontaneous waves of neuronal activity propagating throughout the organoid by d22 with fast tetradotoxin (1 µM)-sensitive signals by d30. To test spontaneous neuronal network activity, BENOs (day 30-60) were subjected to calcium imaging under GABAergic (picrotoxin, 58 µM; saclofen, 330 µM) inhibition. Interestingly, spontaneous Ca2+ signals of synchronized neurons became asynchronous upon GABAR inhibition (2 independent experiments). Antagonist washout restored synchronicity suggesting the presence of functional GABAergic networks. Using a stimulation electrode (injected current: 20-100 µA) we could evoke Ca2+ influx in remote regions (distance from electrode 0.5 to 1.5 mm) suggesting a strong neuronal network that extends throughout the organoid. Multi-pulse stimulation demonstrated a Ca2+ influx pattern similar to paired pulse depression (PPD), indicating short term plasticity. The PPD-like Ca2+ signal pattern was alleviated by a GABA-A inhibition (picrotoxin 58 µM) and was restored upon washout (2 independent experiments). Taken together, BENOs from human pluripotent stem cells contain electrically active neuronal networks that demonstrate typical forms of short-term plasticity mediated by functional interneurons.

TISSUE ENGINEERING

W-2087

HETEROCELLULAR COUPLING MEDIATES PRO-CONTRACTILE EFFECTS OF CARDIAC PROGENITOR CELLS IN HUMAN ENGINEERED CARDIAC TISSUE

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The promising benefits for treating heart failure by delivering human bone marrow-derived mesenchymal stem cells (hMSCs) and c-Kit-positive cardiac progenitor cells (hCPCs)-in isolation or in combination-warrant a better understanding of underlying mechanisms of action. Previous work showed paracrine signaling, not heterocellular coupling, as primarily responsible for hMSC-mediated pro-contractile effects on human engineered cardiac tissues (hECTs); however, the effects of hCPCs on hECTs have not been tested. This study aims to distinguish the relative contributions of paracrine signaling and heterocellular coupling in hCPC-mediated contractility enhancement of hECTs created using cardiomyocytes derived from human induced pluripotent stem cells. We studied three experimental groups in our force-sensing, multi-tissue bioreactor system: (1) control hECTs without hCPC supplementation (-hCPC), or (2 and 3) alternating hECTs without or with hCPC supplementation (10% of total cell number added during tissue creation) cultured in a shared paracrine media bath (p.-hCPC and p.+hCPC, respectively). Functional testing of hECT twitch force at 0.5-Hz electrical pacing was performed on culture day 6. The hECTs directly supplemented with hCPCs (p.+hCPC) had a two-fold increase in developed force ($p<0.05$) compared to non-hCPC-supplemented counterparts sharing the paracrine media bath (p.-hCPC), as well as non-hCPC-supplemented negative controls (-hCPC),

with no significant difference between p.-hCPC and -hCPC groups. The spontaneous beat rate and beat rate variability (analyzed without electrical pacing) showed no significant difference between groups. Similar results were obtained on culture days 8 and 10. In addition, treatment of -hCPC hECTs with hCPC-conditioned media had no significant effect on developed force. Altogether, these findings demonstrate that unlike hMSCs, contractile potency of the hCPC secretome is minimal, whereas direct heterocellular coupling is critical for hCPC-mediated contractile enhancement in human cardiac tissues. These findings may open new avenues of research to exploit the contrasting and possibly synergistic cardioactive mechanisms of hMSCs and hCPCs to improve hECT performance in vitro, and optimize future stem cell based-cardiotherapies in vivo.

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W-2089

HUMAN VENTRICULAR CARDIAC TISSUE STRIPS ENGINEERED FROM PLURIPOTENT STEM CELLS ACCURATELY PREDICT INOTROPIC DOSE RESPONSES IN A BLINDED VALIDATION STUDY

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Traditional discovery and development of novel drugs and therapeutics for heart diseases continue to be an inefficient and expensive process. Although traditional animal models such as rodents, dogs and pigs are accessible, major species differences in anatomy and physiology limit their ability to predict pharmacological responses in humans. Cardiomyocytes derived from human pluripotent stem cells can potentially fill this gap, but conventional 2D cultures and experiments with single cells or disorganized clusters inadequately recapitulate the human cardiac phenotype. In this study, we systematically examined the pharmacological responses of our engineered human ventricular cardiac tissue strips (hvCTS), with a morphological appearance resembling that of native human trabecular muscle, to different categories of well-defined cardioactive drugs. Under baseline conditions, hvCTS developed a mean developed force of 76 micronewtons. As a validation step, we measured developed force in hvCTS (n=70) subjected to 13 drugs with known cardiovascular effects. When treated with known negative inotropes (verapamil, nifedipine, mibefradil and bepridil) and Class I antiarrhythmics (disopyramide and flecainide), the developed forces of hvCTS dose-dependently decreased. By contrast, positive inotropes including beta-agonists and phosphodiesterase inhibitors dose-dependently increased the developed forces. Drugs with no known inotropic effects (procainamide and tocainide) elicited no significant contractile responses. To test the predictive capacity of hvCTS, we next performed a blinded study of 17 drugs on 128 hvCTS, categorizing their effects on developed force into positive, negative or no inotropic effects and estimating their EC50 values. Upon unblinding of the results, 100% of negative inotropes, 86% of positive inotropes and 80% of drugs without known effects were accurately identified. We conclude that hvCTS can recapitulate known effects of cardiovascular drugs with prediction of potency via accurate estimation of EC50, and can be used as a highly efficient in vitro human model for screening of pharmacologically active agents.

W-2091

THE EFFECT OF STRUCTURAL OFFSET AND GRADIENT PORE SIZE ON OSTEOBLAST DIFFERENTIATION IN PCL SCAFFOLDS

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Melt elctrospinning writing (MEW) is a solvent free strategy allowing precise control of the configuration of electrospun fibers in 3D printed scaffolds 1. By controlling fibre diameter and geometry and the distribution and size of pores, scaffolds with increased surface area allowing, greater cell penetration and proliferation, nutrient/waste/gas exchange and improved mechanical properties, can be produced 2. This study describes the effect of 1) pore size, 2) pore size gradient and 3) fibre offset on osteogenesis in MEW 3D -poly caprolactone (PCL) scaffolds. The highest surface area was seen with offset scaffolds and calcium phosphate coating improved scaffold wettability. Mineralization, bone-specific gene and protein expression were assessed following 30 days cell culture with human osteoblasts. The 50% offset scaffold showed higher mineralization in response to osteogenic growth factors while high levels of expression of opn and ocn markers of bone differentiation were observed in the gradient scaffold.

All groups showed upregulation of alp and collagen-I after 14 days of osteogenic differentiation. In conclusion, the pore size gradient and offset scaffolds can be considered good candidates for bone formation studies.

W-2093

CELL-DERIVED MATRIX PROMOTES IN VITRO EXPANSION AND PHENOTYPE RETENTION OF PRIMARY HUMAN CHONDROCYTES

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Cartilage damage as a result of traumatic injury or long-term wear is the major cause of disability worldwide, and a significant health priority for the rapidly aging global population. Cell-based cartilage repairs, such as autologous chondrocyte implantation and cartilage tissue engineering, are promising and have achieved considerable clinical successes. Both procedures, however, demand significant amplification of chondrocytes *in vitro*. During expansion, the dedifferentiation of chondrocytes, loss of the chondrocyte phenotype and the adaption of the fibroblast phenotype, inevitably occurs and contributes to the inferior clinical outcomes. Extracellular matrix (ECM) harbors tissue-type-specific biological cues, which influence cell behavior. We hypothesized that ECM produced by chondrocytes would support the proliferation of chondrocytes without loss of chondrocyte phenotype. In this study, we manufactured a chondrocyte-derived extracellular matrix (CELLvo™ ChondroMatrix) and tested its effect on the dedifferentiation of chondrocyte during in vitro expansion. This matrix, elaborated by chondrocytes is rich in collagen II and GAG, similar to their native microenvironment. Importantly, when seeding cells onto CELLvo™ ChondroMatrix, we observe rapid proliferation, relative to TCP, with total cell number increasing roughly 500-fold within two weeks, versus 50-fold on TCP. More importantly, chondrocytes isolated and expanded on CELLvo™ ChondroMatrix maintain a high ratio of Col2/Col1, in contrast to cells cultured on TCP, which showed decreased expression COL2A1 gene and increased expression of COL1A1. When controlling for population doubling level, the phenotypic differences become more pronounced. Interestingly, when the same cells were isolated and expanded on bone marrow mesenchymal stem cell-derived matrix (CELLvo™ Matrix), proliferation rate was very similar to CELLvo™ ChondroMatrix, but phenotype retention was less profound. This indicates that a tissue-specific microenvironment is critical for phenotype retention. Together, these results suggest that a chondrocyte-specific extracellular matrix drastically improve the expansion of chondrocytes resulting in a higher quality and quantity of cells for therapeutic applications.

W-2095

PEROXIREDOXIN I PARTICIPATES IN THE PROTECTION OF REACTIVE OXYGEN SPECIES-MEDIATED CELLULAR SENESCENCE

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Peroxiredoxin I (Prx I) plays an important role as a reactive oxygen species (ROS) scavenger in protecting and maintaining cellular homeostasis; however, the underlying mechanisms are not well understood. Here, we identified a critical role of Prx I in protecting cells against ROS-mediated cellular senescence by suppression of p16INK4a expression. Compared to wild-type mouse embryonic fibroblasts (WT-MEFs), Prx I-/- MEFs exhibited senescence-associated phenotypes. Moreover, the aged Prx I-/- mice showed an increased number of cells with senescence associated-β-galactosidase (SA-β-gal) activity in a variety of tissues. Increased ROS levels and SA-β-gal activity in Prx I-/- MEFs and reduction with a chemical antioxidant further

supported an essential role for Prx I peroxidase activity in cellular senescence that is mediated by oxidative stress. The up-regulation of p16INK4a expression in Prx I-/- and suppression by overexpression of Prx I indicate that Prx I possibly modulate cellular senescence through ROS/p16INK4a pathway.

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W-2099

THE MOUSE LYMPH NODE: A NOVEL SITE FOR THE GENERATION OF FUNCTIONAL BIOENGINEERED KIDNEYS

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The shortage of kidneys for transplantation is a major problem worldwide. Cell-based therapies and tissue engineering have the potential to produce alternative solutions to current organ replacement methods. However, the cellular complexity of the kidney does not easily lend itself to these therapeutic approaches. When the sophisticated structures of the kidneys are altered during kidney disease, local delivery of specific cell types might fail to restore renal function, as the fibrotic and inflammatory milieu of an injured organ is not a conducive environment for cell survival. Conversely, the rebuilding of a whole-kidney de novo may be a better therapeutic approach to alleviate ESRD. Several research groups have attempted cultivation of renal progenitor cells either isolated from fetal tissues or derived from pluripotent stem cells, with the most exciting advancement of the past decade being the development of organoid systems. Although very promising, this technology faces major challenges, including long-term cell propagation and proper differentiation, both necessary to obtain sufficient laboratory-grown building blocks for therapeutic use. No fully differentiated nephrons have been generated in vitro. Terminal differentiation is linked to an oxygen-rich environment. Thus, strategies to establish blood flow and oxygenation within organoids are being pursued toward generating mature renal tissues. In our lab, we have pioneered an in vivo vascularized tissue-engineering model, in which target cells/tissues are implanted into a mouse lymph node (LN). Upon transplantation into the LN, fragments of mouse and human embryonic kidneys acquired markers of mature renal structures, had functional glomeruli, tubules, and erythropoietin-secreting cells, and produced entrapped fluid that concentrated urea. We now demonstrate that the LN also acts as an innovative bioreactor to organize kidney progenitors into vascularized and functional renal structures. We also show that the LTβR pathway in LN-resident stromal cells supports kidney engraftment and function by promoting excellent vascularization. Our study has a wide-ranging impact for tissue engineering approaches for the rebuilding of functional tissues in vivo including - but not limited to - the kidney.

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W-2101

TISSUE-ENGINEERING TRACHEA FROM A 3D-PRINTED SCAFFOLD ENHANCES WHOLE-SEGMENT TRACHEAL REPAIR IN A GOAT MOEDEL

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Common treatment options for tracheal stenoses often provide short-term results with temporary relief. In some cases the treatment therapies themselves can threaten the life of the patient. Tracheal resection and reconstruction, or "end-to-end anastomosis" alone increases anastomotic tension, causing severe postoperative tearing of the trachea. Novel and more suitable long-term treatments for stenoses are needed. Here, our team used rapid and accurate 3D printing technology, combined with tissue engineering methods to prepare a bionic tissue engineered trachea (TET) and successfully carried out orthotopic transplantation in a goat model. In vitro experiments demonstrated that our TET had good mechanical properties and cell compatibility. Subcutaneous transplantation of the TET in nude mouse illustrated the TET's satisfactory biocompatibility properties, as well as adequate support for the formation of cartilage-like tissue. After orthotopic transplantation the longest survival time in the experimental group reached 98 days, with an average survival time of 65.00324.01 days, a result significantly longer than the control group. Remarkably, autopsy at the time of death revealed that none of the TET-treated goats suffered from tissue necrosis and collapse. This is the first documented scientific report elucidating a novel and successful orthotopic transplantation of whole 3D printed TET in a large animal model. We have reasons to believe this TET-treatment regime has clinical potential to successfully treat airway stenoses in the future following further improvements.

Funding Source: This study was supported by those grants:15411966800, 12ZR1446500,PKJ2012-Y48,20144Y0166, YG2012MS36, YG2016MS28 and the Open Research Fund of State Key Laboratory of Bioelectronics, Southeast University, China.

W-2103

3D BIO-PRINTING OF INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIAC PROGENITORS: A STEP TOWARDS CARDIAC REGENERATION

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It is widely acknowledged that the heart does not have the capacity to regenerate after myocardial infarction due to the low proliferation potential of cardiomyocytes. This can produce further complications such as heart failure, stroke, and pericarditis that can affect people's life expectancy and quality of life. Several approaches are being studied to address the heart's limited regenerative capacity, with stem cell technologies being a promising candidate for regeneration of cardiac tissue. However, the delivery of these cells to the damaged region, retention of cells in the target tissue, and the formation of new functional tissue need to be improved. 3D bio-printing is a new fabrication method that allows the construction of elaborate structures through deposition of cells embedded within a hydrogel, which combined with the deployment of stem cell derived cardiac cells presents an exciting prospect for the generation of functional cardiac tissue. We aim to generate a 3D bio-printed cardiac tissue through the characterisation and selection of a shear thinning biomaterial, encapsulation of pluripotent stem cell-derived cardiac progenitors (PSC-CPC), and printing and maturation of the 3D construct. Rheological characterisation of biocompatible hydrogels was performed to confirm their shear thinning flow behaviour and recovery rate after exposure to high shear rates, which is related to their printing ability. Furthermore, optimal printability parameters were determined based on the hydrogel printing fidelity. The effect of shear stress on cell viability during the extrusion was studied and found to be not significant. Initially, we will utilise the NKX2-5-GFP human embryonic stem cell (hESC) reporter line to isolate cardiac progenitor cells (CPC) from differentiating cultures, and encapsulate these GFP+ cells for the creation of the 3D bio-printed constructs. Knowledge obtained from the behaviour of the tissue construct will shed light on the effect that the interaction between printing, biomaterial, and differentiating cells have on the 3D system performance.

W-2105

AN EFFICIENT PROTOCOL FOR PORCINE LIVER DECELLULARIZATION IN THREE DAYS SUPPORTS SUBSEQUENT RECELLULARIZATION

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Decellularized porcine livers have a wide range of applications in regenerative medicine, however previously existing protocols for organ decellularization still need to be adjusted to a full size porcine liver. To establish a protocol that enables effective decellularization of the entire porcine liver in a short period Livers (n = 3) were perfused with decellularizing solutions through the portal vein during 3 days at room temperature. To analyze the extracellular matrix (ECM), after decellularization, histology and electron microscopy were performed. The vascular tree integrity was evaluated, by magnetic resonance and computed tomography, injecting gadolinium and iodine, respectively. Toluidine blue dye was also used. The presence of residual cells was analyzed by DAPI and quantification of DNA by spectrophotometry. Collagen IV presence was detected by immunohistochemistry. To recelularize 106/ml of HEPG2 cells were cultured over the matrix. Cell presence in the recellularized matrix was visualized by DAPI and secretion of albumin was detected by ELISA. After 3 days, this new protocol, based on the use of sodium-deoxycholate as detergent,

was effective in preserving all ECM structures and the vascular system. DNA quantification indicated that >97% of cells were removed. Electron microscopy showed the presence of collagen in ECM and cells could not be detected. Cell seeding showed that ECM could be recellularized after 15 days. By enabling the achievement of liver scaffolds with preserved tissue integrity in 3 days at room temperature, this novel protocol brings new perspectives to the development of bioartificial livers.

W-2107

3D PRINTING OF STEM CELLS IN LIQUID-LIKE SOLIDS FOR HUMAN NEURAL TISSUE FABRICATION

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As the human population is getting older, neurodegenerative diseases are becoming an increasing threat to human health and a growing burden in terms of economic cost. For decades, these diseases have been studied using animal models and simple cell culture systems. However, both approaches have limitations: the translation of animal experiments to humans frequently fails and simple monolayer cultures are inherently unable to serve as adequate models for live tissues. Three-dimensional (3D) human in vitro models are promising candidates to overcome these limitations because they can be engineered to architecturally and functionally mimic live tissue. The main challenges in creating such models are obtaining high resolution and systematically fine-tuning the cellular microenvironment. It is particularly challenging to fabricate nervous tissue because it is one of the softest tissues (200-1000 Pa). Constructing highly defined structures with such soft materials is extremely demanding, due to post-fabrication deformations from material sag and interfacial tension. We utilized 3D bioprinting in liquid-like solids (LLS), a technology only recently developed, to overcome these challenges. Human neural stem cells (hNSCs) embedded in a hydrogel were printed inside a custom-made LLS support. The results show that we can print highly defined cell-laden structures (resolution below 50 µm) that retain their shape for more than a week. Such long-lasting architectural integrity of soft hydrogels is not achievable with conventional bioprinting methods. Printing two different populations of cells was also accomplished, an important step towards mimicking different parts of the brain in one structure. Furthermore, we are developing poly(ethylene glycol)-based (PEG) hydrogels with tunable physical and chemical properties in order to enhance the differentiation of hNSCs into desired types of neurons. We aim to combine these advanced PEG-based hydrogels with our approach of bioprinting in LLS support in order to create an advanced 3D model of the brain with physical and chemical resemblance of brain tissue. Lastly, extending this method to human induced pluripotent stem cells (iPSCs) would allow us to model Parkinson's disease and even make the approach patient specific.

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ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

W-2109

NATIONAL PLAN TO PROMOTE STEM CELL RESEARCH AND INDUSTRY IN KOREA

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South Korea's commercialization of stem cell technology has been impressive as seen in its launching of the world's first stem cell drug. While its accumulated number of clinical trials using stem cell drug (between 1999 and 2016) is only after the U.S, the country is actively conducting clinical research on stem cell medication. This indicates South Korean government's active pursuit of concentrated investments in stem cell drug, a promising item still in its initiation period being produced with high internal technological capability and potential. South Korea's stem cell application technology has also reached comparatively high level based on the government's active policies to promote the technology. A mid- and long-term stem cell strategy is a ten-year prospective plan to ensure South Korea's flexible response to the rapid changing environment surrounding the technology and reduced technological gap with top players through forward-looking investments. The strategy suggests the future state of South Koran stem cell technology. It also includes a plan to build ecosystems for stem cell research and its industrial application that requires the government support. Thus, this introduces mid- and long-term strategy to promote stem cell research and its industrial application currently under discussion in Korea.

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POSTER ABSTRACTS

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

W-2113

DOSAGE EFFECTS OF ZP2 AND ZP3 HETEROZYGOUS MUTATIONS CAUSE HUMAN INFERTILITY

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The zona pellucida (ZP) is an extracellular matrix universally surrounding mammalian eggs, which is essential for oogenesis, fertilization, and pre-implantation embryo development. Here, we identified two novel heritable mutations of ZP2 and ZP3, both occurring in an infertile female patient with ZP-abnormal eggs. Mouse models with the same mutations were generated by CRISPR/Cas9 gene editing system, and oocytes obtained from female mice with either single heterozygous mutation showed approximately half of the normal ZP thickness compared to wild-type oocytes. Importantly, oocytes with both heterozygous mutations showed a much thinner or even missing ZP that could not avoid polyspermy fertilization, following the patient's pedigree. Further analysis confirmed that precursor proteins produced from either mutated ZP2 or ZP3 could not anchor to oocyte membranes. From these, we conclude that ZP mutations have dosage effects which can cause female infertility in humans. Finally, this patient was treated by intracytoplasmic sperm injection (ICSI) with an improved culture system and successfully delivered a healthy baby.

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W-2115

GLOBAL TRENDS OF CLINICAL TRIALS FOR ARTICULAR CARTILAGE REPAIR BY CELL THERAPY

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Regeneration of articular cartilage is an important thesis in advanced nations entering the aging society. Several early cell therapy products have been already developed and available in the market. In this study, we examined the clinical research trends related to cell therapy products in the cartilage repair field based on data obtained from the ClinicalTrial.gov website. Although this website does not provide comprehensive results of clinical trials, it offers information on prospective clinical trials, including work in progress, and thus allows chronological analysis of the data. We selected 180 studies related to the field of cartilage regeneration from ClinicalTrial.gov. The results showed a shift in the clinical translational trend in cells used in such research from cartilage- and bone marrow-to adipose tissue-based cells. Whereas the studies that used cartilage as the cell source included many Phase III trials, fewer studies using bone marrow and adipose tissue cells progressed to Phase III, suggesting that most clinical developments using the latter sources have not been successful so far. Two products covered the development period from start of Phase I to completion of Phase III, with time to completion of more than 100 months. Sub-analysis of each generation of autologous chondrocyte implantation showed that the first generation has been abandoned since 2010, while the second and third generations have been actively studied since 2006. The use of ClinicalTrials.gov as the sole data source can yield a perspective view of the global clinical translational trends.

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W-2117

STEM CELL THERAPY FOR THE TREATMENT OF SEVERE TISSUE DAMAGE AFTER RADIATION EXPOSURE

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The late adverse effects of pelvic radiotherapy concern 5 to 10% of patients, which could be life threatening. However, a clear medical consensus concerning the clinical management of such healthy tissue sequelae does not exist. Our group has demonstrated in preclinical animal models that systemic MSC injection is a promising approach for the medical management of gastrointestinal disorder after irradiation. We have shown that MSC migrate to damaged tissues and restore gut functions after irradiation. The clinical status of four first patients suffering from severe pelvic side effects was improved following MSC injection in a compassional situation. A quantity of 2x106 - 6x106 MSC /kg were infused intravenously to the patients. Pain, hemorrhage, frequency of diarrheas and fistulisation as well as the lymphocyte subsets in peripheral blood were evaluated before MSC therapy and during the follow-up. Two patients revealed a substantiated clinical response for pain and hemorrhage after MSC therapy. In one patient pain reappeared after 6 months and again substantially responded on a second MSC infusion. The frequency of painful diarrhea diminished from 6/d to 3/d after the first and 2/d after the 2nd MSC injection in one patient. A beginning fistulisation process could be stopped in one patient resulting in a stable remission for more than 3 years of follow-up. In all patients, prostate cancer remained in stable complete remission. A modulation of the lymphocyte subsets towards a regulatory pattern and diminution of activated T cells accompanies the clinical response. MSC therapy was effective on pain, diarrhea, haemorrhage, inflammation, fibrosis and limited fistulisation. No toxicity was observed. For patients with refractory chronic inflammatory and fistulising bowel diseases, systemic MSC injections represent a safe option for salvage therapy. A clinical phase II trial will start in 2018.

W-2119

NON-CLINICAL STUDY OF HUMAN IPSC-DERIVED DOPAMINERGIC PROGENITORS FOR PARKINSON'S DISEASE PATIENTS

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In Japan, we plan to start a clinical trial of allogeneic transplantation of human induced pluripotent stem cell (iPSC) -derived dopaminergic progenitors (DAPs) for Parkinson's disease (PD) patients. In the clinical trial, we plan to use a cell line from "iPSC stock", which is established from peripheral blood of HLA-homozygous volunteer donor. The donor iPSC-DAPs will be grafted to PD patients' putamen. Here, we describe the results of non-clinical safety studies required by Japanese Pharmaceuticals and Medical Devices Agency (PMDA), and efficacy studies. For safety study, the DAPs were transplanted into striatum of immunodeficient (NOD/Shi-scid,IL-2Rγ KO Jic: NOG) mice and observed for life-long period. During the observation period, no mouse died from the cause related to the graft although some mice died from senility. As another safety study, "spike test" was performed to exclude the risk of residual undifferentiated iPSCs in DAPs. In that test several different numbers of undifferentiated iPSCs were spiked into DAPs and Matrigel and injected to subcutaneous space of NOG mice that were observed for 26 weeks after injection. No tumor was observed in the group of DAPs without iPSC. For efficacy study, the DAPs were transplanted into striatum of 6-hydroxydopamine (OHDA) lesioned nude rats (F344/NJcl-rnu/rnu) that were observed for 20 weeks after transplantation. The methamphetamine-induced rotational behavior test showed improvement of the motor symptoms and the histological analysis proved the graft survival of almost all rats. The utility and the limitation of these non-clinical studies will be discussed.

Funding Source: Japan Agency for Medical Research and Development (AMED)

W-2121

AUTOLOGOUS HUMAN STROMAL VASCULAR FRACTION INJECTION IN POST-BURN HYPERTROPHIC SCAR; A DOUBLE-BLINDED PLACEBO-CONTROLLED CLINICAL TRIAL

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Post-burn hypertrophic scar (HTS) is a common complication of burn injury which results from prolonged inflammatory response, and leads to cosmetic, functional, and psychological complications. Adipose-derived stromal vascular fraction (SVF) contains heterogenous cells with regenerative, anti-inflammatory and anti-fibrotic properties. In this phase I randomized placebo-controlled clinical trial, we aimed to evaluate the safety and potential efficacy of intra-dermal injection of autologous SVF in HTS. We enrolled 20 patients who had HTS for more than 1 year with Vancouver scar scale (VSS) of ≥ 4. In each patient, two 6 cm2 scar sites were randomly received 2 ml of SVF (1×106 cells/cm2) or normal saline. Patients were followed till 4 months post-transplantation. Our primary endpoint was the number and severity of adverse events. Our secondary endpoints were alterations in VSS score, skin thickness (dermal and epidermal layers) measured using high resonance ultrasound, patient self-assessment of HTS improvement based on visual analog scale (VAS), and level of expression of FOX-p3 and TGF-β assessed by immunohistochemical assay. Safety evaluations showed no major and serious adverse events in treated patients. The mean VSS scores of the treatment and placebo sites before the injection were 8.031.2 and 7.3 31.6 which respectively decreased to 6.431.4 and 6.731.7 at 4 months following cell transplantation. The mean VAS at 4 months after cell transplantation in SVF and control groups was respectively 3.232.2 and 0.731.8 (P-value<0.05). The mean skin thickness in SVF group decreased from 2.930.7 mm to 2.730.6 mm, while in control group was respectively 2.8 30.7 mm and 2.830.8 mm, before and 4 months after the treatment (P-value<0.05). Significant increase in the expression level of FOXP3 and TGF-β was observed in SVF group compared to placebo group, 4 months after cell injection. In this study, we demonstrated safety and tolerability of autologous SVF intra-dermal injection in HTS. Besides, our study results indicated the potential efficacy of SVF injection in HTS probably via the anti-inflammatory and regenerative properties of SVF cells. However, this needs to be confirmed performing further investigations.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

W-2123

MODELING LINEAGE SPECIFICATION DURING HUMAN PREIMPLANTATION DEVELOPMENT

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Our aim is to decipher how cell fate decisions are driven during preimplantation development in human. To do so, we performed a bioinformatics analysis of recent datasets complemented by our own data set. Our dataset corresponds to embryos that have been followed by time-lapse microscopy prior to analysis, guaranteeing us perfect staging using the Gardner and Schoolcraft grading system (2011). Notably, we have set up an automated image analysis pipeline to stage human embryos, allowing us to precisely discriminate embryos from day 5 and day 6: B2 (early blastocysts just after blastulation), B3 (just before zona pellucida thinning) and B4 (late blastocyst, beginning of expansion). This is of utmost importance as lineage specification in human occurs between the B2 and the B4 stage which is achieved within 24h. We generated a pseudo-time model showing perfect correlation between the pseudo-time and stage of the embryos. Moreover, we have positional information of sequenced cells showing proper separation of mural TE from the polar TE, PE and EPI cells. Based on our model, we propose a hierarchy of human lineage specification. Specifically, we identified a transient cell state undergoing specification at the early blastocyst stage (B2 to B3 stage), within a window of only 12h. Our model accounts for the establishment of the 3 cell types in human blastocysts: EPI, PE and TE. The combination of our sample-based and gene-based analysis yields multiple hypothesis: our model shows that EPI is the first cell type established in human, followed by TE. PE comes later, possibly from the differentiation of EPI cells. We will refine our model and complement it by tracking spatiotemporal expression of the identified genes during human preimplantation development by immunofluorescence and single cell proteomics.

W-2125

CHARACTERIZATION OF METASTABLE UNDIFFERENTIATED CELL STATES WITHIN THE MALE MOUSE GERMLINE

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The role of stem cells in adult tissue maintenance is widely appreciated and simple hierarchical models of stem cell self-renewal and differentiation often proposed. Stem cell activity in the male germline is restricted to undifferentiated A-type spermatogonia (A_{undiff}) however only a fraction of this population act as stem cells in undisturbed testis and A_{undiff} hierarchy remains contentious. Through newly developed compound reporter mice, we define molecular signatures of self-renewing and differentiation-primed adult A_{undiff} fractions and dissect A_{undiff} heterogeneity by single cell RNA-Seq analysis. We uncover an unappreciated population within the self-renewing A_{undiff} fraction marked by expression of embryonic patterning genes and the homeodomain transcription factor PDX1. Importantly, we find that PDX1 marks a population with potent stem cell capacity that is unique to mature, homeostatic testis and demonstrate dynamic interconversion between PDX1+ and PDX1- A_{undiff} states upon transplant and *in vitro* culture. We propose that A_{undiff} can exist in a series of metastable states with distinct functional potential and provide evidence that stability of such states is dictated by niche-derived cues.

W-2127

NICHE ECM MEDIATES THE EXPRESSIONS OF PLURIPOTENT TRANSCRIPTION FACTOR OCT4 THROUGH IGF-IR ACTIVATION IN MOUSE GERMLINE STEM CELLS

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Stem cell niche is known to regulate stem cells self-renew and differentiation. Our previous studies have demonstrated that the niche hypoxia maintains the Oct-4 level through HIF-2α-IGF-1R signal loop in mouse germline stem cells. However, how the niche extracellular matrix cooperates with hypoxia-derived signals in stemness maintenance of stem cells still remains largely unknown. In this work, we found that laminin significantly increase the alkaline phosphatase (AP) activity and the expressions of stemness-related gene like Oct4, Nanog, and Sox2 in CD49f+AP+ germline stem cells (GSCs). Meanwhile, the IGF-1 dose-dependently increases the expressions of OCT4, and importantly, the CD49f. Blockage of IGF-IR signaling either by using IGF-IR shRNA or IGF-IR-Akt-mTOR signaling inhibitors of PPP, LY294002, and Rapamycin not only effectively suppressed the expressions of Oct4, but also dramatically suppressed the CD49f expressions. CD49f knockdown significantly suppressed the expression of IGF-IR and Oct4. Further, double knockdown of IGF-IR and CD49f synergistically suppressed the expressions of Oct4 in mouse CD49f+AP+GSCs evidenced by western blotting and immunocytochemical staining combined with confocal image. Together with these results demonstrated that laminin-CD49f-IGF-IR signaling loop maintains the Oct4 expression in early AP+GSCs. Findings in this study would provide insights into niche extracellular matrix and endocrinology underlying the early pluripotent germ line development.

TECHNOLOGIES FOR STEM CELL RESEARCH

W-2129

IN SEARCH OF THE ELUSIVE TISSUE-RESIDENT "MESENCHYMAL STEM CELL": USING MULTICOLOUR FLOW CYTOMETRY TO FACILITATE CHARACTERISATION OF MESENCHYMAL CELL POPULATIONS IN HUMAN DISSOCIATED TISSUE

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Mesenchymal stem/stromal cells (MSCs) are widely reported to be multipotent precursors present in the stromal fraction of many adult tissues. For this reason, they have been the subject of intense investigation, especially in the context of regenerative medicine. However, their anatomical location, in vivo phenotype, and functional properties are one of the most controversial and confusing areas in stem cell biology. For example the cell surface phenotype of human "MSCs" is dramatically affected by cell culture conditions so studies on freshly isolated mesenchymal cells are now necessary to accurately correlate molecular phenotypes with functional properties. In addition, evidence of multi-lineage differentiation, and importantly, clinical translation potential amongst these tissue cultured "MSC" lines is weak, suggesting that populations that might reasonably be termed MSCs are rarer than previously appreciated. In an effort to discriminate between mesenchymal cells with stem cell properties and those that are more differentiated, we have been using high dimensional multicolour flow cytometry (in

combination with immunofluorescence microscopy) to define the primary (uncultured) mesenchymal cell populations present in several human tissues. Multicolour flow cytometry is a powerful method to detect multiple cell populations simultaneously in a complex sample, such as digested human tissue. We have developed and optimised a 17-colour flow cytometry panel (CD45, CD34, CD31, CD144, CD146, CD73, CD90, CD26, CD105, HLADR, CD141, CD36, CD271, Podoplanin, FAP, CD117, DAPI) and surveyed populations in human lymph nodes, stromal vascular fraction (adipose tissue) and dermis (skin). Flow cytometric characterisation has indeed revealed striking heterogeneity for a number of cell surface receptors suggesting there are likely to be sub-populations with differing capacity to differentiate. Subsequently, multicolour fluorescence-activated cell sorting (FACS) can be used to isolate these sub-populations to more precisely assess their functional (differentiation) and molecular (single cell RNAseq) characteristics. Knowledge from these studies will help inform predictions of the potential impact of the various types of "MSC" products being administered in current clinical trials.

W-2131

GMP COMPLIANT EXPANSION OF MESENCHYMAL STROMAL CELLS (MSC) USING THE CLINIMACS PRODIGY® AND 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. To ensure highest quality and safety of the resulting cellular products, MSCs have to be maintained under standardized cultivation conditions and procedures. To this, we have developed our xeno-free MSC-Brew GMP medium following the recommendations of USP <1043> on ancillary materials, thus enabling isolation and expansion of MSCs from various tissue sources (e.g. human bone marrow, adipose tissue and umbilical cord) for clinical research use. To increase the level of process standardization and product safety we developed a procedure for cultivation of MSCs using the integrated cell processing platform CliniMACS Prodigy®. MSCs from human bone marrow samples were isolated via density gradient centrifugation and subsequent adherence to plastic using automatic feeding and harvesting procedures in a closed, single use tubing set under adherent culture conditions. Subsequently, these cells could be replated and expanded within the closed system, illustrating the feasibility of an automated cell production for clinical applications. 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.

W-2133

EFFICIENT STRATEGIES FOR SCALE-UP OF PLURIPOTENT STEM CELLS

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Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While numerous feeder-free monolayer media systems exist, the scale at which PSCs can be efficiently scaled using this approach is limited without significant increase in hands on time and effort, as well as increased risk for contamination. Therefore, to fully realize the potential of PSCs in downstream applications where large numbers of cells are required, such as cell therapy and high-throughput screening applications, alternative expansion methodologies may be beneficial. Here we present comparison of scale-up strategies for utilizing adherent monolayer cell factories and spinner flask systems for generation of greater than a billion PSCs while maintaining pluripotency and normal karyotype of PSCs. Critical parameters for culture optimization, methods for downstream characterization of expanded cell populations, and considerations for downstream cryopreservation and differentiation are highlighted. Through implementation of these strategies one can successfully scale-up production of PSCs with minimal hands on time, decreased reagent consumption, and thus decreased cost to achieve greater than a billion PSCs.

W-2135

INTERPLAY OF PROTEIN SIGNATURES IN SUBTYPES OF BREAST CANCER STEM/ PROGENITOR CELLS

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Many panels of surface markers including $D44^{high}/CD24^{low}$, aldehyde dehydrogenase ($ALDH^{high}$), are currently being used to enrich for cancer stem cells (CSCs). However, these markers do not always correlate with aggressiveness of all tumors. Breast cancer is a heterogeneous disease and therefore we hypothesized that alternative markers could be used for the different subtypes of breast cancer. In this study we aimed at identifying novel protein biomarkers that are specific to triple negative breast cancer cells. Cells from triple negative breast cancer cells (MDA-MB-231, SUM159PT, & BT-549) were Flow Cytometry-sorted using combinations of CD44/24 surface markers. The cells were sorted into three sub-populations including cancer stem cell enriched (Stem), differentiated-like enriched (differentiated) and bulk of cancer cells. The phenotypic characteristics of these cell populations were subsequently evaluated by Waters Synapt G2- label-free quantitative Nano-liquid chromatography tandem mass spectrometry expression analysis (LC-MS/MS). An average of 1080 proteinshave been identified from each of the different cell lines representing the breast cancer subtypes. Over 270 proteins were significantly differentially expressed between the different sub populations of the analyzed cell lines and many of them are membrane/stem cell-related proteins. A review of some of these CSCS specific protein have shown an overlap between a panel of 8 unique CSCs protein with previously reported proteins characterized from embryonic stem cell line (F9) thus indicating their potential for the identification of novel triple negative CSCs markers. We have identified unique proteins that might be specific markers for identification of cancer stem cells in the triple negative breast cancer cells. Some of them have been implicated as putative stem cell markers.

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W-2137

COMBINED META-ANALYSIS OF PRE-CLINICAL STEM CELL THERAPY STUDIES SHOWS OVERLAPPING EFFECT MODIFIERS FOR MULTIPLE DISEASES

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Stem cell therapy has been studied extensively in many different research domains, but cellular replacement or repair of damaged solid tissues such as brain, heart or kidneys, is yet to be fully understood. Systematic review and meta-analysis are widely used to aggregate data and find important patterns of results within research domains. In this study, we use these tools across research areas to look for common biological denominators affecting therapeutic effect size in preclinical stem cell therapy studies for renal, neurological and cardiac disease. We used datasets of five previously published meta-analyses to study the impact of stem cell biology and experimental design of chronic kidney disease, spinal cord injury (SCI), stroke, and ischemic heart disease (MI). To do this, we transformed all primary outcome measures to ratios of means to permit direct comparison across disease areas. Pre-specified variables of interest were species, use of immunosuppression and the characteristics of the cellular interventions themselves. The five datasets from 506 publications yielded data from 13,638 animals. Overall, stem cell transplantation appear to improve outcome in all diseases models with the highest effect seen in SCI, which is also the largest dataset (effect size 27.3 %; 95%CI 25.1-29.4% 319 comparisons in 5,736 animals). Most heterogeneity between experiments was accounted for by facets of stem cell biology. Cell type, and source seemed to influence efficacy, with no clear trend for certain cell types being superior across all disease models. Biological plausibility was supported by the presence of a dose-response relationship where increase in dose resulted in higher effect size in all but cardiac stem cells in MI dataset. Immunosuppression showed a negative influence in SCI and possible positive effects when introduced genetically in MI. All the studies seem to be affected by similar decrease in effect size when using larger animals compared to rodents. This has important implications for the translation of cellular therapeutics

to the clinic. In conclusion, all disease models in which stem cell therapy is tested, seem affected by the same phenomenon. This has important implications for the studied therapy, but also for translational science and animal models that we all use.

W-2139

METHODS FOR RAPID ESTABLISHMENT AND SCALE UP OF IPSC

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The development of reprogramming technologies for the generation of induced pluripotent stem cells (iPSCs) has catalyzed powerful possibilities in the field of stem cell research and regenerative medicine. Successful establishment of iPSCs consists of three critical steps, including initial colony selection, early clonal expansion, and subsequent scale up of cells for use in further applications. The main challenges encountered are identifying and selecting high quality colonies, and manual passaging of early clones, both of which require a high degree of technical expertise and time commitment. Simplification and consistency of this process is necessary for rapid generation and expansion of iPSCs for use in downstream applications. In this study we report the development of a cell passaging reagent effective for establishment of early iPSC clones. This reagent shows consistent performance across different matrices, medias and user experience levels. Cells harvested using this reagent show efficient detachment with uniform clump size and consequently show better attachment and faster growth rates when reseeded for further culture. Additionally, very early passage clones, that often need to be manually passaged using mechanical methods, can be subjected to bulk harvesting using this reagent without compromising quality or survival. Unlike EDTA, a commonly used passaging solution for iPSCs, this reagent can be used to passage cells across a broader incubation time window and for varying densities of cells. These features collectively enable the use of this cell harvesting reagent across the entire iPSC workflow from early iPSC clonal establishment to scale up in cell factories. The ability to successfully scale up early iPSC clones with minimal manual manipulations streamlines the bioprocessing workflow for both basic and translational research.

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W-2141

DEVELOPMENT OF NOVEL QUALITATIVE AND QUANTITATIVE METHODS TO ASSESS MATURATION STATUS OF CARDIOMYOCYTE DERIVED FROM MOUSE PLURIPOTENT STEM CELLS

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Recent progresses on cardiac differentiation from pluripotent stem cells (PSCs) have fostered a foundation for disease modeling and pharmaceutical application. To this end, PSC-derived cardiomyocytes (PSC-CMs) should be matured as adult cardiomyocytes rather than embryonic or neonatal ones, and some hormones and extracellular matrices (ECMs) were reported as possible enhancer for cardiomyocyte maturation. Nonetheless, quantitative assessments of cardiomyocyte maturity were lacked and it is still unclear which factor is superior and if any treatment can fully maturate PSC-CMs. Therefore, we aimed to develop novel qualitative and quantitative methods to assess the effects of hormones and ECMs on cardiomyocyte 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 that is upregulated postnatally. Unless the cells differentiate to mature cardiomyocytes, no RFP can be detectable. After 2-3 weeks, weak RFP became visible in approximately 30% of PSC-CMs. To test the effects of hormones and ECMs, we first examined if reported hormones and ECMs could enhance RFP expression of the maturation reporter line. Approximately 50% of PSC-CMs were RFP positive after treatment either with glucocorticoid and thyroid hormones or with matrigel, and approximately 80% of PSC-CMs were RFP positive with the combination of both. In the latter condition, cells displayed more developed and organized sarcomeres that were visualized by RFP fused with a sarcomere gene. These data suggest that hormones and ECMs can enhance maturation of PSC-CMs. For a quantitative method, we collected a reference transcriptome dataset from embryos to adults with a cost-effective transcriptome method using next generation sequencing. We can compare transcriptome of PSC-CMs to that of in vivo counterparts and quantitatively assess the maturation status of PSC-CMs according to the similar approach with microarray-based method as we demonstrated in our previous paper (Uosaki, Cell Rep, 2015). We will analyze and quantitate the maturation of PSC-CMs with the treatment of hormones and ECMs using the novel transcriptome method.

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W-2143

FUNCTIONAL INTACT ACTIVITY OF HUMAN PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS ON WOUND HEALING AFTER CDY1 AND VISIBLE LIGHT TREATMENT

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Cell therapy with human mesenchymal stem cells (hMSCs) is one of the promising cures to repair damaged tissues in a variety of clinical applications. Due to their wide variety of tissue sources and relatively easy in vitro expansion, therapeutic application of hMSCs has been actively evaluated worldwide. Recently, new techniques have been established for deriving hMSCs from human embryonic stem cells (hESCs). Unlike hMSCs from adult tissues, hESC-derived MSCs not only show high proliferative ability but also can be used for producing unlimited numbers of hMSCs with consistent characteristics. However, despite a number of advantages of hESC-derived MSCs, cell therapy based on hESCs would not be free of the risk of teratoma formation, which results from the residual undifferentiated hESCs. Our previous studies have shown that pluripotent stem cell (PSC) selective fluorescent probe, CDy1, not only selectively stains PSCs but also efficiently kills PSCs through its photodynamic property. Herein, we examined whether CDy1 (with visible light) treatment would interfere with the functional characteristics of hESC-derived MSCs. Treatment conditions of CDy1 and visible light, which are able to inhibit teratoma formation, did not affect the multi-differentiation potential and wound healing activity of hESC-derived MSCs. Finally, CDy1 and visible light treatment is new technique that is teratoma-free stem cell therapy without affecting other differentiated cells.

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W-2145

GENERATION OF STEM CELL SHEETS USING PHOTOTHERMAL EFFECT

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Sheets of mesenchymal stem cells (MSCs) can be efficiently generated by Near-infrared (NIR) light photothermal effect of a conductive polymer surface. The harvested ADMSCs exhibited high cell viability and a normal karyotype. Furthermore, the ADMSCs were positive for CD73, CD90, CD105 of MSC markers and differentiated into osteoblasts, chondrocytes, adipocyte. We analyzed secretory factors of sheet-type MSCs for wound healing. The MSCs significantly produced many growth factors including bFGF, TGF-β1, EGF, HGF, PDGF, and VEGF useful for would healing. In animal study, the ADMSC sheet treated group showed significantly higher wound closure effect, compared with that of a control and injection group. These results indicate that ADMSC sheets could be applied to several diseases, especially in wound healing.

W-2147

EXPANSION OF PLURIPOTENT HPSC IN VARIOUS VITRONECTIN-BASED CULTURE SYSTEMS

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Human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC), collectively referred as human pluripotent stem cells (hPSC), are able to differentiate into the three germ layers of the human embryo, and are presumed to have the capacity for self-renewal in vitro. Consequently, they possess great potential for cell-based therapy and differentiation studies. Culture conditions, including culture media and matrix, have a substantial effect on pluripotency. The most common feeder-free matrices are Matrigel and recombinant proteins that support hPSC self-renewal such as laminin isoforms and vitronectin. NutriStem® V9 XF is a defined, xeno-free and serum-free medium specially designed to support the growth and expansion of hPSC using vitronectin matrix. The medium contains low concentration of human proteins and only the essential components required for long-term maintenance of hPSC. The present study evaluates long term expansion of hPSC using NutriStem® V9 XF on Vitronectin ACF. Two Vitronectin-based culture systems were tested: pre-coated plates as well as precoating-free procedure by direct addition of the Vitronectin ACF to the culture medium. Results show that NutriStem®

V9 XF medium enables high proliferation rate in long-term culture, while maintaining stable karyotype, high pluripotency marker expression, and preservation of tri-lineage differentiation potential of hPSC.

W-2149

BART-SEQ: COST-EFFECTIVE MASSIVELY PARALLEL TARGETED SEQUENCING FOR SINGLE CELL TRANSCRIPTOMICS

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The introduction of techniques for single cell transcriptomics by next-generation sequencing (NGS) has accelerated our understanding of stem cell regulation. Majority of these techniques, however, suffer from shallow coverage because they are unbiased (global) and thus require numerous reads to cover the gene repertoire. Targeted approaches can remove this bottleneck by lowering the number of analyzed genes, but current methods are not compatible with gene expression measurements of single cells, and are costly when applied to hundreds/thousands of samples. We developed the first highly sensitive, quantitative, and inexpensive barcoding technique for enriching selected cohorts of transcripts from thousands of single cells, and multiplexing them for conventional NGS. The novel workflow, named Barcode Assembly foR Targeted Sequencing (BART-Seq), is based on a simple method for synthesizing virtually unlimited matrices of differentially barcoded forward and reverse primer sets, which generate sample-specific combinatorial amplicon labels. Moreover, we developed tools for designing the primers and barcodes, and decoding the barcoded NGS reads. We demonstrated the technique by quantitative analysis of pluripotency genes in bulk cell preparations and thousands of single cells. The preliminary results revealed potential effects of commonly used maintenance media (mTeSR, E8, and bFGF) on the state of pluripotency and early differentiation. Specifically, the use of BART-Seq indicated the influence of different media on the multilineage differentiation capacity of pluripotent stem cells, likely related to de novo methylation. Taken together, BART-Seq will complement low-sensitivity global single cell transcriptomics approaches for discovering the mechanisms of stem cell regulation, by cost-effective analysis of thousands of single cells across the dynamic range of gene expression.

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W-2151

ELECTROPHYSIOLOGICAL PHENOTYPE CHARACTERIZATION OF HUMAN IPSC-DERIVED DOPAMINERGIC NEURONAL LINES BY MEANS OF HIGH-RESOLUTION MICROELECTRODE ARRAYS

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High-resolution-microelectrode-array (MEA) technology enables to study neuronal dynamics at different scales, ranging from axonal physiology to network connectivity (Müller et. al, Lab on a Chip, 2015). We have used this MEA technology to characterize and compare the electrical phenotypes of commercially available human dopaminergic neurons (iCell DopaNeurons, MyCell DopaNeurons A53T α-synuclein, Cellular Dynamics International, Madison, WI, US). Furthermore, we have studied the effect of human astrocytes (iCell Astrocytes, Cellular Dynamics International, Madison, WI, US) on neural culture development. Astrocyte/neuron co-cultures showed higher signal amplitudes and higher firing rates than neural cultures without astrocytes. Adding astrocytes to neural cultures changed the whole culture morphology by promoting cell clustering. Interestingly, astrocyte/neuron co-cultures showed a lower sample-to-sample variability across multiple MEA recordings compared to neural cultures without astrocytes. We compared action potential propagation velocities along axons between dopaminergic A53T α-synuclein neurons and the wild-type isogenic control cell line. We found that in both, wild-type and disease model neurons, axonal action potential propagation velocities were lower than in rat primary cortical neurons. Furthermore, we found different axonal action potential velocity development profiles of A53T α-synuclein dopaminergic neurons and the wild-type counterpart. Finally, we were able to precisely evoke action potentials in individual single human neurons by subcellular-resolution electrical stimulation. 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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W-2153

DEVELOPMENT OF GESICLES PLATFORM FOR GENE AND CELL THERAPY

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Gesicles are small cellular vesicles containing, across their membrane, the envelope glycoprotein of the vesicular stomatitis virus (VSV-G). In presence of polybrene, gesicles were able to deliver plasmids in several animal cells. Unfortunately, their manufacturing, characterization and use for nucleic acid delivery are still poorly documented. We propose here to develop the gesicle platform. First, HEK293 cell line was used for the first time to produce gesicles by means of polyethylenimine (PEI)-mediated transient transfection. This method is efficient and cost effective for transient gene expression. A simple protocol for gesicles purification has also been successfully designed using combination of iodixanol density gradient and dialysis. Interestingly, transmission electron microscopy of purified preparations revealed spherical particles containing VSV-G with membrane mimicking enveloped viruses. Dynamic light scattering analysis reported that gesicles have a mean size of 140 nm in a PBS solution. Gesicle proteomic analysis identified 877 human proteins originating from the producer cells. These proteins are mainly represented by enzymes (52%), cytoskeleton (19%), receptors (15%) and ribosomes (12%). Then, parameters involved in the DNA-Gesicles delivery such as component concentrations, incubation time, assembling order were studied to obtain optimal transfection efficiency. Transfections experiments delivered plasmids in 70% of HEK293 cells, 55% of HeLa cells and 22% of hard-to-transfect human myoblasts without cytotoxicity. For the first time, gesicles were used for delivery of a large plasmid (18-kb) with 42% of efficiency and for gene silencing (up to 60%) with siRNA. Finally, freezing/thawing cycles and storage at +4 °C, -20 °C and -80 °C did not reduce gesicles' ability to transfer plasmid DNA. In conclusion, gesicles represent attractive bioactive agents with great potential to improve the research field of nucleic acid trafficking which could be useful for cell and gene therapies.

W-2155

TOWARDS DEVELOPING A GLOBAL PLURIPOTENT CELL LINE TO SERVE ALL HUMANKIND

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There is enormous potential in regenerative cell therapies, but creating cell lines for each patient will be unrealistic given the costs, risks, and regulatory requirements. The most efficient solution will be to source cells from HLA-mismatched (allogenic) donors, but the challenge is that they will be immune rejected. Immunosuppressive drugs reduce this risk, but they are often life-long, do not always work, and increase the risk of life-threatening infections and cancer. Here, we show that the expression of eight local-acting, immunomodulatory transgenes introduced into embryonic stem (ES) cells is sufficient to protect cell derivatives against rejection in allogenic, immune-competent recipients. Allografts survive long-term, in different MHC-mismatched recipients, and without immunosuppressive drugs. Recipients of these engineered cells do not have suppressed systemic immunity and can simultaneously reject non-edited allografts. The immunomodulatory genes used here have roles in the regulation of immune suppression, cancer immune escape, fetal tolerance, and the spread of infectious cancers in the Tasmanian devil and canines. The use of many transgenes that target both innate and adaptive immune pathways was rationalized based on the complexity of allograft rejection, which involves many cross-regulated immune cell types. These genes can modulate antigen presentation and the initiation of adaptive immunity, as well as the cytotoxic leukocytes that destroy allogenic cells. The solution described here satisfies the needs of an off-the-shelf approach to cell therapy, where one tested cell line could be used in genetically-unrelated recipients. It does not require suppressive drugs, recipient-specific cell lines, secondary suppressive cells, or bone marrow transplantation. Yet given the risks that come from evading the immune system, realizing these benefits is only possible if the cells also contain a FailSafe system (Monetti abstract) that eliminates the possibility of tumor formation. We are now translating our proof-of-principle from the mouse into human cells, as well as testing in-vivo applications

that leverage these cell's properties (Payne abstract). Ultimately, the approach here could promote the wide-scale implementation of regenerative cell therapies into the clinic.

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W-2157

A PROTOCOL RESULTING IN A CLINICALLY COMPLIANT GMP HUMAN EMBRYONIC STEM 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 now present our protocol for generating new clinically compliant GMP hESC lines derived under defined and xeno-free GMP conditions. We have previously published xeno-free and defined protocols for derivation and culture but several of the components did not hold GMP standards when analyzed in detail. All the components in the original protocol are now 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 working with the first lines at Vecura, a GMP facility within Karolinska University hospital, and our facility will produce cell lines and provide clinical grade GMP hESCs for experimental research and for clinical applications in regenerative medicine.

W-2159

SMALL HYPOXIA-PRIMED MESENCHYMAL STEM CELLS ATTENUATE GRAFT-VERSUS-HOST DISEASE

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Mesenchymal stem cells (MSCs) are of particular interest for the treatment of immune-related diseases due to their immunosuppressive capacity. Here, we show that Small MSCs primed with Hypoxia and Calcium ions (SHC-MSCs) exhibit enhanced stemness and immunomodulatory functions for treating allogeneic conflicts. Compared with naïve cultured human umbilical cord blood-derived MSCs, SHC-MSCs were resistant to passage-dependent senescence mediated via the monocyte chemoattractant protein-1 and p53/p21 cascade and secreted large amounts of pro-angiogenic and immunomodulatory factors, resulting in suppression of T-cell proliferation. Genome-wide DNA methylome and transcriptome analyses indicated that genes related to immune modulation, cell adhesion, and the cell cycle were up-regulated in SHC-MSCs. MSCs overexpressing polo-like kinase-1 (PLK1), zinc-finger protein-143, dehydrogenase/reductase-3, and friend-of-GATA2 exhibited enhanced self-renewal, migratory, pro-angiogenic, anti-inflammatory, and T-cell suppression capacities, indicating that these proteins play a key role in the beneficial effects of SHC-MSCs. Administration of SHC-MSCs or PLK1-overexpressing MSCs significantly ameliorated symptoms of graft-versus-host disease (GVHD) in a humanized mouse model, resulting in significantly improved survival, less weight loss, and reduced histopathologic injuries in GVHD target organs compared with naïve MSC-infused mice. Collectively, our findings suggest that SHC-MSCs can improve the clinical treatment of allogeneic conflicts, including GVHD.

W-2161

SPATIO-TEMPORAL CONTROL OF GENOME EDITING IN CELLS AND ANIMALS

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CRISPR-Cas9-based genome editing has accelerated the pace of the drug discovery process from generation of disease models to identification and validation of drug targets. However, there is a need for a rapid system for temporal and spatial interrogation of mammalian genome readily applicable to different cellular and animal models, particularly in the early drug development cascade. Here we describe the ObLiGaRe Dox Inducible Cas9 (ODIn-Cas9) a cross-species homology independent strategy to insert an all in one, tight, inducible Cas9 construct in the safe harbor loci of the murine and human genome. This approach allows efficient gene targeting in human and mouse cell lines with temporal control of gene manipulation in mammalian cell lines *in vitro* and in adult mice *in vivo*. We also observed previously underscribed toxicity of Cas9 overexpression in mouse embryo and in iPSC. Together our data suggest that the ODIN-Cas9 system is very tight and useful for rapid generation of mouse models and to study the Cas9 overexpression in vivo.

PLURIPOTENCY

W-2163

GRID CULTURE OF HPSCS FOR MASS PRODUCTION

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Human Pluripotent Stem Cells (hPSCs) can be propagated and expanded in vitro indefinitely, and provide a potentially inexhaustible source of stem cells for human therapy. Various cell types derived from hPSCs would provide robust cellular platforms for disease modeling, drug screening, and toxicological testing. The key issue for their future clinical and pharmacological applications is the generation of large numbers of clinical-grade hPSCs through in vitro cell culture. Many efforts to produce large numbers of hPSCs for cell therapy and drug development have developed but the current culture methods are not satisfying all requirements for simple, robust, cost-efficient and safe production of hPSCs. Each hPSC colonies also show very heterogeneous growth speed since they have different colony aggregation by each cell viability on the broad surface of dish. therefore, who culture hPSCs are difficult to decide the subculture time. To overcome these above fundamental limitations, we deploy grid culture system. these square grids are printed with 3D printer and each grid has various size such as 300, 500, 1000, and 2000 micrometer. these are also coated by matrigel. when single hPSC seeded onto each grid about 2000 cells and they are formed colonies in the grid. the colony formation is depended on the seeding cell number and the grid size. Among the three kinds of grids, 1000 is best for showing rapid growth speed and there are filled hPSC colonies over 90% of the grids at the same time. An optimal size for hPSC culture is 1000. In addition, hPSCs culture in the grids with 1000 results most likely in the increase of beginning ratio of cell attachment, cell viability, and pluripotency gene expression such as Oct4, Nanog, and tra1-60. They can be differentiated into various cell types in vitro and in vivo. Here, we demonstrate a direct correlation between colony formation and grid size. Our study will fulfill all requirements such as simple, robust, cost-efficient and safe production of hPSCs by taking advantage of grid culture in automated GMP facility.

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W-2165

BIOLOGICAL CHARACTERIZATION OF HUMAN ADIPOSE TISSUE-DERIVED PERICYTES AND REPROGRAMMING INTO INDUCED PLURIPOTENT STEM CELLS

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Pericytes are multipotent stem cells that play an important role in the development and maintenance of vascular integrity. Pericytes reside in the vascular tissue walls, and have been isolated from different vascular tissues as the brain, kidneys, retina, and ears. In this study, we aim to isolate pericytes from human adipose tissue, and investigate their biological characteristics as a novel source of multipotent stem cells for regenerative medicine. Pericytes were isolated from human adipose tissue and characterization by flow cytometry, immunofluorescence staining and conventional PCR. Isolated cells showed positive expression of the pericyte-markers, α smooth muscle actin (α-SMA) and desmin, mesenchymal stem cell markers CD73 and CD105, while lacked the expression of hematopoietic stem cells markers CD45, HLADR, CD11b and CD45. Upon stimulation with the appropriate differentiation factors, pericytes showed adipogenic, osteogenic, myogenic, and vascular differentiation, confirming their multipotent potential. Scanning electron microscopy and transmission electron microscopy confirmed that the ultrastructure of adipose-derived pericytes was similar to other tissue-pericytes, including prominent eccentric nucleus, cytoplasmic processes and composite caveolae. Pericytes reprograming into induced pluripotent stem cells (iPSCs) was carried out by transfection with bacterial plasmids 27077 (pCXLE-hOCT3/4-shp53-F), 27078 (pCXLE-hSK), and 27080 (pCXLE-hUL) via

electroporation. Colonies of iPSCs were observed after 24 hours of transfection. Reprogrammed cells showed positive expression for embryonic stem cell markers SSEA-4, Oct-4 and Nanog, and positive expression for nuclear beta catenin at day 5 indicating early stage reprogramming. Our study showed that adipose tissue represents an available source for multipotent pericytes. iPSCs successfully reprogrammed from pericytes may provide a readily accessible source of cells for regenerative medicine.

Funding Source: The Science and Technology Development Fund (STDF) project #5300, and the Academy of Scientific research and Technology (ASRT), Ministry of Scientific Research; Egypt.

W-2167

ESTABLISHING THE MECHANISM BY WHICH OCT4 IS BOTH REQUIRED FOR, AND ANTAGONISTIC TO, NAÏVE PLURIPOTENCY IN MOUSE EMBRYONIC STEM CELLS.

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All cells of the mammalian embryo originate from a small group of pluripotent stem cells found in the late blastocyst which possess the capacity to form all somatic and germ lineages. This state of naïve pluripotency is therefore crucial in developmental biology. Oct4 is a core transcriptional regulator of the pluripotent state; it is essential for the establishment and maintenance of pluripotency in vivo and in vitro. Either overexpression or loss of Oct4 results in destabilization of the naïve pluripotent state and drives cells to differentiate. Recent reports have shown that cells with a lower than wild-type level of Oct4 have a more robust pluripotency transcription network and resist differentiation. This leads to an interesting dichotomy: how is it that Oct4 is required in pluripotency while a low level is optimal for maintaining a strong pluripotency network? Here we utilize a novel rapidly-degradable Oct4 transgene to investigate the requirement for Oct4 for the expression of pluripotency associated genes, explaining the requirement for this unique gene. We also demonstrate that Oct4 negatively affects the binding of other pluripotency factors to the genome. This establishes a negative feedback loop in the naïve pluripotency transcription network. Not only does this work provide new insights into the role of Oct4 in the naïve state, it also suggests a mechanism through which pluripotent cells may remain competent to exit pluripotency and differentiate.

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W-2169

COPS2 ANTAGONIZES OCT4 TO ACCELERATE THE G2/M TRANSITION OF MOUSE EMBRYONIC STEM CELLS

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Proper regulation of the cell cycle is essential to safeguard the genomic integrity of embryonic stem cells (ESCs), while maintaining the fast proliferation rate. The pluripotency factor Oct4 has been shown to inhibit Cdk1 activation, thus preventing mitotic entry and facilitating the maintenance of genomic integrity. Yet, how ESCs enter mitosis in the presence of Oct4 remains unclear. We previously reported that Cops2 promotes the progression through the G2/M phase of ESCs. In this study, through co-immunoprecipitation and mass spectrum analysis, we found that Cops2 interacts with Oct4 and Cdk1. We further demonstrated that Cops2 stimulates the activity of Cdk1/Cyclin B only when Oct4 is present. Consistently, Cops2 promotes the G2/M transition only in the presence of Oct4 in Hela cells. Mechanistically, Cops2 attenuates the interaction between Oct4 and Cdk1, by sequestering Oct4 and by forming a Cops2/Cdk1 complex, thus blocking the inhibitory effect of Oct4 on Cdk1 activation.

W-2171

POST-TRANSLATIONAL REGULATION OF KLF4 MODULATES EXIT FROM NAÏVE PLURIPOTENCY

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Pluripotency is regulated by an interconnected network of transcription factors including OCT4, SOX2, KLF4 and NANOG. Although differentiation of embryonic stem (ES) cells to specific cell types occurs over several days, during which Sox2 and Oct4 expression are maintained, disruption of KLF4 and NANOG function occurs within 24hr and is critical for pluripotency exit. We identified distal regulatory regions that regulate Klf4 transcription, as homozygous deletion caused a 95% decrease in Klf4 transcript; however, surprisingly only a 50% decreased in protein was observed suggesting KLF4 protein is highly stable in ES cells. Determination of protein half-life revealed KLF4 has a half-life >12hr in ES cells. KLF4 stability is disrupted within 24hr of differentiation, evidenced by a shift to a half-life of <2hr. Next we investigated the effects of individual signalling pathways on KLF4 stability and observed that removing MEK pathway inhibition or LIF/STAT3 activation had the greatest effect on KLF4 destabilization. MEK-ERK

activation causes KLF4 phosphorylation at S132 which we show leads to KLF4 nuclear export. We observed that constitutively nuclear KLF4 S132A mutant retains stability after differentiation and conversely a constitutively cytoplasmic KLF4 NLS (nuclear localization) mutant is unstable in undifferentiated conditions. As nuclear residence caused KLF4 stabilization we hypothesized that DNA binding and interaction with transcriptional complexes was important for KLF4 stabilization; in support of this a KLF4 ZNF mutant which is unable to bind DNA is unstable. Next we investigated the role of protein-protein interactions in stabilizing KLF4 and determined that STAT3 phosphorylation causes interaction with nuclear KLF4 and increased KLF4 stability. Conversely, SOX2 knockdown, due to enhancer deletion, caused reduced KLF4 stability. Mutations that increased KLF4 stability blocked differentiation of ES cells and caused the sustained expression of pluripotency factors in the absence of LIF/2i. These data indicate KLF4 function is modulated by post-translational mechanisms rather than transcriptional mechanisms during pluripotency maintenance and disruption to KLF4 stability is critical for differentiation.

W-2173

MODELLING AN L-PROLINE-ACTIVATED MTOR AND MAPK REGULATORY NETWORK IN MOUSE EMBRYONIC STEM CELLS

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Mouse embryonic stem cells (ESCs) derived from the developing mouse blastocyst can be differentiated in vitro to early primitive ectoderm-like (EPL) cells in the presence of exogenously added L-Proline. This amino acid has been demonstrated to have novel growth factor-like properties throughout development - from promoting blastocyst development to driving neurogenesis. We show that EPL cells express genes comparable to the in vivo primitive ectoderm and primed epiblast stem cells (EpiSCs). EPL cells downregulate the ESC marker Rex1 and upregulate FGF5 9.1+/-32.0 fold (SEM, P<0.01, n=9) and Dnmt3b 2.4+/-31.2 fold (SEM, P<0.0001, n=8). Further differentiation with L-proline induced gene expression consistent with neurectoderm differentiation. As ES cells differentiated to EPL cells, the cells underwent a morphological change, increased growth rate at 48 h by 1.9+/-30.14 fold (SEM, P<0.5, n=4), and reduced apoptosis at 48 h by 26.1+/-31% (SEM, P<0.05, n=3) as measured by Annexin V assays. BrdU incorporation assays showed that the proliferation rate did not change between ES and EPL cells. L-Proline acutely activated the MAPK, mTOR and PI3K pathways, and these pathways were constitutively active during differentiation to EPL cells. Inhibition of PI3K, mTORC1 complex, P70S6 Kinase, MEK1 and FGFR often showed transient reduction in phosphorylation of downstream kinases, such as RPS6 and ERK1/2. Combinations of inhibitors was required to maintain long-term reduction in RPS6 and ERK1/2 phosphorylation, indicating substantial cross talk within these pathways. When inhibitors were added individually alongside L-Proline to ES cells, they reduced the efficiency of differentiation, but the cells maintained some characteristics of an EPL-cell population. Combinations of inhibitors which suppress mTOR and MAPK signalling were able to block differentiation to EPL cells. The contribution of individual pathways can be mathematically modelled to assess pathway interactions and downstream functions such as differentiation, morphology, growth, proliferation and apoptosis. L-proline acts through mTOR and MAPK to drive differentiation of ESCs to pluripotent EPL cells. Understanding this mechanism would provide insight into pluripotency regulation and the role for amino acids in development

W-2175

A FEEDER-INDEPENDENT CULTURE SYSTEM TO CONVERT AND MAINTAIN HUMAN PLURIPOTENT STEM CELLS IN A NAÏVE-LIKE STATE

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Human pluripotent stem cells (hPSCs) within the developing embryo progress through a spectrum of pluripotent states as they transition from naïve to a more lineage-restricted, primed state. Capturing these states in vitro requires specialized culture media and protocols. To date, conditions that maintain hPSCs in naïve-like states have depended on the use of feeder cells for robust long-term expansion. RSeT™ Feeder-Free (RSeT™-FF) is a defined medium that supports the reversion of primed hPSCs to a naïve-like state and supports their long-term feeder-independent maintenance. To revert primed hPSCs cultured in mTeSR™1 or TeSR™-E8™, colonies were seeded as small clumps on Corning Matrigel® coated plates. After 24 hours, the medium was changed to RSeT™-FF supplemented with 0.2% Matrigel® and hPSCs were cultured for 5 days with medium changes every other day. hPSCs maintained in RSeT™-FF + 0.2% Matrigel were dissociated to single cells and re-seeded at a density of 2.1 × 104 cells/cm2 on Matrigel®-coated plates every 4-6 days. Colony morphology and cell expansion was assessed at each passage (P). Transition to a naïve-like colony morphology was observed in

cultures as early as P1, with colonies possessing a highly homogeneous naïve-like morphology with >90% of colonies displaying a domed morphology, and extremely low levels of background differentiation (n=5 hPSC lines). On average, hPSCs cultured in RSeT™-FF expand by 3.0 3 0.9-fold per passage (n=4); comparable to hPSCs cultured in RSeT™ Medium on feeders. Cells from naïve-like colonies (>P5) expressed markers associated with undifferentiated hPSC such as OCT4, SSEA4, TRA-1-60 and ALP. Importantly, as in RSeT™ Medium on feeders, naïve-like hPSCs cultured in RSeT™-FF Medium showed upregulation of genes commonly enriched in the naïve-state (KLF2, KLF4, KLF17, TFCP2L1, STELLA, and DNMT3L1) compared to mTeSR™1-cultured primed hPSCs. Additionally, naïve-like hPSCs maintained in RSeT™-FF Medium are capable of direct differentiation to all somatic lineages using the STEMdiff™ Definitive Endoderm, Mesoderm Induction, and SMADi Neural Induction Kits. In summary, we have developed RSeT™-FF, a defined medium that promotes robust conversion of primed hPSCs to a naïve-like state and the continuous maintenance of these cells in a feeder-independent culture system.

PLURIPOTENT STEM CELL DIFFERENTIATION

W-3001

SINGLE CELL PROTEIN EXPRESSION MAP OF DEVELOPMENTAL REGULATORS DURING EXIT FROM THE HUMAN PLURIPOTENT STATE

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A pluripotent stem cell (PSC) maintains its identity by the stable expression of a suite of transcription regulators, which synergistically maintain a gene expression program ready for differentiation. This is not a binary function. Instead, the absolute and relative abundance of such regulators (e.g. OCT4, SOX2, NANOG, etc.) are modulated in response to extrinsic differentiation signals, ultimately yielding changes in other key regulators who enforce a more restricted cell identity. Effort has been spent to better understand the combination and sequence of extrinsic factors needed to maintain and specify human pluripotent cells into distinct embryonic tissue. However, there is very little data on the simultaneous, single cell expression dynamics of developmental transcription regulators during embryonic stem cell maintenance and specification. To address this gap, we established a single cell proteomic platform where more than 20 developmental regulators with established function in embryonic development (e.g. OCT4, SOX2, NANOG, ISLET1, GATA4, PAX6, etc.) can be simultaneously quantified, in combination with comprehensive cell identity, as (h)uman PSCs undergo multi-lineage differentiation in vitro. To that end we combined specific metal conjugated antibodies for each regulator with probes for cell-cycle, apoptosis, and phenotype (cell identity), to pair with single cell mass-cytometry (CyTOF). hPSCs directed to embryonic mesoderm, endoderm, or ectoderm over 7 days were used as a model to understand pluripotency exit and commitment. With this system we can now identify a rule-set of the combinatorial single cell protein expression of key regulators across all three embryonic lineages as cells emerge from the pluripotent state. For instance, we observe opposing dynamic expression of both transcription factors PBX1 and OCT4 in cells directed to mesoderm or endoderm. Additionally, multidimensional clustering and visualization tools highlight hierarchies of key regulators, such as the cell-state specific activation of EOMES, FOXA2 and finally GATA4 during endoderm specification. A holistic view of this system will be presented which should serve as a template for engineering directed differentiation as well as understanding early human embryogenesis in the future.

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W-3003

HUMAN FIBRINOGEN COATING FOR GENERATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED RETINAL PIGMENT EPITHELIUM

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The differentiation of induced pluripotent stem cells (iPSCs) requires adhesion to tissue culture surfaces. Most often this is accomplished by coating with an extracellular matrix protein. As iPSC derived cells enter human clinical trials there is a need to scale-up their growth and, wherever possible, to use non-xenogeneic materials. P reviously, we have shown that iPSC-derived retinal pigment epithelium (RPE) can be grown on fibrin hydrogels. From this work, we hypothesize that fibrinogen coated surfaces could be

used to differentiate iPSC-RPE. To test this hypothesis, various concentrations of fibrinogen solution were used to coat tissue culture polystyrene plates by incubating 0.3 mL/cm2 overnight at 4°C. Partially-differentiated RPE (LAgen Laboratories, Rochester MN) were then plated onto the fibrinogen at a density of 0.4-0.5 x 106 cells/cm2 and cultured with differentiation media, up to 12 weeks. After 4 weeks, RPE appeared pigmented and in the characteristic cobblestone pattern. Western blot analysis (Wes, ProteinSimple, San Jose CA) for RPE markers showed expression of Best1, RPE65, CRALBP, and MERTK as early as week 6. Immunofluorescent staining for ZO-1 demonstrated formation of tight junctions, for Ezrin demonstrated formation of microvilli and for Best1 confirmed basolateral expression. ELISA analysis (DuoSet Kit, RND Systems, Minneapolis MN) of 24 hr conditioned media demonstrated secretion of PEDF, 9.631.6µg/mL, and VEGF, 3.630.1ng/mL. Human fibrinogen-based reagents can be produced and easily scaled for both research and clinical demands while maintaining cost-effectiveness, stability and ease of use. In conclusion, fibrinogen coated surfaces are suitable for use in differentiation of iPSC-derived cells.

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W-3005

GENERATION OF A NOVEL POPULATION OF PANCREATIC MULTIPOTENT PROGENITOR CELLS EXPRESSING NKX6.1

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Diabetes is a metabolic disease caused by the loss or impaired function of pancreatic β-cells. Different therapeutic strategies aim to restore the endogenous production of insulin rather than the cornerstone insulin injections. hPSCs make an invaluable tool for cell-based therapy for diabetes. However, most of the previous studies generated immature beta cells that failed to respond to glucose. The coordinate expression of specific transcription factors (TF) in distinct stages governs the differentiation of hPSCs into beta cells. The dual expression of PDX1 and NKX6.1 during multipotent progenitor cell (MPC) stage is important for guiding the cells towards functional beta cells. However, cells expressing PDX1 but lack NKX6.1 expression tend to take the poly-hormonal path. This guided the differentiation protocols to focus on enriching PDX1+/NKX6.1+ MPCs. The aim of this study was to further explore different MPC populations in terms of PDX1/NKX6.1 expression. We used two different differentiation protocols to differentiate hPSCs into MPCs. The mRNA and protein expressions of the generated MPCs were analyzed using different techniques. Our results showed that hPSCs were successfully differentiated into the conventional (PDX1+/NKX6.1+) and (PDX1+/NKX6.1-) MPCs. Interestingly, our results showed the generation of a novel population expressing NKX6.1 in the absence of PDX1 (PDX1-/NKX6.1+). Culture modifications in our optimized protocol have led the uncharacterized subset of MPCs to exhibit a pattern of 3D aggregates that were consistently PDX1-/NKX6.1+. To understand and characterize this unique population and its fate, we examined the expression of endocrine precursors markers, including CHGA, NGN3, and NKX2.2. Interestingly, while CHGA and NGN3 were negative, some cells of these 3D aggregates co-expressed NKX2.2, suggesting that this population may have an undefined role in the development of MPCs into endocrine progenitors. Additionally, the expression of ductal epithelium marker cytokeratin 19 was negative for the 3D structures. These findings showcase a novel population of NKX6.1 expressing MPCs and suggest the possibility for differentiation trajectory for the development of MPCs into functional beta cells.

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W-3007

PEROXIREDOXIN REGULATE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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In vitro differentiation of stem cells provide valuable surrogate for understanding molecular and cellular mechanisms in the developmental process of organism and invaluable source for regenerative medicine. Among the research results provided recently, growing number of evidences suggest that reactive oxygen species (ROS) play critical roles in various cell fate decisions such as osteogenesis, chondrogeneis and adipogenesis. Although peroxiredoxin (Prx) well known maintain cellular homeostasis as ROS scavenger, I investigated the significance of Prx in the differentiation potential using Wild Type, Prx I-/- and Prx II-/- mouse embryonic stem cells (mESCs). Interestingly, here I reveal that Prx I and II exhibit distinct functional properties in regulating differentiation propensities of mESCs. Prx I-/- mESCs showed marked increase of pluripotent genes compared to WT mESCs. Genes related to ectoderm and endoderm (Tubb3 and Gata6, respectively) revealed similar patterns across all mESCs but mesodermal gene, Brachyury was highly up-regulated in Prx-/- cells. In addition, Prx I and Prx II appear to have a tight association with the mechanism underlying the protection of ESC stemness in developing teratomas. Therefore, our findings have important implications for understanding of maintenance of ESC stemness through involvement of antioxidant enzymes and may lead to development of an alternative stem cell-based therapeutic strategy for production of high-quality neurons in large quantity.

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W-3009

THE ROLE OF CHEMOKINE GPCR, CCR7 IN HUMAN EMBRYONIC STEM CELL DIFFERENTIATION TO TROPHOBLAST

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Although G-protein coupled receptors (GPCRs) are the largest receptor family, they have not been considered as major regulators of embryogenesis. Recent findings by our group in zebrafish, implicate chemokine GPCR signaling as a negative regulator of β-catenin levels during embryonic axis formation. Our current studies are directed at testing whether this function of chemokine GPCRs is conserved in human embryonic stem (hES) cells and during early stages of their differentiation to trophoblast. Immunoblotting, immunocytochemical, RT-PCR analyses and published RNA-seq data sets from human embryos revealed that the chemokine GPCR, CCR7 and its ligands, CCL19 and CCL21, are expressed in H9 and H1 hES cells, and early human embryos. Preliminary data suggest that as H9 cells differentiate into embryoid bodies, the expression of both ligands is up-regulated and the levels of CCR7 are down-regulated. Exposure of H9 hES cells to CCL19 or CCL21 inhibited cell proliferation. These data support the notion that CCR7 signaling may possibly affect differentiation in vitro and during embryogenesis. Since CCR7 expression and immunoreactivity has been identified in human trophoblasts, our hypothesis is that CCR7 signaling regulates trophoblast differentiation from hES cells. We have generated CCR7 -/- mutants in H9 and H1 hES cells, by bi-allelic mutation using CRISPR/Cas9 technology. We are differentiating wild-type H9/H1 and CCR7-/-H9/H1 cells into trophoblast cells including isolation of cytotrophoblasts (CTBs) and further differentiating them to syncytiotrophoblasts (STBs) and extravillous trophoblasts (EVTs). Expression of markers such as CDX2, TEAD4, p63, EGFR, KR17, GATA3 in the absence of the mesoderm marker Brachyury (T), as well as functional assays is used to assess the efficiency of STBs differentiation. Secretion of hCG or immunoreactivity of this hormone and MMP2 expression will support the presence of CTBs and EVTs, respectively. We are also testing whether the expression pattern of trophoblast markers and thus differentiation is affected by treatments with recombinant CCL19 or CCL21 ligands in wild-type H9/H1 cells. The results from our study will advance our understanding of the role of CCL19/CCL21/CCR7 signaling axis in human trophoblast differentiation in vitro.

W-3011

IDENTIFYING REGULATORS OF POSTERIOR HOX GENE ACTIVATION AND AXIS ELONGATION

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Hox genes are evolutionary conserved master regulators of patterning along the anterior-to-posterior (A-P; head-to-tail) axis during development. In almost all species, multiple Hox genes are found adjacent to one another at distinct genomic loci (Hox clusters), a feature that facilitates coordinated expression. In vertebrates, the strict timing of Hox gene activation based on their position along the chromosome translates into the ordered spatial pattern required to pattern the axis. While the Hox genes are the ultimate effector molecules, it is the signals and molecules controlling Hox expression that orchestrate how the main body axis is laid down. Revealing the identity of these signals, and the cis-regulatory elements through which they act, are fundamental to our understanding of developmental and evolutionary mechanisms. We have previously shown that miR-196, a family of microRNAs genomically embedded within Hox gene clusters that targets multiple Hox genes, is an essential regulator of vertebral number and identity in mouse. Importantly, we found that not only does miR196 post-transcriptionally regulate known direct Hox target genes, but remarkably also indirectly controls the timing of posterior Hox gene activation in a developmental process known as trunk-to-tail transition. Using mouse induced pluripotent stem cells (miPSCs) in combination with in vitro differentiation we have established that Gdf11 signalling is also required for activation of the Hox genes in a timely manner. We are now employing sophisticated genomic sequencing to investigate the genomic dynamics around the Hox gene clusters during gene activation, to identify novel regulators that activate the 5'Hox genes. Our preliminary data indicates that miR196 and Gdf11 signalling synergize/works together to activate the posterior Hox code and thus are necessary for facilitating the trunk-to-tail transition and the cessation of axis elongation.

W-3013

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO RENAL PROGENITORS FOR RESEEDING ON PORCINE KIDNEY MATRICES

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Regenerating solid organs for transplantation would overcome the scarcity of viable human organs available for donation. Here, we investigate the efficacy of kidney regeneration using human induced pluripotent stem cells and 3D culturing on extracellular matrix scaffolds derived from porcine kidney. We differentiated human induced pluripotent stem cells into kidney progenitor cells by recapitulating embryonic kidney development through mesoderm, intermediate mesoderm, and metanephric mesenchymal induction in vitro. Using a specific combination of small molecules and growth factors, we report a cell population that expresses mesoderm (BRY+), intermediate mesoderm (PAX2+, LIM1+), and metanephric mesenchyme (SIX2+) markers following each stage of differentiation. We also decellularized sections of porcine kidney for subsequent use in culturing renal progenitor cells, which would provide the specific microarchitecture and biochemical signals for renal progenitor cells to migrate and proliferate. We have found that low concentrations of SDS can efficiently decellularize sections while preserving extracellular matrix proteins and glycosaminoglycans necessary to create a renal progenitor cell niche. In these preliminary studies, we aim to examine the role of cell and extracellular matrix interactions in the further differentiation of renal progenitor cells. By using human induced pluripotent stem cells and extracellular matrix scaffolds, we have the technical means to create a 3D cell culture system for drug screening, and eventual whole kidney regeneration.

Funding Source: Ontario Research Foundation.

W-3015

DISTINCT STAGE DEPENDENT REQUIREMENTS FOR RUNX1 AND GROUP F SOX GENES DURING HUMAN HAEMATOPOIESIS

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We explored the generation of blood cells during human haematopoiesis using in vitro differentiation of human pluripotent stem cell (hPSCs) reporter lines to follow endothelial (SOX17) and hematopoietic (RUNX1C) development. Modeling extra-embryonic yolk sac-like haematopoiesis using the blast colony forming cell (BL-CFC) assay, revealed that SOX17-CD34+CD43- endothelial-like cells were the major source of haematopoietic progeny while SOX17+CD34+CD43- cells predominantly gave rise to endothelium. Deletion of RUNX1 permitted a single wave of primitive erythropoiesis from BL-CFCs but abrogated all myeloid and subsequent erythroid clonogenic cells. Conversely deletion of SOX17 or all three Group F SOX genes did not influence the production of BL-CFCs, or their endothelial or haematopoietic progeny. Differentiation of SOX17- or GroupF SOX- deficient cell lines towards intra-embryonic haematopoiesis revealed increasingly severe defects in vascular organization and reduced haemogenic capacity of the endothelium. Our data indicate distinct haemogenic and endothelial biased precursors in human yolk sac-like cultures and characterize the requirements for RUNX1 and SOX genes during extra- and intra-embryonic human haematopoiesis.

W-3017

DISSECTION OF ENDOTHELIAL-TO-HAEMATOPOIETIC TRANSITION AT THE SINGLE CELL LEVEL IDENTIFIES CELL-CYCLE REGULATION AS A DETERMINANT OF HAEMATOPOIETIC COMMITMENT

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Haematopoietic stem cells (HSC) are responsible for the maintenance of blood homeostasis and are involved in a diversity of diseases including leukemia. Understanding their embryonic development is essential to produce these cells in vitro from human pluripotent stem cells (hPSC), but also to develop new therapies. During development, they first arise in the aorta-gonad-mesonephros (AGM) region of the embryo from a population of haemogenic endothelial cells lining the ventral portion of the dorsal aorta which undergo endothelial-to-haematopoietic transition (EHT). During this process, both erythro-myeloid and lymphoid progenitors are produced, ultimately culminating with the generation of the first HSCs capable of multilineage differentiation and long-term engraftment. Little is known about the molecular mechanisms driving this process, especially in human where the AGM region is not easily accessible in vivo. Here we used differentiation of hPSCs as a model system to uncover the mechanisms by which haemogenic endothelium generates early HSCs. By combining this approach with single cell RNA-sequencing (scRNAseq), we were able to dissect this fundamental stage of development and gain information about cell state and dynamics during EHT. Of particular interest, we identified that cell-cycle progression is essential for endothelial cells to undergo the transition to the haematopoietic fate. Specifically, we demonstrated that most of the endothelial cells at this stage reside in the G1 phase, with a direct correlation between an active cell-cycle and their ability to engage cell fate decision towards the haematopoietic fate. We also confirmed the importance of the cell-cycle by functional validations and identified CDK4/6 and CDK1 as key molecules affecting this process. Finally, we used scRNAseq to identify potential pathways disrupted upon inhibition of these regulators. Ultimately, we propose here a direct connection between cell-cycle machinery, cell fate decision and capability of haemogenic endothelial cells to undertake the haematopoietic fate during EHT. These results will have major impact in the improvement of protocols for the production of functional HSCs in vitro, but also for understanding key mechanisms regulating HSCs, therefore helping with the development of new therapies.

W-3019

HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATE WHILE ENCAPSULATED, AND EXPRESS INSULIN MRNA AND C-PEPTIDE WHEN TRANSPLANTED INTO DIABETIC MICE.

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Differentiated human stem cells (embryonic and induced) are a potential source of surrogate β-cells for treatment of Type 1 Diabetes (T1D). Microencapsulation of the differentiated cells is a means of protection during differentiation and once transplanted into a mouse model of T1D. We aimed to determine if microencapsulation prior to differentiation would produce a superior outcome after encapsulated cells were transplanted. Endeavour 1 human embryonic stem cells (hESCs) and induced pluripotent stem cells (IPSCs) from a Type 1 Diabetic

were encapsulated in 500μm alginate microcapsules (2% w/v UPMVG; ProNova) for differentiation to the endocrine pancreatic lineage. This process was verified using RT-qPCR at days 0,3,6,9 by measuring the expression of specific markers for definitive endoderm, primitive foregut and pancreatic endoderm. NOD/SCID mice were made diabetic with streptozotocin and implanted intraperitonealy with encapsulated hESCs. Body weight and blood glucose concentrations were measured up to 83 days. Animals were then euthanized and capsules were collected to measure cell viability, mRNA expression and insulin secretion upon glucose stimulation. Plasma was assayed for human C-peptide, as a measure of insulin production from the transplanted cells. At 83 days, hESCs expressed mature β-cell mRNAs including INS. Circulating human C-peptide at days 30 and 83 were 3.130.5 and 4.130.1pmol/L, respectively. Implanted mice gained weight without requiring exogenous insulin while the streptozotocin treated, blank capsule controls lost weight (≥10%) despite receiving regular insulin. Implantation of encapsulated hESCs, however, did not reduce blood glucose levels. Our data confirm that encapsulated hESCs undergo appropriate lineage commitment in vitro. In vivo, hESCs continued to differentiate to express insulin and secrete C-peptide. Our findings present a streamlined and scalable method for differentiation of stem cells for the treatment of Type 1 Diabetes.

Funding Source: CSIRO - Stem Cells and Diabetes Group; Australian Foundation for Diabetes Research - Stem Cells and Diabetes PhD Scholarship; JDRF Australia - PhD Top-Up Scholarship.

W-3021

HUMAN EMBRYONIC STEM CELL IN VITRO DIFFERENTIATION INTO PROXIMAL RESPIRATORY AIRWAY EPITHELIUM - TOOLS FOR PROTOCOL OPTIMIZATION AND TARGET CELL ISOLATION

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Artificial trachea is proposed to improve the life quality of patients received trachea surgery. Other than biomaterials to build windpipe, the cellular component making the artificial trachea functions appropriately is also important. We propose to study the procedure to differentiate human embryonic stem cell (hESC) into ciliated respiratory epithelial cells for artificial trachea construction. This project looks into the possibility to introduce human pluripotent stem cells as the cellular source. The in vitro differentiation protocol optimization and the resulting cell product analyses lay the foundation for its future regenerative medical applications. In this project, we will use the CRISPR/Cas9 technology to generate lineage-specific reporter hESC lines to delineate the details of hESC in vitro ciliated respiratory epithelial differentiation. Feeder-independent and serum-free hESC culture will be introduced. Anterior foregut endoderm (AFE) and ciliated cell lineage will be focused.

W-3023

FOOTPRINT-FREE RNA-BASED GENERATION AND DIFFERENTIATION OF PLURIPOTENT STEM CELLS USING GRAPHENE OXIDE NANOPARTICLES AND SYSTEMATIC ANALYSES OF THEIR GENOMES, TRANSCRIPTOMES, AND PROTEOMES

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Clinical applications of induced pluripotent stem cells (iPSCs) require development of technologies for the production and differentiation of footprint-free (gene integration-free) iPSCs, which avoid the potential risk of insertional mutagenesis in humans. We developed an RNA delivery system employing graphene oxide (GO) nanoparticle complexes for the efficient generation and differentiation of footprint-free iPSCs. GO nanoparticle complexes were found to be very effective for loading mRNA or total RNA of reprogramming or differentiation transcription factors, protection from RNA degradation by RNase, and suppression of RNA-mediated immune response. The iPSCs showed all the hallmarks of pluripotent stem cells including expression of pluripotency genes, epigenetic reprogramming, and differentiation into the three germ layers. We also could differentiate the iPSCs into adipocytes and mesenchymal stem cells using the GO nanoparticle-RNA-based differentiation methods. Moreover, we conducted single cell genome sequencing analysis and systemic analysis to compare the transcriptomes and proteomes of RNA-induced iPSCs or differentiated cells using microarray and LC-MS/MS, respectively. Single cell

genome sequencing and comprehensive transcriptome and proteome analyses showed significant similarities in transcript and protein expression patterns of the iPSC and ESC for genes involved in pluripotency and epigenetic reprogramming, compared to the original somatic cells. Importantly, the genes related to genotoxicity (DNA damage or repair, apoptosis, carcinogenesis, growth arrest etc.) showed that the expression of genotoxicity genes was decreased in RNA-induced iPSCs or differentiated cells. Taken together, transient delivery of exogenous RNA into cells provides a safer reprogramming and differentiation system to transgenic approaches that rely on exogenous DNA vectors, confirming that RNA-based reprogramming and differentiation technology may be more suitable for clinical applications.

W-3025

ENRICHMENT OF LEFT VENTRICLE CARDIOMYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

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Cardiovascular disease is one of the leading causes of death around the world. Myocardial infarction results in dramatic loss of cardiomyocytes, particularly in the left ventricle (LV). As a result, tissue damage occurs and the heart is remodelled, usually resulting in impaired function. Adverse remodelling might be ameliorated if the LV were to be repopulated by functional cardiomyocytes with the electrophysiology properties of the left ventricle. To this end, attempts are under way to reprogram, in an efficient manner, endogenous or exogenous cells to a LV identity. To better understand the formation of the LV in vivo, mouse hearts from several embryonic developmental time-points were micro-dissected into distinct anatomical regions and analysed by mRNA-sequencing. In combination with spatial transcriptomic analysis of embryonic mouse hearts, we have identified candidate genes and signalling pathways which are specifically up- or down- regulated in the LV myocardium during heart development. Some of these identified candidate genes, which represent an array of biological functions, have been modulated to optimise in vitro differentiation of hESC into a homogenous LV cardiomyocyte fate.

Funding Source: Work supported by British Heart Foundation (FS/12/37/29516) and the Francis Crick Institute which receives core funding from Cancer Research UK (FC001157), UK Medical Research Council (FC001157), and the Wellcome Trust (FC001157).

W-3027

TET1 DEFICIENCY IMPAIRS THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO NEUROECTODERM

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Tet family of 5-methylcytosine (5mC) dioxygenases play critical roles in development by modifying DNA methylation. While the expression of Tet1 is the highest in human embryonic stem cells (hESCs), its function in hESCs is unclear. Using CRISPR, we mutated both alleles of Tet1 gene in H9 hESCs by introducing stop codons in its catalytic domain. H9 cells with mutated Tet1 exhibited normal morphology, expressed pluripotency markers and were able to differentiate to cells of all three germ layers in serum-containing medium, even though the level of hydroxymethylcytosine (5hmC) decreased to 20% of that in parental H9 cells. Neural differentiation induced by double SMAD inhibitors was not significantly affected by loss of Tet1 activity. However, in morphogen-free condition, Tet1 deficiency significantly reduced the generation of SOX1+PAX6+Nestin+ neuroectoderm cells from 70% in parent H9 cells to 20% in the mutant H9 cells. This was accompanied by a 20 fold reduction in the expression level of PAX6 and a significant decrease in the amount of 5hmC on PAX6 promoter. Our results suggest that loss of tet1 catalytic function impairs the intrinsic ability of hESCs to differentiate to neuroectoderm, possibly by decreasing the expression of PAX6, a key transcription factor in the development of human neuroectoderm.

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W-3029

DIFFERENTIATION OF HUMAN iPSC LINES CARRYING BRCA MUTATIONS INTO BREAST EPITHELIAL LINEAGES

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Breast cancer is one of the most common cancers affecting women, with an expected lifetime risk of 12%. While breast cancer is predominantly caused by sporadic mutations, up to 10% of all cases can be attributed to mutations in the BRCA gene family. Carriers of BRCA mutations have an expected lifetime risk of 45% (BRCA2) to 65% (BCRA1) of developing breast cancer. The only currently available prophylactic therapies are drugs that are not suitable for all women, or complete mastectomy. The aim of this project is to develop a differentiation protocol to drive cells to mammary epithelial cell types from induced pluripotent stem cell (iPSC) lines derived from women with a BRCA mutation. These cells can then be used to screen for novel therapeutics to prevent malignant transformation of breast epithelial cells carrying BRCA family mutations. iPSC lines were generated from tissue samples donated by women undergoing either prophylactic mastectomy or breast reduction surgery and who were either BRCA 1 or 2 carriers or controls. All cell lines generated were characterised for pluripotency markers, karyotypic normality and ability to form teratomas. The iPSC lines are differentiated using a substrate/media driven approach to drive cells into a putative mammary epithelial fate. Differentiated cells have been generated that express the breast luminal progenitor cell markers EpCAM, CD49f, cytokeratin 8/18 and are negative for the basal cell marker cytokeratin 14 and pluripotency markers OCT4 and PCDH1. These cells also exhibit luminal morphology in colony formation assays, and further functional characterisation is underway. We have shown that this protocol gives rise to putative mammary epithelial cells of human origin. We anticipate using these cells to search for novel compounds to prevent malignant transformation of breast epithelial cells carrying BRCA family mutations as well as providing a tool to assess the mechanisms leading to transformation.

W-3031

DEVELOPING A VISUAL MODEL AND GENOMIC MAP FOR CARDIOMYOCYTE DIFFERENTIATION

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The Allen Institute for Cell Science is creating a dynamic visual model of cell organization from pluripotency to differentiation using pluripotent iPSCs and cardiomyocytes derived from them. Using the WTC hiPSC line and CRISPR/Cas9, we have fluorescently tagged ~25 target genes representing key cellular organelles including some cardiac-specific genes. Here we present our optimized cardiac differentiation methods and discuss metrics for determining the efficiency and quality of the resulting cardiomyocyte populations, including myofibril contraction, cardiac protein expression, and transcriptome profiling. We will also present the gene editing strategy used to generate the cardiac-specific gene-tagged hiPSC lines. We have initiated 3D live cell imaging of the major organelles, including the sarcomeres (ssTNNi1, ACTN2, Titin), in cardiomyocytes derived from these structure-tagged hiPSC lines. While our current focus is on imaging cardiomyocytes on days 12-26 post differentiation, we are developing methods to enable high resolution 3D live imaging of the differentiation process starting from pluripotent hiPSCs through mesoderm induction to cardiomyocytes; the goal is to better understand the changes in organization and dynamics of the major cellular structures during this differentiation. In parallel to live imaging, we have initiated single cell transcriptomics analysis of cardiomyocytes to explore the heterogeneity within and across cell populations derived from various differentiation conditions and time points. We will present our initial scRNAseq analysis indicating the presence of various sub-populations of cardiomyocytes. We will expand this analysis with additional time points to identify the key molecules and pathways that drive state/cell type decisions during the differentiation process using live imaging and FISH. Using this dual approach of single cell genomics and live cell imaging, we plan to build integrated and predictive models of stem cell states ranging from pluripotency to differentiation.

W-3033

GENERATION OF EN1 POSITIVE RHOMBOMERE 1 SPECIFIC RADIAL GLIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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It has been hypothesized that engrafting oligodendrocyte progenitor cells (OPCs) contributing to myelinating oligodendrocyte could recover the demyelinating diseases such as multiple sclerosis; however, conventional strategies to generate oligodendrocytes have been mainly focused on direct differentiation into forebrain and spinal cord specific oligodendrocytes. With advantages of recently established culture system, we generated long-term expandable rhombomere 1 specific En-1 positive Radial glial cells. Radial glial cells exhibited

a rosette like structure and expressed FABP7 (also known as BLBP), Sox9 as well as Sox1, Sox2, and Nestin. Furthermore, the radial glial cells can be propagated as a highly homogenous population with no evidence of chromosomal instability. Notably, during spontaneous differentiation, the radial glial cells mostly differentiate into GABAergic neurons whereas astrocytes and oligodendrocytes differentiation was only observed by sphere formation. Importantly, differentiated neurons, astrocytes, and oligodendrocytes co-expressed En-1 following GABA, GFAP, and MBP, respectively. In conclusion, we identified that En-1 positive radial glial cells can differentiate into neurons, astrocytes, and oligodendrocytes, while retaining En-1 expression, allowing to find the route or mechanism of nervous system development in human hindbrain.

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W-3035

STK40 FUNCTIONS AS A NOVEL REGULATOR OF SKELETAL MUSCLE DIFFERENTIATION AND MESODERM DIFFERENTIATION

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Skeletal muscle differentiation and mesoderm differentiation are precisely coordinated process and the molecular mechanism regulating the process remains incompletely understood. Here, we report the identification of serine/threonine kinase 40 (Stk40) as a novel positive regulator of skeletal myoblast differentiation in culture and fetal skeletal muscle formation in vivo. We show that the expression level of Stk40 increases during skeletal muscle differentiation. Down-regulation and overexpression of Stk40 significantly decreases and increases myogenic differentiation of C2C12 myoblasts, respectively. In vivo, the number of myofibers and expression levels of myogenic markers are reduced in the fetal muscle of Stk40 knockout mice, indicating impaired fetal skeletal muscle formation. Mechanistically, Stk40 controls the protein level of histone deacetylase 5 (HDAC5) to maintain transcriptional activities of myocyte enhancer factor 2 (MEF2), a family of transcriptional factor important for skeletal myogenesis. Together, our study reveals that Stk40 is required for fetal skeletal muscle development and provides molecular insights into the control of the HDAC5-MEF2 axis in skeletal myogenesis. Besides, deletion of Stk40 enhances of the migration and proliferation of mesoderm cells, represses the mesoderm related markers and the Brachyury-GFP cells. This indicates that Stk40 is also required for the mesoderm differentiation.

PLURIPOTENT STEM CELL: DISEASE MODELING

W-3037

DISEASE MODELING USING INDUCED PLURIPOTENT STEM CELLS OF PATIENTS WITH SYSTEMIC SCLEROSIS

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Systemic sclerosis (SSc) is a very rare and intractable autoimmune disease. SSc patients suffer from progressive skin thickening and fatal systemic complications. However, SSc population is too small to study pathophysiology or develop medicine for cure. Shortage of patient number and patients-derived biomaterials prevents scientists from investigating SSc delicately. One of strategy for overcoming these limitations, induced pluripotent stem cells (iPSCs) emerges as a promising alternative. Patient-derived iPSC is spotlighted for their potential to recapitulate disease phenotypes in a dish. Here, we generated iPSC lines from 6 SSc patients and 3 healthy controls (HC). Keratinocytes and fibroblasts are main cell component of epidermis and dermis, respectively. SSc-iPSCs were differentiated into skin component cells including SSc-iPSC-derived keratinocytes (SiK) and fibroblasts (SiF). SiK and SiF proliferated more actively than HC. Three dimensional co-culture of SiK and SiF formed SSc-iPSC skin organoid. Skin layers of SSc skin organoid were much thicker than HC. The expression level of extracellular matrix-collagen and alpha smooth actins was more increased in SSc than HC. NOD/SCID mice which engrafted with skin organoid mimics thickened skin phenotype of SSc patients. In this study, we revealed that SiK, SiF, and SSc skin organoid revealed scleroderma-like disease phenotype in a dish and xenograft model. SSc-iPSC-derived fibroblast was used in a high-throughput platform to novel drug screening. More than 800 FDA approved drugs were tested for their competence against fibrotic proliferation. At initial screening, 48 drugs showed effective suppression of SiF proliferation. Revalidation test with ELISA revealed 13 drug were really efficient for reduce proliferation of SiF and synthesis of ECM protein. Interestingly, raloxifene which is a well-known anti-osteoporotic drug showed a potent anti-fibrotic effect. Raloxifene is very safe drug and here was first known to inhibit fibrosis of SSc. In conclusion, iPSC-based SSc platform was useful for disease modeling, drug screening platform, and drug repositioning.

W-3041

MODELING ALZHEIMER'S DISEASE PATHOPHYSIOLOGY USING ISOGENIC HUMAN APOE IPSC-DERIVED NEURAL CELLS

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The apolipoprotein ε4 allele (apoE4) is a well-established genetic risk factor for late onset Alzheimer's disease (AD), while the apoE2 allele provides protection against AD development. However, the exact molecular mechanism by which apoE plays its role in AD pathogenesis remains largely unknown. Aiming to investigate the role of apoE in AD, isogenic lines for its different isoforms were generated from four human induced pluripotent stem cell (iPSC) donors within the framework of the IMI2 project ADAPTED. The lines from the first donor were used to derive cortical progenitor cells that can be differentiated into cortical neurons and astrocytes. After characterization of these cells, they were further utilized to study phenotypic differences induced by the various apoE isoforms. It had been previously reported that human apoE might play a role in the inflammatory response of cells. Since inflammation and neurodegeneration are closely linked, we aimed at analyzing the role of apoE in modulating the inflammatory response of isogenic astrocytes. Indeed, stimulating these astrocytes with a cytokine cocktail triggered a differential inflammatory response depending on the apoE genotype. ApoE4 carrying astrocytes displayed stronger pro-inflammatory responses compared to apoE3 cells indicating a potential role for neuro-inflammation in AD. In this study the analysis of these differences was extended to additional apoE genotypes, like apoE2/2 and apoE3/4. ApoE has also been reported to influence the complexity of neuronal networks denoted by the neuron's ability to grow and extend neurites. However, this has not been tested in human-derived cells. Therefore, we investigated the impact of the various apoE isoforms on the differentiation and maturation of cortical neurons under basal conditions and in the presence of various stressors. Since apoE4 is a significant factor in the development of late onset AD, we believe that our mechanistic studies help understand its role in the disease course, and ultimately bring us closer to developing impactful treatments.

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W-3043

INDUCED PLURIPOTENT STEM CELLS FROM AZOOSPERMIA PATIENT

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Non-obstructive azoospermia (NOA) is the most severe form of male infertility, which is defined as zerosperm count in the ejaculates due to failure of spermatogenesis. Induced pluripotent stem cells (iPSCs) are patient-specific and have been reported to be capable to differentiate into a wide spectrum of cell types including gamete cells. Here we worked to generate iPSCs from NOA patients as these cells may serve as translational tools to investigate the impaired spermatogenesis process in these patients. Peripheral blood mononuclear cells (PBMC) were harvested from two patients with azoospermia (AZ) in the IVF clinic at Mackay Memorial Hospital (Taipei, Taiwan). This work was approved by Institutional Review Boards (IRB) at Mackay Memorial Hospital. PBMCs were infected by Sendai virus carries reprogramming factors (OCT4, SOX2, KLF4, and hc-Myc). After 14-18 days of reprogramming, the colonies formed and were subcloned to several lines. These iPSCs from AZ patients (AZiPSCs) showed typical characteristics of pluripotency, such as alkaline phosphatase activity, expression of TRA-1-60 and SSEA4. Embryoid body (EB) assay showed that these AZiPSCs are capable of differentiating into all three germ layers in vitro. The present work reports successful derivation of patient-specific iPSCs from NOA patients. Further genetic screening and germ cell differentiation will reveal the undefined factors for human spermatogenesis. Our results may provide new resources for treating infertility patients and regenerative medicine.

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W-3045

DETAILED CHARACTERISATION OF IPSC LINES AND iPSC DERIVED MOTOR NEURONS HARBOURING A 1.35 MB COMPLEX INSERTION

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Family-54 is a large Australian family with an autosomal dominant form of distal hereditary motor neuropathy (DHMN1: OMIM %182960) - a group of length-dependent neurodegenerative disorders affecting the lower motor neurons leading to chronic disability. We recently reported a novel 1.35 Mb complex insertion within the DHMN1 locus on chromosome 7q34-q36 after having excluded all coding mutations in the linkage region. We hypothesise the DHMN1 insertion is likely to cause disease by dysregulating the expression of one or more nearby genes. Studying gene dysregulation in peripheral nerve disease is challenging as the relevant tissues (spinal cord and peripheral nerve) are not easily accessible from living patients. Therefore, alternative strategies are needed to elucidate the disease mechanisms and pathways involved in peripheral nerve degeneration. To address this problem, we have outsourced reprogramming of fibroblasts from patients harbouring the DHMN1 complex insertion into induced pluripotent stem cell (iPSC) lines (Cellular Dynamics International). The DHMN1 iPSC lines were shown to maintain the rearrangement post-reprogramming and pluripotency was confirmed using a combination of immunofluourescence, Western blot and qRT-PCR analyses. DHMN1 iPSC lines were differentiated into motor neurons (iPSCDHMN1). Motor neuron identity was confirmed by staining with NF68, TUJ1 as well as pan-neuronal markers ISLET1 and HB9. Using the iPSCDHMN1 model, we will perform RNA-seq and qPCR validation experiments to compare differential gene expression between patients and controls to identify disease-relevant gene dysregulation in neural tissue. This model will shed light on the pathogenic mechanisms underlying the DHMN1 insertion and provide useful insights of pathways leading to peripheral nerve degeneration which is essential for the development of treatment therapies.

Funding Source: Pamela Jeanne Elizabeth Churm Postgraduate Research Scholarship (Sydney Medical School Foundation, University of Sydney).

W-3047

DEVELOPMENT OF A NOVEL HUMANIZED MOUSE MODEL OF NEUROINFLAMMATION

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Microglia play a critical role in the development and progression of many brain disorders including Alzheimer's disease (AD) and the majority of AD risk genes are highly expressed by microglia. Recently, we developed a fully-defined protocol to efficiently generate human microglia from induced pluripotent stem cells (iPSCs) to study these AD risk genes. Our two-step approach involves the differentiation of iPSCs in to CD43+ primitive hematopoietic progenitors (HPCs) and then exposure of these HPCs to growth factors and cytokines implicated in the differentiation and maturation of microglia within the brain. Although iPSC-microglia represent a useful approach to study human microglia in vitro, it is important to also be able to study the interactions between microglia, neuropathology, and other brain cells in vivo. Here we describe the development of a novel chimeric mouse model of neuroinflammation in which robust engraftment of human microglia can be achieved by transplanting iPSC-derived HPCs into the brains of P1 MITRG mice. An important component of this model is the expression of humanized CSF1 which we find is necessary for human microglial survival. Newborn MIRTG pups received intraventricular and cortical injections of human CD43+ HPCs generated from green fluorescent protein (GFP)-expressing iPSCs and brains were collected two months later. Immunoflourescent and confocal analysis demonstrated robust engraftment of GFP expressing donor-derived cells throughout injected mice brain. Furthermore, human HPCs differentiated in vivo into the three yolk-sac derived CNS myeloid lineages: microglia, perivascular macrophages, and meningeal macrophages as confirmed by specific markers and morphological features. GFP expressing human cells were then sorted by flow cytometry and mRNA isolated for RNA-sequencing analysis to determine whether the transcriptome of in vivo matured iPS-microglia is similar to that of human brain-derived microglia. In ongoing studies we will now utilize this model to examine the effect of peripheral LPS stimulation on human microglia in vivo.

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W-3049

MODELLING OBESITY WITH HUMAN STEM CELL-DERIVED POMC HYPOTHALAMIC NEURONS

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Obesity is now a global epidemic after exponentially increasing over the past few decades in the United States. The onset and progression of obesity is heavily influenced by several brain circuits, one of which is the hypothalamic-pituitary axis. The hypothalamus regulates metabolism and energy expenditure, and harbors two types of neurons identified to play a crucial role in controlling appetite. These are the anorexigenic Proopiomelanocortin (POMC) neurons, and the orexigenic Agouti-related protein (AgRP)/neuropeptide Y (NPY) neurons. The activity of these hypothalamic cells regulates appetite suppression and stimulation respectively. Activation of POMC neurons suppresses appetite, leading to reduced food intake and decreased levels of obesity. For this reason, murine POMC knockout models display severe obesity. To date it has been intractable to study human hypothalamic neurons, but human induced pluripotent stem cells (iPSCs) provide a tool to study this tissue. Here we report a human in vitro model of POMC neurons by efficient, pure and scalable differentiation. Using CRISPR/Cas9 genome editing we have generated a POMC-GFP reporter cell line, allowing us to FACS-purify POMC-positive neurons and maintain them in culture for downstream assays. Our 50-day differentiation protocol produces mature POMC hypothalamic neurons, which upon further purification can be nearly homogenous for POMC expression. Our studies include leptin- and insulin-response assays, as well as neurophysiological characterization of these cells. Leveraging genome editing we have created leptin receptor knockout POMC neurons, and will use single-cell RNA-Seq to assay their response to insulin and leptin. This will be the first time human-specific data is generated on POMC neuron response to leptin and insulin. This discovery effort will investigate the insulin and leptin pathways in hypothalamic neurons, shedding light on possible mechanisms of obesity. We believe our model presents an ideal opportunity for dissecting regulatory pathways involved in feeding mechanisms, allowing us to understand the molecular basis of appetite regulation in humans. The model we have created here can be an avenue for the discovery of novel therapeutic targets to treat and cure obesity.

W-3051

A PATIENT IPSC DERIVED KIDNEY ORGANOID DISEASE MODEL OF NPHP FUNCTIONALLY VALIDATES A CILIARY PHENOTYPE.

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Up to 50% of patients with genetic renal disease remain undiagnosed following genomic sequencing. Novel genomic variants of unknown significance (VUS) require validation by functional genomic platforms, which have previously included animal models and human, non-renal, two-dimensional, cellular models. Kidney organoids derived from patient induced pluripotent stem cells (IPSC) represent a previously unreported, complex multicellular, three-dimensional, patient derived tissue for renal disease modelling. By examining kidney organoids derived from a patient with a previously validated genotype, this 'proof of concept' study demonstrates the ability of kidney organoids to express a ciliopathic disease phenotype. An eleven-year-old girl with nephronophthisis and retinitis pigmentosa was diagnosed with compound heterozygous mutations in IFT140 by trio whole exome sequencing. Simultaneous reprogramming and CRISPR/Cas9 gene-editing of patient fibroblasts yielded uncorrected patient and isogenic, gene-corrected IPSC. Both IPSC

lines were simultaneously differentiated to kidney organoids. Tubules in patient organoids demonstrated shortened, club shaped primary cilia whilst tubules in gene corrected organoids rescued this phenotype. Differential expression analysis of epithelial cell fractions isolated by magnetic cell sorting from patient and gene-corrected organoids demonstrated downregulation of genes involved in apicobasal polarity, cell-cell junction and dynein motor assembly pathways. In Matrigel cyst culture, patient epithelial cells were less able to form polarised spheroids than gene-corrected controls. This study, presented from bedside to bench, validates the combined use of patient IPSC-derived kidney organoids and gene correction technologies as a novel, faithful and patient-specific approach for the validation of novel genomic VUS as well as for the further study of inherited renal disease within regenerated, human, in vitro tissue.

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W-3053

USING A CRISPR-CAS9 GENETICALLY MODIFIED ABCA7 KNOCKOUT HUMAN IPSC LINE TO MODEL LATE ONSET ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) affects more than 44 million people and is the 7th leading cause of death worldwide. Late onset Alzheimer's disease (LOAD), which affects 95% of AD patients, is understudied in comparison to early onset AD. This is largely due to the fact that in addition to genetics, environment and aging play a role in LOAD making it difficult to study. Present technology has allowed for the conduction of large genome wide association studies (GWAS) to identify late-onset Alzheimer's disease risk variants over ethnically diverse populations. One specific gene identified, ATP binding cassette transporter subfamily A member 7 (ABCA7), is expressed in neurons and in neuronal support cells, which are often overlooked in neurodegenerative diseases. This study aims to unravel the cellular processes that may be altered in variant ABCA7 cell types and how they play a contributing role in LOAD. Most of the ABCA7 LOAD risk variants identified result in loss of function, which is why we used CRISPR/CAS9 technology to generate an ABCA7 knockout human iPSC line, as a means to further model and investigate LOAD. Single stranded guide RNAs were selected to target exon 4 of the ABCA7 gene. The targeting oligomers were then annealed and inserted into a pSpCas9 plasmid, with a GFP reporter. The plasmid was nucleofected into cells and transfected. GFP+ cells were FACS sorted. Individual cells were plated at a single cell density to allow for the creation of isogenic colonies. The colonies were picked and placed in a 96 well plate. Cell lines were expanded, DNA was isolated, PCR amplified, and sent for sequencing. Sequence analysis identified multiple cell lines that appeared to have a genome edit that would cause an early stop codon. Currently, we have 18 clones with edits in ABCA7 that we are interested in and we are validating the lines to confirm the genotype. Future studies will differentiate the generated ABCA7 knockout iPSC line into neuronal cell types, which will allow for the investigation of the effect ABCA7 LOAD risk variants have on astrocytes, microglia, and neurons. This study aims to illustrate a model system that can be used to validate a multitude of other LOAD risk variants. Due to the impending number of AD reported cases and deaths worldwide, it is of urgency to take advantage of a human model system to advance our knowledge of LOAD.

Funding Source: California Institute for Regenerative Medicine (CIRM).

W-3055

A HUMAN IPSC-BASED VASCULAR MODEL TO STUDY THE HDAC9 GENETIC VARIANT ASSOCIATED WITH LARGE-VESSEL STROKE

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A common variant in the Histone Deacetylase 9 (HDAC9) gene has been identified in GWAS studies as the strongest genetic risk for large-vessel stroke to date and further associated with promoting carotid atherosclerosis. The mechanism linking HDAC9 variant with increase risk of stroke is still unknown due to the lack of suitable models. Since HADC9 levels were found up-regulated in carotid plaques, we suggest that the HADC9 stroke-associated variant may affect gene expression and this may promote vessel damage, which contributes to the pathophysiology of ischaemic stroke. To test this hypothesis, we have developed a vascular model using human induced pluripotent stem cells (iPSC), by differentiating iPSC carrying the HDAC9 risk variant into vascular smooth muscle cells (SMC) of neural crest origin, which mimic the blood vessel of the brain, using a well-defined protocol. Increased expression of HDAC9 was observed in the iPSC line with the HDAC9 stroke-associated variant compared to the WT iPSC used as control. Vascular SMC, differentiated from the stroke risk iPSC line, also expressed high levels of HDAC9. Moreover, proliferative and apoptotic studies showed that these SMC have lower proliferative rate and increased apoptosis levels compared to WT iPSC-derived SMC. Finally, the use of sodium valproate, which

is known to inhibit HDAC activity, was able to reduce the cell death rate of SMC harbouring the HDAC9 risk variant. Our iPSC-based vascular model has showed that the stroke-associated variant in HDAC9 is likely to cause the up-regulation of HDAC9 gene expression, and this in turn could affect the proliferative activity and apoptotic rate of SMC. This indicates that our human iPSC-derived SMC model offers a powerful tool to study how HADC9 stroke-associated variant affects the vascular SMC phenotype, with the aim to identify new pathways/targets for therapeutic development and test existing compounds as well as newly developed drugs in a human system.

Funding Source: British Heart Foundation (BHF); Stroke Association; Rosetrees Trust.

W-3057

A SCALED FRAMEWORK FOR CRISPR EDITING OF HUMAN PLURIPOTENT STEM CELLS TO STUDY PSYCHIATRIC DISEASE

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Scaling of CRISPR-Cas9 technology in human pluripotent stem cells (hPSCs) represents an important step for modeling complex disease and developing drug screens in human cells. However, variables impacting scaling efficiency of gene editing in hPSCs remain poorly understood. Here, we report a standardized CRISPR-Cas9 approach, with robust benchmarking at each step, to successfully target and genotype a set of psychiatric disease-implicated genes in hPSCs and provide a resource of edited hPSC lines for six of these genes. We found that transcriptional state and nucleosome positioning around targeted loci was not correlated with editing efficiency. However, editing frequencies varied between different hPSC lines and correlated with genomic stability, underscoring the need for careful cell line selection and unbiased assessments of genomic integrity. Together, our step-by-step quantification and in-depth analysis provides an experimental roadmap for scaling CRISPR editing in hPSCs to study psychiatric disease, with broader applicability for other polygenic diseases.

Funding Source: Stanley Center for Psychiatric Research.

W-3059

INVESTIGATING MECHANISMS UNDERLYING ENDOTHELIAL CELL (VASCULAR CELLS) DYSFUNCTIONS THAT COULD LEAD TO STROKE-LIKE EPISODES IN MELAS

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Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a mitochondrial disorder that is characterised by a myriad of multi-organ symptoms such as seizures, headaches, lactic academia, dementia, aphasia, diabetes myopathy and muscle weakness. One of the causal genetic mutations of MELAS is the m.3243A>G mutation in the MT-TL1 gene encoding the mitochondrial tRNA(Leu(UUR)) leading to stifled mitochondrial protein synthesis, ultimately resulting in impaired mitochondria energy metabolism. Early clinical data has already reported that MELAS individuals have altered cerebral vasculature with clinical studies demonstrating the presence of cerebral infarcts and even stroke-like lesions, which are similar to ischemic stroke. However, the underlying metabolic dysfunctions caused by mitochondrial defects resulted in the observed vascular pathologies remains unclear. Using iPSCs derived from a MELAS patient, we generated CD31+ endothelial cells (ECs) and SMA+ SMMHC+ smooth muscle cells (SMCs) to model the vascular pathologies in MELAS syndrome and to investigate the underlying vascular perturbations that led to disrupted blood reperfusion and angiopathy in these patients. Herein, we present novel data illustrating that MELAS ECs exhibited altered EC functionalities and displayed altered inflammatory response in the presence of inflammatory stimuli, LDL metabolism and LDL-dependent inflammation. MELAS neuroectoderm-SMCs failed to differentiate in vitro and MELAS SMCs also displayed molecular perturbations. Collectively, our data suggests that the underlying mitochondrial disorder in MELAS patients is associated with clear dysfunctions in vascular cell types that could potentially address the pathogenesis of stroke-like episodes in these patients.

W-3061

ATOH1 AND RFX TRANSCRIPTION FACTORS FACILITATES THE DIFFERENTIATION AND CHARACTERIZATION OF INNER EAR HAIR CELL-LIKE CELLS FROM PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Degeneration or loss of inner ear hair cells (HCs) is irreversible and results in sensorineural hearing loss (SHL). Human induced pluripotent stem cells (hiPSCs) have been employed in disease modelling and cell therapy. Most studies on the differentiation of HC-like cells from pluripotent stem cells have used chicken utricle stromal cells which may cause problems for subsequent examination of the induced HC-like cells. Herein, we propose a transcription factor (TF)-driven approach using regulatory factor of x-box (RFX) and ATOH1 TF genes to differentiate HC-like cells from hiPSCs. Our results suggested that ATOH1/RFX1/RFX3 could significantly increase the differentiation capacity of iPSCs into mCherryMYO7A(+) cells, upregulate the mRNA expression levels of HC marker genes

(MYO7A, and ESPN) and promote the differentiated HCs with more mature stereociliary bundles on the cell surface than ATOH1 alone on Day 42. For modelling of molecular and stereociliary structural changes involved in HC dysfunction of SHL, we further used ATOH1/RFX1/RFX3 to differentiate HC-like cells from the iPSCs of patients with myoclonus epilepsy associated with ragged-red fibres (MERRF) syndrome, which is caused by mitochondrial DNA A8344G mutation and characterised by myoclonus epilepsy, ataxia, and SHL. Compared with isogenic iPSCs, MERRF-iPSCs possessed about 42-44% mtDNA A8344G mutation and exhibited significantly elevated reactive oxygen species (ROS) production, CAT gene expression, and differentiated morphology. Furthermore, MERRF-iPSCs-differentiated HC-like cells (MERRF-HC-like cells) also exhibited significantly elevated ROS levels and MnSOD and CAT gene expression. MERRF-HC-like cells that had more single cilia with a shorter length could be observed only by using the non-TF method, but those with fewer stereociliary bundle-like protrusions than isogenic iPSCs-differentiated-HC-like cells could be further observed using ATOH1/RFX1/RFX3 TFs. In conclusion, our ATOH1/RFX1/RFX3 TF-driven approach to the differentiation of HC-like cells from iPSCs is efficient and promising for disease modelling of SHL and can be employed in future therapeutic strategies against SHL.

Funding Source: Ministry of Science and Technology, Taiwan.

W-3063

MODELING OF CARDIAC FIBROSIS FOR DRUG SCREENING USING SIMULATED EXTRACELLULAR MATRIX IN HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVED CARDIAC CONSTRUCT

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Cardiac fibrosis is one of major etiologies in heart failure and development in new medications targeted to the cardiac fibrosis is crucial for the treatment of heart failure. However assay system for discovery in new medications targeted fibrosis mainly depend on conventional assay system using heart failure animal model in which some drawbacks such as high cost and prevention of cruelty to the animal exist and new in vitro assay system using human cells should be developed for effective drug screening. We hypothesize cardiac tissue using human induced pluripotent stem cell derived cardiomyotes (hiPSCs-CMs) may provide the useful tools for in vitro drug screening targeted to cardiac fibrosis. Cardiomyogenic differentiation was induced in hiPSCs to produce cardiac tissue like structure (CTS) including iPSC-CMs and fibroblasts. After stimulation to CTS by pro-fibrotic protein such as TGF-β, gene expression of Extracellular matrix (ECM; Collagen type I, Collagen type III, Fibronectin), proteases (MMP-2, MMP-13), and pro-fibrotic cytokine (CTGF; connective tissue growth factor) was significantly increased as assessed by quantitative PCR. In addition, treatment of TGF-β decrease the cardiac contractile/relaxation velocity (without vs with TGF-β; 27.436.1 vs. 20.934.3, 8.433.0 vs. 3.931.5μm/sec, respectively; $P < 0.05$) and contractile/relaxation force (2.430.6 vs. 1.530.4, 1.430.5 vs. 0.630.3μm, respectively; $P < 0.05$), as assessed by image-based motion analysis. Furthermore, we analyzed whether CTS could response to anti-fibrotic factor, HGF. As a result, treatment of HGF decreased the gene expression of ECM and increased the contractile/relaxation property. Finally, we generated the different ratio (50, 60, 70 and 80%) of cardiomycoytes in CTS to compare sensitivity to pro-fibrotic stimulation. As a result, the increase of the gene expression of fibrotic marker was more abundant in CTS containing 50 to 70% cardiomyocytes. We concluded that CTS generated by iPSC-CM and fibroblast response to both pro- and anti- fibrotic stimulation and have a possibility to propose simulated cardiac ECM in vitro, suggesting that this system may provide the useful tool for anti-fibrotic drug screening.

W-3065

IMPAIRED OSTEOGENESIS OF COSTELLO SYNDROME IPSCS-DERIVED MESENCHYMAL STEM CELLS

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Costello syndrome (CS) is caused by HRAS mutations in RAS/MAPK signaling pathway. Approximately 90% of CS patients have bone abnormalities such as craniofacial malformation, kyphoscoliosis, osteoporosis, and short stature. However, molecular mechanisms about how HRAS mutation accounts for aberrant bone development are poorly understood. iPSCs derived from fibroblasts of a CS patient (CS-iPSCs) normally differentiated into mesenchymal stem cells (MSCs) that expressed MSC-positive markers such as CD73+, CD90+, and CD105+. Intriguingly, CS-MSCs exhibited decreased alkaline phosphatase (ALP) activity and mineralization during osteogenic differentiation. Hyperactive HRAS was observed in CS-MSCs and CS-osteoblasts, and resulted in elevated p-ERK activity. Treatment with a farnesyl transferase inhibitor led to increments of ALP activity and mineralization in CS-osteoblasts. The results demonstrate that hyperactive HRAS cause impaired osteogenesis in CS-MSCs.

REPROGRAMMING

W-3067

RAPID CHANGES IN THE EPIGENETIC LANDSCAPE FACILITATED BY MEF2C UNDERLIE 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 and occurs through largely unknown underlying mechanisms. To understand the explicit transcriptional dynamics associated with direct cardiac reprogramming from a fibroblast to a cardiomyocyte driven by Gata4, Mef2c and Tbx5 (GMT), we evaluated RNA expression dynamics using single-cell RNA sequencing (scRNA-seq). 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 inhibition of TGF and WNT signaling limits acquisition of an alternative cell fate characterized by an immune cell type signature. Examination of chromatin accessibility at similar time points revealed widespread changes in the epigenetic landscape that correlated with the expression dynamics at early time points. In accordance with our scRNA-seq data, the presence of TGF and WNT inhibitors during reprogramming was associated with a reduction in chromatin accessibility at regulatory elements associated with the immune system, providing evidence that epigenetic remodeling facilitates repression of pathways that can lead to the improper acquisition of alternative cell fates in our system. A random-forest classifier built on the observed gene expression dynamics predicted Mef2c and Tbx5 were robust drivers of this cell fate conversion process. DNA binding profiles generated by ChIP-sequencing within 48 hours revealed isolated Mef2c binding sites were associated with regions with increased chromatin accessibility while changes in chromatin at Gata4 and Tbx5 bound sites required binding of at least one additional reprogramming factor. Interestingly, unlike during embryonic cardiac development, GMT was infrequently bound together, confirming the predictions of the classifier that suggests Mef2c works independently early during reprogramming, while Tbx5 is associated with the Smad2 repressor Tgif1. Collectively, these results reveal novel mechanisms by which transcription factor function can be exploited to induce new cellular identities for therapeutic purposes.

W-3069

INTEGRATIVE MOLECULAR ANALYSES REVEAL DISTINCT REPROGRAMMING TRAJECTORIES INTO STATES OF NAIVE AND PRIMED HUMAN INDUCED PLURIPOTENCY?

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In 2006 Shinya Yamanaka demonstrated that mature somatic cells can be reverted back to a pluripotent state by the forced expression of four transcription factors; Oct-4, Sox2, Klf4 and c-Myc (OSKM). These so called induced pluripotent stem cells (iPSCs), like embryonic stem cells (ESCs), can give rise to any cell types of the body, however, they are free of the ethical constraints surrounding the generation of ESCs. Furthermore, iPSCs carry the promise of personalized regenerative medicine and hold tremendous potential for applications such as cell replacements therapeutics, disease modelling and in vitro drug screening. In order for this reprogramming technology to fulfil this potentials, the basic mechanisms of nuclear reprogramming needs to be fully understood to further improve the related technologies. Here we investigate the molecular events underlying the reprogramming of human fibroblasts into naïve and primed pluripotent states. We show that the human reprogramming pathways (a 4-week process) can be broken up into a discreet series of cellular transitions. Furthermore, we observe distinct molecular trajectories of direct reprogramming into naïve and primed pluripotent states and further characterisation of intermediate population revealed specific molecular signatures associated with the respective pluripotent states. Altogether, our study unveils the molecular roadmaps for direct reprogramming into naïve and primed pluripotent states and provides crucial insights for future studies aimed at optimising implementations of iPSC technology and in turn accelerate its therapeutic translation.

W-3071

A HIGH-EFFICIENT REPROGRAMMING METHOD OF HUMAN SOMATIC CELLS BY SENDAI VIRUSES

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Somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs) by introduction of 4 factor genes, OCT3/4, SOX2, KLF4 and c-MYC. Although several reprogramming methods have been reported using retrovirus, lentivirus, and episomal vectors, the efficiency of reprogramming in human cells is quite low. It is already known that various types of cells exist during reprogramming stage, but this cell heterogeneity is a serious obstacle for the understanding of cellular reprogramming mechanism. In this research, we tried to increase the reprogramming efficiency using Sendai virus with different combination of three mediums (DMEM with 10% FBS, StemFit AK03N and StemFit AK03N without basic fibroblast growth factor (bFGF)). To check the reprogramming efficiency, the ratio of TRA-1-60, human pluripotent cell marker, was tracked at day 7, 11, and 16 after introduction of Sendai viruses and the number of iPSC-like colonies was counted at day 16. Finally, we found an optimal reprogramming method of human dermal fibroblast cells by introduction of 4 factors with AK03N-bFGF, and changed medium to AK03N at day 7. Previous reprogramming method using episomal vectors needs at least 20 days to appear iPSC-like colonies, but our novel method needs 4 to 7 days to get iPSC-like colonies. We confirmed the expression of Nanog, early marker for reprogramming, at day4. Moreover, percentages of TRA-1-60 expressing cells were increased up to 40% and 90% at day 7 and 11, respectively. In view of the results, we could develop the novel and highly efficient method for reprogramming using the combination of Sendai viruses and StemFit AK03N, and hypothesize that bFGF accelerates the induction of iPS-like colony formation.

W-3073

FUNCTIONAL ROLES OF SIRTUINS AND THEIR DOWNSTREAM TARGETS ON METABOLIC REPROGRAMMING DURING HUMAN INDUCED PLURIPOTENCY

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Metabolic reprogramming (known as "Warburg effect") from OXPHOS toward glycolysis is a hallmark of cancer cells as well as pluripotent stem cells. We recently found that SIRT1 upregulation and SIRT2 downregulation is a molecular signature of human pluripotent stem cells. In addition, we showed that the miR-200c-SIRT2 axis play an important role for metabolic switch from OXPHOS to glycolysis by controlling the acetylation levels of many glycolytic enzymes. To further understand molecular mechanisms underlying metabolic reprogramming, we investigated specific roles that are coordinated by SIRT1 and other sirtuins and their potential downstream targets and pathways. In addition, we explored how various microRNAs can regulate sirtuins, leading to metabolic reprogramming and control pluripotent stem cell fate and function. We will discuss our novel findings regarding the potential functional roles of sirtuins and microRNAs as regulators of metabolic reprogramming during human induced pluripotency and pluripotent stem cell function.

W-3075

GSK3B-INDEPENDENT WNT ACTIVATION IMPELS TO RAPID AND EFFICIENT GENERATION OF INDUCED PLURIPOTENT STEM CELLS

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The Wnt signaling pathway is well known for maintaining self-renewal of pluripotent stem cells (PSCs) and usually activated by inhibitors of Gsk3β to enhance reprogramming in both mouse and human. However, considering Gsk3β is also involved in various signaling such as Insulin, Receptor Tyrosine Kinases, and Hedgehog signaling, Gsk3β inhibition for Wnt activation may cause unexpected or complicated phenomena. To understand the precise mechanistic basis, we focused on the modulation of Wnt signaling using different Wnt activating small molecules to separate Wnt activation from Gsk3β inhibition. Here, we showed that the S compound (SC), a Gsk3β-independent Wnt activator through disturbing the interaction between β-Catenin and Axin, a component of Wnt destruction complex, significantly enhances the kinetics of reprogramming which resulted in rapid formation of intact iPSC colonies around day six which is two times faster than control. We found SC stabilized β-Catenin and up-regulated pluripotency gene expression promptly. Interestingly, there were no granular intermediate cells during reprogramming. We found that this rapid process resulted from active Gsk3β which decomposes Snail then promotes MET process. Of note, CHIR99021, a Wnt agonist which inhibits Gsk3β did not recapitulate this acceleration of SC treatment. Global gene expression analysis revealed that SC also activated Mapk/Erk signaling which is necessary for efficient iPSCs reprogramming through control of mitochondrial fission. These results indicate that SC-mediated Gsk3β independent Wnt activation as well as induction of MET and Erk signaling synergistically improve iPSC reprogramming. We finally confirmed that the SC promoted the reprogramming in human. We also questioned SC-effect on self-renewal of PSCs. However, SC could not replace Gsk3β inhibitor, CHIR99021. Based on our results, we concluded that Wnt activation without Gsk3β inhibition guides to a more faster route to reach the pluripotent state during reprogramming as well as Wnt activation with Gsk3β inhibition is mainly beneficial to support self-renewal of PSCs.

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W-3077

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM INSULIN RESISTANT PATIENTS

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Insulin resistance (IR) is an accelerating factor for the greatest health challenge facing the world today, Type 2 diabetes (T2D). Psoriasis, is an immune-mediated, long-term inflammatory skin disorder characterized by its strong genetic predisposition. The patients with psoriasis for more than two years are found to be more susceptible to developing IR and diabetes. However no studies reported the relationship between IR, T2D, and epidermal dysfunction using induced pluripotent stem cells (iPSCs). Our aim in this study was to generate patient-specific hiPSCs from the peripheral blood mononuclear cells (PBMCs) of IR Qatari patients (associated with psoriasis or T2D) and from healthy individuals to differentiate them into insulin target cells. PBMCs were reprogrammed through forced expression of the pluripotency factors; OCT4, SOX2, C-MYC, and KLF4 using non-integrating Sendai viral vectors. About 20 days following transduction, the healthy undifferentiated colonies of defined border and high nuclear to cytoplasmic ratio were manually picked up for expansion and maintenance. The newly generated hiPSC colonies were extensively examined for the pluripotency characteristics using several techniques including RT-PCR, immunostaining, western blotting, alkaline phosphatase assay, embryoid body (EB) formation, karyotyping, and hPSC Score-Card (TaqMan) assay. The human embryonic stem cell (H1-hESC) line was used as a positive control. All hiPSC lines showed typical hESC morphology, normal karyotype and stained positive for alkaline phosphatase activity. The hiPSC clones highly expressed the pluripotency markers, such

as OCT4, SOX2, NANOG, KLF4, C-MYC, SSEA4, TRA-60, TRA-81, REX-1, DPPA4, and TERT at mRNA and protein levels. Our generated colonies are able to differentiate spontaneously and directly into endodermal (SOX17+/FOXA2+), mesodermal (BRACHYURY+) and ectodermal (NESTIN+) lineages. This multi-lineage differentiation potential was validated by the hPSC Score-Card assay in vitro. We will differentiate these fully reprogrammed hiPSCs into insulin target cells to identify signaling pathways involved in the development of inherited form of IR and to understand the genetic link between IR, T2D, and psoriasis.

W-3079

OCT4 ENHANCES THE NEUROGENIC POTENTIAL OF HUMAN DENTAL PULP STEM CELLS

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Human dental pulp stem cells (DPSC) harvested from the pulp tissue of adult human teeth are multipotent stem cells that possess neurogenic potential. They exert positive effects in the injured or diseased brain via a number of molecular and cellular mechanisms, and are potential candidates for cell-based therapies for treatment of neurological diseases. In the adult brain there are neurogenic niches in which reside neural stem cells (NSC). These NSC have a role in repairing the brain, making them ideal for brain repair, however they are present in limited numbers. Recently, cellular reprogramming has been used to convert more accessible cell types (e.g. fibroblasts) to NSC. Studies have demonstrated the successful reprogramming of cells to NSC and neuronal cells using pluripotency-associated (OCT4, SOX2) and neuronal (BRN2, ASCL1, MYT1L) transcription factors. DPSC may be a suitable cell type from which to obtain NSC. DPSC have been differentiated into neurons and have been reprogrammed to pluripotent cells. Overexpression of OCT4 in DPSC has been shown to enhance proliferation, pluripotency and multilineage differentiation, though neural differentiation has not been assessed. OCT4 has also been used to reprogram fibroblasts and blood cells to NSC. The role of OCT4 may be to induce plasticity and revert cells to an earlier stage in their development. The aim of this study is to investigate the ability to reprogram DPSC into NSC using OCT4, and to examine the neurogenic potential of these modified DPSC in vitro and in vivo. Human DPSC transduced with OCT4 via lentivirus were cultured in NSC conditions before being transferred to neuronal maturation conditions. They were analysed for gene and protein expression of neural stem and progenitor cell markers, the ability to self-renew, and differentiate into neurons, astrocytes and oligodendrocytes. Cells displaying a neuronal morphology underwent electrophysiological analysis to examine functionality. In vivo analysis was carried out to examine their ability to survive, integrate and differentiate in an avian developmental model. We hypothesise that DPSC overexpressing OCT4 will display enhanced neurogenic properties in vitro and in vivo. Human DPSC may provide an alternative source of NSC suitable for cell-based therapies for the treatment of neurological diseases.

W-3081

NONVIRAL DIRECT REPROGRAMMING TO DOPAMINERGIC NEURONS: A BIOMATERIALS-BASED THERAPY FOR PARKINSON'S DISEASE

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Patients with Parkinson's disease may see temporary improvements with pharmacologic intervention or deep brain electrical stimulation, but these clinical benefits inevitably decline with the progressive loss of dopaminergic activity. Transdifferentiation of resident glia to dopaminergic neurons would be an attractive therapeutic alternative to the transplantation of induced or differentiated cells. The feasibility of converting astrocytes to neurons in situ has been reported in vitro and in vivo using lentiviral delivery of transcription factor genes. Now, we have developed a virus-free, nanoparticle-based reprogramming protocol that eliminates the risk of deleterious genetic integration inherent with viral methods. We delivered cocktails of transcription factor and microRNA constructs packaged in polymer nanoparticles derived from the synthetic bioreducible poly(amidoamine) p(CBA-ABOL). Programmed degradation of the polymer backbone releases the payload efficiently, while diminishing toxicity of the carrier so as to enable repeated application without compounding cytotoxicity. Using a serial dosing strategy, we could efficiently convert human astrocytes to Tuj1+/TH+ cells. Conversion efficiencies were assayed using high content imaging and automated analysis, which also allowed us to quantify neuronal morphometrics including as neurite number, length, and branching. These nonvirally-induced dopaminergic neurons (NiDAs) were further validated for expression of MAP2, DAT, synaptophysin, and synapsin I, as well as electrophysiological function. Our virus-free approach produces NiDAs from human astrocytes with favorable efficiency and quality, thus lowering the barrier to the safe and effective translation of direct reprogramming to dopaminergic neurons.

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W-3083

YAP PROMOTES PLURIPOTENCY INDUCTION VIA A CELL NON-AUTONOMOUS MECHANISM

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Cell fate decisions in vivo are known to be instructed by microenvironmental or niche factors. Whether these factors similarly impact in vitro pluripotency induction is normally disregarded. Here we report a unique niche-like function, driven by the transcriptional coactivator YAP, on the reprogramming of somatic cells to induced pluripotency. YAP can influence cell fate by relaying extracellular chemical and mechanical cues to the nucleus. While a large body of work establishes YAP as a stem cell factor, promoting dedifferentiation or pluripotency, our work reveals that YAP exclusively accomplishes this function in a cell non-autonomous manner. Specifically, while YAP-overexpression in cells undergoing reprogramming is inhibitory to their entrance to pluripotency; YAP-overexpression in somatic cells of the same culture promotes nearby pluripotency induction. Therefore, YAP exhibits dual functionality on somatic cell reprogramming - cell autonomous inhibition and cell non-autonomous promotion. Using a co-culture system in which cells are kept physically separate, we validated that cell-cell contact is not necessary for YAP's cell non-autonomous activity. Additionally, medium conditioned by YAP-overexpressing feeder cells possesses similar reprogramming-enhancing function, further supporting that the YAP-mediated cell non-autonomous promotion of reprogramming is facilitated by soluble factors. Ultimately, we present a novel cell non-autonomous process for the enhancement of pluripotency induction. As we demonstrate, the neighboring cells that elude reprogramming are not merely passive bystanders, but rather create a functional microenvironment akin to the in vivo stem cell niche to influence cell fate decisions. Further understanding of the cell non-autonomous influences on cell fate conversion by microenvironmental factors can be employed for the improved derivation and manipulation of desired cell fate transitions that are otherwise difficult or invasive to achieve.

W-3085

ROLE OF TRANSGENE EXPRESSION IN MOUSE INDUCED HEPATOCYTES FOR MAINTENANCE OF HEPATIC IDENTITY

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Recent studies have demonstrated the direct conversion of fibroblasts into induced hepatocyte-like cells (iHeps) which share key hepatic features with in vivo hepatocytes. Previously, we described the generation of iHeps using single hepatic transcription factor, Hnf1a together with small molecules which represent the more mature hepatic state compared with iHeps generated with multiple hepatic factors. However, the underlying mechanism of hepatic conversion such as transgene dependency of established iHeps is largely unknown. Here we describe the generation of Hnf1a-derived iHeps (e-iHeps) using episomal vector with small molecules. In contrast to Hnf1a-derived r-iHeps (using retrovirus), e-iHeps lost their typical morphology with rapid down regulation of hepatic markers upon withdrawal of small molecules. However, hepatic identity of e-iHeps could stably be maintained by ectopic expression of Hnf1a even in the absence of small molecules. Taken together, our data indicate that single factor-derived iHeps is metastable and its hepatic identity could only be maintained by continuous assistance of either small molecules or master hepatic factor, Hnf1a.

W-3087

MOUSE COLONIC SECRETORY CELLS DE-DIFFERENTIATE INTO INTESTINAL STEM CELLS AND PROMOTE MUCOSAL REPAIR THROUGH ACTIVATION OF NF-KB SIGNALING

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Intestinal epithelial cells (IECs) consist of intestinal stem cells (ISCs), progenitor cells and differentiated secretory or absorptive cells. Studies have revealed the plasticity of secretory-committed IECs, showing that these IECs can revert back to ISCs under massive loss of the ISC pool. However, it remains mostly uncertain how these cells functionally exhibit their de-differentiation ability, possibly due to the difficulty in precisely identifying those events in vivo. Therefore, in this study, we aimed to visualize the de-differentiation events of secretory-committed IECs, and reveal the regulatory mechanism of this process. Accordingly, we established a mouse model which allowed us to perform lineage tracing of Atoh1+ cells (Atoh1-crePGR; ROSA26-LSL-tdTomato; Atoh1tdTomato). In Atoh1tdtomato mice, we found that a rare population of Atoh1+ IECs could re-construct crypt-villus units under a completely homeostatic condition. Also in the organoid culture system, we confirmed that a rare population of Atoh1+ organoid cells convert to ISCs under a standard culture condition. However, when we traced the fate of Atoh1+ IECs in DSS-induced colitis mice, we found that the frequency of Atoh1+ IEC de-differentiation significantly increases in the inflamed colon. Microarray analysis of Atoh1+ IEC-derived colonocytes showed that those IECs in the DSS-induced colitis mice acquire enhanced expression of NF-kB related genes, and also acquire ISC-like gene expression profile. Consistently, Atoh1+ IEC-derived colonocytes of DSS-induced colitis mice showed enhanced ability to reconstruct organoids from single isolated IECs. In addition, we added pro-inflammatory cytokines and bacterial components to colonic organoids derived from the Atoh1tdTomato mice and found that de-differentiation of Atoh1+ IECs can be promoted in vitro by the induction of NF-kB signaling. Specific blockade of the NF-kB pathway clearly canceled the promotion of Atoh1+ IEC de-differentiation. Consequently, we found that a sub-population of Atoh1+ IECs could de-differentiate into ISCs under a homeostatic condition, and could also contribute to mucosal repair in colitis through activation of intrinsic NF-kB signaling. These results provide new insights into the mechanism of intestinal homeostasis maintenance.

LATE BREAKING ABSTRACTS

W-4001

GENETIC MANIPULATION OF CAVEOLAE MEDIATED β-CATENIN SIGNALING REGULATES ADIPOGENIC DIFFERENTIATION OF HUMAN ADIPOSE DERIVED MESENCHYMAL STEM CELLS

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Caveolae are cholesterol rich lipid rafts which are small invaginations of the cell membrane formed by major scaffolding protein caveolin-1 (CAV-1). CAV-1 has been shown to bind with a variety of client signaling proteins to regulate signal transduction pathways. Little is known about its role in mesenchymal stem cells. In this study, we investigated the role of CAV-1 on adipogenesis in human adipose derived mesenchymal stem cells (hASCs). We show that lentiviral over expression of full length human CAV-1 cDNA in hASCs significantly decreased adipogenic differentiation as evident by adipocyte specific PPARγ and EBPα gene expression and Oil Red O staining compared to controls. Conversely, shRNA mediated inhibition of endogenous CAV-1 significantly enhanced PPARγ and EBPα gene expression confirming the inhibitory role of CAV-1 on hASCs adipogenesis. Mechanistically, western analysis demonstrated, over expression or knockdown of CAV-1 using lentiviral vectors significantly increased or decreased β-catenin expression respectively suggesting that CAV-1 can modulate hASCs adipogenesis through direct regulation of β-catenin signaling. In summary, this study identifies CAV-1 as a potent cellular target which can be used to manipulate β-catenin signaling dependent adipogenesis in human stem cells and would be a novel platform for soft tissue reconstruction therapies.

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W-4005

CANCER-ASSOCIATED STROMAL FACTORS - NOVEL TARGETS FOR THE TREATMENT OF COLORECTAL CANCER?

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Despite colorectal cancer (CRC) remaining a prominent cause of cancer death worldwide, there is little known about how the surrounding connective tissue "stroma" encourages poorer prognosis. While many studies have attempted to interrogate the molecular cross-talk between the tumour and its stroma, the most important tumour-promoting interactions remain ill-defined. We are yet to adequately exploit these pathways for the prevention and treatment of cancer. Tumour development is often likened to the "organ that never develops", as common developmental programs appear to be shared with tumourigenesis. We therefore postulated that by interrogating all the differentially expressed genes in the stroma during colonic development and tumourigenesis, compared to the normal homeostatic colon, we might identify the common, and thus the most biologically relevant, factors associated with CRC progression - the 'Activated Stromal Signature' of CRC. This list was obtained via RNA sequencing (RNAseq), of which the preliminary findings will be presented. We will also explore how the top prognostic stromal hits will be investigated, predominantly via organoid culture and orthotopic murine CRC models. We expect to not only identify a network of factors involved in CRC progression, but to suggest novel targets for the prevention and treatment of CRC.

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W-4007

IDENTIFICATION OF A NOVEL PEPTIDE AND TARGET FOR THE TREATMENT OF CARDIAC ISCHEMIA

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During cardiac ischemia, the accumulation of acidic metabolic byproducts results in extracellular acidification reaching levels as low as pH 6.5. This severe acidosis significantly impacts cardiac function and is a leading cause of heart tissue damage during ischemia. From a clinical perspective, cardiac ischemia-acidosis is a serious problem during acute myocardial infarction and donor organ preservation for heart transplantation. A better understanding of the mechanisms that govern responses to ischemia and the development of new cardioprotective therapeutics would have profound benefits to cardiovascular medicine and patient prognosis. With this knowledge, we identified and investigated the role of a putative therapeutic target expressed in cardiomyocytes. We also characterized the cardioprotective effects of a potent and specific peptide inhibitor of the target which was previously identified by our team. Cardiomyocytes that were derived from human induced pluripotent stem cells (hiPSC-CMs) and then cultured under combined acidic and hypoxic conditions (0.5% O2 with pH 6.0 or 5.0) underwent significant cell death. Treatment with the peptide inhibitor (10-100 nM) led to complete rescue of cell viability at pH 6.0 and nearly complete rescue at pH 5.0. Live calcium imaging of hiPSC-CMs revealed a pH-induced spike in intracellular calcium that was markedly reduced by treatment with the peptide, suggesting an effect on calcium transients. To assess the efficacy of the peptide on a whole organ level, we used a Langendorff-based ischemia reperfusion mouse model. Hearts treated with the peptide showed improved function after injury including increased left ventricular developed pressure and decreased end diastolic pressure compared to control hearts. Treated hearts also exhibited increased coronary flow during the first five minutes of reperfusion. Collectively, these data facilitate a better understanding of cell death in response to ischemia/acidosis and suggest a novel therapeutic approach for cardioprotection following cardiac ischemia.

W-4009

NON-REDUNDANT AND CELL-TYPE SPECIFIC FUNCTIONS OF YAP AND TAZ

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It is generally accepted that YAP and TAZ, two major effectors of the Hippo signaling pathway, play overlapping roles in the regulation of mammalian tissue homeostasis. To date, however, it remains unclear whether YAP and TAZ may play non-redundant roles in specific cellular contexts in humans. Here, we generated isogenic YAP knockout and TAZ knockout human embryonic stem cells (hESCs), mesenchymal stem cells (hMSCs), and endothelial cells (hECs) by CRISPR/Cas9-mediated gene editing. While dispensable for hESC pluripotency, TAZ and YAP played essential roles in maintaining the activity of hECs and hMSCs, respectively. TAZ was required for hEC proliferation and angiogenesis, but YAP was geroprotective for hMSCs through transcriptional upregulation. Overexpression of YAP suppressed hMSC senescence, as well as restored bone density and attenuated the development of osteoarthritis in mouse models. Our study not only demonstrated the functional non-redundancy and cell-type specificity between YAP and TAZ, but also identified YAP pathway as a novel aging regulatory axis, which could potentially be targeted to alleviate age-related disorders.

W-4011

SINGLE CELL RNASEQ ANALYSIS OF MURINE SALIVARY GLAND DEVELOPMENT

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Regenerative approaches to repair exocrine glandular tissues, requires that we understand progenitor cell interactions during development and how homeostasis is maintained in adult glands. Therefore, understanding the interactions between stem and progenitor cells will provide targets to repair or regenerate damaged tissues. For example, salivary glands are damaged after irradiation resulting in loss of secretory function. We propose to characterize different cell populations in the developing and adult murine salivary glands; to identify markers that could be used to isolate specific cell populations; and to investigate their function during regeneration. Here we characterized cell types in developing murine submandibular glands by single cell RNA-seq using the 10xGenomics platform to investigate lineage relationship between cell developing populations. We isolated salivary glands from developing embryos at E12, E14, E16, postnatal P1, and adult (P30). The tissues were dissociated into single cells and analyzed by single cell RNAseq. In total, ~5000 cells were sequenced with an average read depth of over 1 million reads and ~2000 genes/cell. Unsupervised clustering with 10xGenomics Loupe browser identified multiple mesenchymal and epithelial populations. To further characterize these clusters, we searched for the expression of genes involved in acinar and intercalated duct cell differentiation and proliferation. We initially focused on identifying and characterizing subpopulations of proacinar, acinar and proliferating acinar cells that express genes such as Mist1, Ccnd1, Aqp5, and Prol1. We also identified subpopulations of intercalated duct cells that express Krt5, Krt14, Ccnd1 and SMGc. We found that subpopulations within these clusters express genes that have been shown to be involved in regulating progenitor cell function such as Kit, Sox10, Lpo, and Itga6. Our goal is to further characterize these subpopulations using surface markers that could be used to FACs isolate them to investigate their function during regeneration. Identifying markers involved in progenitor cell lineage and expansion will useful to study progenitor cell function and may inform strategies to regenerate salivary glands after either irradiation damage or for bioengineering of salivary tissue

W-4013

TO TWEET OR NOT TO TWEET: ETHICAL CONSIDERATIONS IN ASKING PARTICIPANTS TO NOT TWEET THEIR STEM CELL CLINICAL TRIAL EXPERIENCES DURING THE RESEARCH PERIOD

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Social media is gaining attention as a globally important information resource, and discussions about stem cell research in particular can create stem cell hype and optimistic mass media reports. There are concerns in the field about information leaks from research participants. For example, participants may give interviews to mass media without asking researchers or post about their experiences on their social network system (SNS) even during the clinical research period. When participants give consent, they are asked to follow instructions, for example the research schedule, diet, and medicine restrictions. Confidentiality during the research period is seldom mentioned because several international ethical guidelines do not include the responsibilities of participants regarding confidentiality. Thus, we will present the ethical justifications of requesting participants to refrain from posting during the research period to maintain trial confidentiality. The most important point is that a participant's SNS post regarding clinical trial participation during the research period may damage the social value of the research. If the research aims to benefit the public, it should be acceptable to request that the participant protect and respect the social value

of the research. Even if the participants' publication of their experiences/information is only temporary and contain only part of the research results, the information may affect future participants and distort the research results. We will discuss the type of information or data that we need to ask participants not to post. Additionally, participants should be informed that their actions may undo the benefits of their participation in the research. In conclusion, to protect the social value of the stem cell research and the patients' participation efforts, we need to at least kindly request that participants be careful not to post about their trial experiences on their SNS. In this presentation, we will present drafted notices to participants regarding the risks of SNS posting.

Funding Source: This research was supported by AMED under Grant Number JP17bm0504010.

W-4015

THE TETRASPANIN CD9 AS A MARKER FOR MURINE AND HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Hematopoietic Stem Cells (HSCs) that give rise to all lineages of blood are defined as a lineage-negative (Lin-), c-Kit+, Sca-1+ (LSK) population in the mouse. The expression of Flt3 on LSK cells is accompanied by loss of self-renewal capacity and transition to lymphoid-primed multipotent progenitors (LMPPs). LMPPs are a heterogeneous population of cells and while these mainly give rise to common lymphoid progenitors (CLPs) and common myeloid progenitors (GMPs), a small fraction also have the potential to generate progenitors within the megakaryocyte-erythrocyte (MK/E) lineage. Here we have investigated the heterogeneity of the Flt3+ population by a combination of single-cell methods and show that the tetraspanin CD9 could be a promising marker to discriminate the MK-generating subset from those with exclusive lymphoid- and myeloid-producing capacity. Moreover, there are species-specific differences in expression of CD9 in hematopoietic lineages such that while CD9 is abundantly expressed within murine HSCs, it is expressed at a low level within human cord blood HSCs. However, CD9 is highly upregulated in aged bone marrow as well as in myeloid leukemia.

W-4017

CPNE7 INDUCES DIFFERENTIATION OF MESENCHYMAL STEM CELLS INTO ODONTOBLASTS

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Our previous study demonstrated that Cpne7, identified in preameloblast-conditioned medium, induces odontoblast differentiation and mineralization in vitro, and promoted tertiary dentin formation in beagle dog IPC (indirect pulp capping) model. The aim of this study is to investigate the effects of CPNE7 in odontoblast differentiation and dentin formation in vitro and in vivo. We also investigated biological function and mechanisms of Cpne7 in regulation of dental and non-dental mesenchymal cell differentiation into odontoblasts via epithelial-mesenchymal interaction. We observed tertiary dentin formation in both the hypersensitivity and DPC model. The regenerated dentin showed the characteristics of physiologic dentin. In hypersensitivity model, dentinal tubule structure was clearly observed beneath the remaining dentin. In DPC model, tubular dentin structure was observed instead of bone-like osteodentin commonly formed in MTA capping. Cpne7 also induced odontoblast differentiation and promoted formation of dentin/pulp-like tissue with formation of tubular dentin in vivo in dental or non-dental mesenchymal cells. These results suggest Cpne7 is a diffusible signaling molecule that is secreted by preameloblasts and regulates the differentiation of mesenchymal cells into odontoblasts. Our findings suggest the novel concept that Cpne7, a dental epithelium-derived protein, plays an essential role in commitment of odontoblasts, odontoblast differentiation, and dentin formation. Therefore, Cpne7 could be used as a novel therapeutic approach for the treatment of diseased dentin-pulp complex.

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W-4019

BRAIN PERICYTES FOLLOWING CEREBRAL INFARCTION HAVE MORE NEURONAL DIFFERENTIATION POTENTIAL THAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are multipotent stem cells that reside in perivascular regions of various organs, including bone marrow (BM). Although increasing evidence shows that MSCs are likely identical to pericytes (PCs), we have recently demonstrated that brain PCs following ischemia acquired the traits as multipotent stem cells that differentiate into various cells, including neural and vascular lineage cells. However, similarities and differences between ischemia-induced multipotent stem cells (iSCs) and BM-derived MSCs (BM-MSCs) remain unclear. In the present study, we compared the features of iSCs with BM-MSCs. Permanent focal cerebral ischemia was produced by occluding the middle cerebral artery of CB-17 adult mice (6-week-old, male). iSCs were extracted from post-stroke areas. BM-MSCs were isolated from BM of CB-17 adult mice. Then, we examined whether iSCs and BM-MSCs have neural and/or mesenchymal stem cells markers and differentiate into neural and/or mesoderm lineages, respectively. Furthermore, to investigate whether iSCs originate from circulating BM-MSCs, GFP+ BM-MSCs were intravenously injected one hour after ischemia and investigated their localization in CNS. Immunohistochemistry showed that iSCs and MSCs had several pericyte markers, including PDGFRβ, NG2 and αSMA, and both of them had MSC markers, such as CD29, CD44, CD73, CD105 and CD106. Not only iSCs but also MSCs differentiated into mesoderm lineage, including osteopontin+ osteoblasts, FABP4+ and Oil red+ adipocytes, and collagenII+ chondrocytes. However, neural stem cell marker nestin was highly expressed in iSCs than MSCs. In addition, not MSC- but iSC-derived spheres differentiated into Tuj1+ functional neurons. Furthermore, although a few GFP+ MSCs were present within and around post-stroke area, we did not evidence that GFP+ MSCs transdifferentiated into iSCs. These results show that iSCs and BM-MSCs have several common markers and differentiate into multiple cells of mesenchymal lineages. However, differentiation activities to functional neurons were observed only from iSCs, suggesting that locally-induced multipotent stem cells, which likely originate from brain PCs following ischemia, have potential to repair neuronal loss after ischemic stroke.

W-4021

CLINICOPATHOLOGICAL CORRELATION (CPC) SINGLE-CELL ASSAYS, IMMUNE SIGNATURES PANELS, AND TECHNOLOGIES FOR MONITORING IMMUNE SYSTEM - PARKINSON'S DISEASE

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"The gold standard for diagnosis of Parkinson's disease has been the presence of SNpc [substantia nigra pars compacta] degeneration and Lewy pathology at post-mortem pathological examination." (Kalia 2015) We hypothesize the neurovascular compartment plays a critical role in neurodegeneration. Parkinson's disease (PD) is diagnosed using clinical features (Frasier 2014) and there is no test for definitive diagnosis of PD at early stages of the disease (Kalia 2015). Biomarkers are critically needed for diagnosing patients with PD, as well as the subclinical PD cohort in whom early interventions could be made, preserving neurologic function. Our research objectives include the development of assays for diagnostics applications - including both sophisticated high-throughput applications (comprehensive panel of markers) and point-of-care (POC) diagnostic assay development. To identify a connection between putative biomarkers and clinical manifestations (such as degeneration, inflammation, structural alterations), we propose single-cell and cytometry assay development (Genomic and Proteomic Single-Cell Arrays) to potentially reveal a connection to clinical manifestation of disease phenotype. Impact. We performed comprehensive bioinformatics analysis and identified novel single-cell subsets (immune single-cell signature)/panel of potential diagnostic markers (genomic signatures) - present at early stages of the disease. The results of the project can provide insight on the potential critical role of neurovascular dysfunction (as evidenced by the analysis of novel biomarkers and the manifestation of ocular symptoms); and provide new perspectives on the cellular and molecular mechanisms underlying PD (early stages [preclinical and prodromal] and disease progression). This knowledge will afford the development of diagnostic assays and inform on potential therapeutic targets.

W-4023

GENERATION OF PSC-DERIVED NEURAL ORGANOIDS USING AN ALGINATE-BASED SCAFFOLD.

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Organoids are stem cell-derived microtissues composed of multiple cell types that are typically arranged with in vivo-like patterning and recapitulate organ function, in vitro. As such, these complex models are quickly becoming a favored system to study embryonic development and disease. However, producing sufficient quantities of these organoids remains a significant hurdle. Here we demonstrate the utility of an animal origin-free, chemically-defined, alginate-based 3D scaffold to generate increased numbers of neural organoids. For this approach we used pluripotent stem cells to form embryoid bodies seeded directly into a preformed alginate 3D scaffold followed by neural induction. Neurospheres subsequently isolated from this scaffold continued to grow and undergo self-directed neural differentiation. These neural organoids formed multiple rosette-like structures that express progenitor markers such as SOX1, SOX2 and FOXG1, whereas the surrounding cells expressed the cortical markers SATB2 and CTIP2 as well as other neuronal markers such as MAP2 and NeuN. Additional gene expression analyses also indicated these organoids expressed many neuronal and cell type-specific markers suggesting these organoids are composed of multiple neural cell types. In all, our data suggest that the application of 3D alginate scaffolds are an effective means to generate neural organoids and may help overcome the issue of scale.

W-4025

CLINICAL APPICABLE HUMAN ES CELL-DERIVED HEPATOCYTES, WHICH ENHANCED HEPATIC METABOLIZING ACTIVITY USING REPEATED EXPOSURE TO XENOBIOTICS

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As a promising cell source for clinical application and in vitro drug screening, hepatic functions of human stem cell-derived hepatocyte-like cells (HLCs) should be increased comparable to human primary hepatocytes. However, the expression and activity of hepatic metabolizing enzymes of final HLCs are usually much lower than primary human hepatocytes. Here, we showed significantly increased hepatic metabolizing enzymes (Phase I, II and nuclear receptor) using an optimized hepatic differentiation protocol; three-dimensional (3D) spheroidal culture and repeated exposure to xenobiotics. Prior to generate highly functional HLCs, >90% albumin-positive HLCs were produced by using our hepatic differentiating protocol without purification. Then we generated 3D spheroidal aggregates of hepatoblast, which have high viability and proliferation ability. Our data showed that the 3D hepatoblast-spheroids expressed significantly increased activity of hepatic enzymes, compared to 2D-cultured HLCs. Furthermore, when the 3D hepatoblast-spheroids were repeatedly exposed to xenobiotic (acetaminophen, rifampicin, and phenobarbital), the expression of hepatic enzymes were much more increased, compared to non-exposed group. In conclusion, our repeated exposure to xenobiotics for maturing hepatic functions of HLCs would be a new method to produce highly functional HLCs for clinical and in vitro drug screening.

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W-4027

THE FUNCTIONAL ROLE OF CIRCRNAS IN NAIVE TO PRIMED PLURIPOTENCY STATE TRANSITION

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Abstract: Preimplantation epiblast derived embryonic stem cells (ESCs) and postimplantation epiblast derived epiblast stem cells (EpiSCs) represent the naïve and primed states of pluripotency, respectively. The function of transcriptional factors, such as Otx2, is addressed in naïve to primed pluripotency states (N-to-P) transition. However, the role of RNA molecules involved in N-to-P transition is less clear. Here we showed that the ectopic expression of circular RNAs (circRNAs) circF, circS, and circT promote the N-to-P transition. On the other hand, disruption of circF, circS, and circT hampered N-to-P transition in vitro and postimplantation epiblasts in vivo. Together, we showed that circRNAs were involved in the pluripotency states transition.

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W-4029

THE LYSINE SPECIFIC DEMETHYLASE LSD1/ KDM1A PRIMES ENDODERMAL COMMITMENT OF HUMAN EMBRYONIC STEM CELLS

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The incidence of glucose metabolism disorders, such as type 2 diabetes (T2D) and insulinresistance (IR), is rising worldwide fueled by the increased prevalence of obesity and unhealthy lifestyles. Recent observations point to aberrant epigenetic marks deposition as a key mechanism responsible for defective pancreatic β-cell maturation during organogenesis that could lead to higher T2D and IR susceptibility risks later in life. The aim of our study is to elucidate the epigenetic role of the lysine specific demethylase LSD1 during specification of human embryonic stem cells (hESCs) into endodermal lineages including glucose sensitive cells such as pancreatic β-cells and hepatocytes. LSD1 is a histone 3 demethylase whose function is finely tuned during human development. LSD1 is progressively induced during differentiation of hESCs into pancreatic β-cells and hepatocytes. Our studies on CRISPR-derived LSD1-depleted WA01 (H1) hESCs (LSD1-/-) have shown an induced expression of the specific endodermal markers FOXA2, SOX17 and HNF4, albeit the stemness properties of LSD1-/-hESCs are maintained. These data suggest that the absence of LSD1 primes the endodermal commitment of hESCs, by unlocking the transcription of poised master regulators of endoderm differentiation. Consistently, our preliminary results indicate that, already at the pluripotent stage, LSD1 occupies the promoter of key transcription factors driving the differentiation into glucose sensitive cells. Altogether our findings suggest a critical role for LSD1 in tuning the differentiation of hESC into glucose sensitive endodermal lineages.

W-4031

MSX2 INITIATES AND ACCELERATES MESENCHYMAL STEM CELL SPECIFICATION OF HPSCS BY REGULATING TWIST1 AND PRAME

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The gap in knowledge of the molecular mechanisms underlying directed differentiation of hPSCs into the mesenchymal cell lineages hinders the application of hPSCs for cell-based therapy. In this study, we identified a critical role of muscle segment homeobox 2 (MSX2) in initiating and accelerating the molecular program that leads to mesenchymal stem/stromal cell (MSC) differentiation from hPSCs. Genetic deletion of MSX2 impairs hPSC differentiation into MSCs, while ectopic expression of MSX2 enhances and accelerates MSC differentiation of hPSCs. When aided with a cocktail of soluble molecules, MSX2 ectopic expression induces hPSCs to form nearly homogenous and fully functional MSCs within a week. Mechanistically, MSX2 induces hPSCs to form neural crest cells, an intermediate cell stage preceding MSCs, and further differentiation by regulating TWIST1 and PRAME. Our findings provide novel mechanistic insights into lineage specification of hPSCs to MSCs and effective strategies for applications of stem cells for regenerative medicine.

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W-4033

ADDICTION ASSOCIATED N40D MU-OPIOID RECEPTOR VARIANT DIFFERENTIALLY REGULATES SYNAPTIC TRANSMISSION IN HUMAN NEURONS

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The mu opioid receptor (MOR) mediates the most powerful addictive properties of opiate alkaloids. Because of its critical role in mediating reward and positive reinforcement, MOR is also an indirect target of alcohol, nicotine, and other drugs of abuse. MOR mediated synaptic alterations in reward-associated brain regions may represent a key underlying mechanism of the reinforcing aspects of drug abuse. Furthermore, human genetic studies suggest that functional genetic variation at the MOR are thought to impact individual responses to opioid drugs. In fact, the most common SNP in the MOR (OPRM1 A118G, rs1799971) replaces an asparagine with an aspartate at position 40 (MOR N40D) of MOR, and has been associated with opioid and alcohol addiction, but its mechanism has remained unclear. The limited understanding of the cellular and synaptic function of the MOR in human neurons has hindered the development of effective opioids that minimize addictive tendencies.To provide new cellular and molecular insight into functional consequences of OPRM1 A118G, we generated homozygous N40D induced inhibitory neuronal cells (iNs) from human subject derived induced pluripotent stem cells (iPSCs). We found that D40 expressing iN cells consistently exhibit a stronger suppression of inhibitory synaptic release by a MOR-specific agonist, DAMGO, compared to N40 iN cells in multiple human subject derived cell lines. We then used CRISPR/Cas9 gene editing to generate two sets of isogenic human stem cell lines carrying homozygous N40 or D40 alleles, which recapitulate the synaptic phenotype of the subject derived iN cells, demonstrating the observed MOR-mediated effect is due to the N40D variant as opposed to secondary background genetic variation in the human subjects. The observed functional differences are mediated by a more robust decrease in neuronal excitability compounded by decreased synaptic release in D40 MOR versus N40 MOR carrier iN cells mediated by MOR signaling. We provide direct evidence that common MOR genetic variation encodes functional variability at the level of synaptic transmission. The use of patient-derived stem cells to unravel the impact of OPRM1 gene variants and may ultimately provide the necessary insight to develop patient-specific, precision medical interventions for drug and alcohol dependence.

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W-4035

CHARACTERIZATION OF TRANSGENIC PORCINE EMBRYONIC STEM CELL LINES FOR IN VITRO BRAIN TUMOR MODEL

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Pigs are brain tumor models more suitable than mice. The pig's brain is larger than the mouse and more similar to humans because it has an anatomical structure. In this study, we are using CreERT2 (a Cre recombinase fused to the mutated ligand-binding domain of the human estrogen receptor) induction system to establish the in vitro brain tumor model. First, we made a vector structure. This is a 2A peptide-dependent polysistronic expression construct carrying the DsRed, SV40LT and HrasV12 genes after CreERT2-related recombination. Whether the introduction of the transgenes are confirmed by PCR and a fluorescence activated cell sorter. Second, the somatic cell nuclear transfer (SCNT) was performed using the brain tumor model transgenic (TG) cell line and then the whole seeding was performed using SCNT TG blastocysts. As a result, two TG porcine embryonic stem (pES) cell lines were established. TG pES cell lines are showed primed and bFGF dependent characteristics. These characteristics are consistent with other previously reported pES cell lines. We confirmed the insertion of a brain tumor-inducing gene through genomic DNA PCR. Immunohistochemical staining results showed that both cell lines expressed Oct4, pluripotent marker. Alkaline phosphatase (AP) staining results showed that AP positive and embryoid formation (EB) were also confirmed by immunohistochemical staining. In conclusion, we established embryonic stem cells in an in vitro brain tumor model through SCNT. Further studies are needed to confirm the in vivo teratoma formation using immunodeficiency (SCID:Severe Combined ImmunoDeficient syndrom) mouses.

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W-4037

DECIPHERING THE ROLE OF NOTCH SIGNALING IN Y TO PDA TRANSDIFFERENTIATION IN VIVO IN C.ELEGANS

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Cell differentiation is a key process in the development of multicellular organisms. In the last ten years, efforts towards understanding how a cell acquires and maintains a stable differentiated identity led to the demonstration that differentiated cells could dedifferentiate both in vivo as a natural process and in vitro by forcing the expression of pluripotency transcription factors. This process in vitro is only fully effective in a restricted number of cell types, i.e is cell context-dependent. We use an exceptional in vivo event of cell identity change during C. elegans development to understand what makes a cell prone to change its identity. This process where a rectal cell (called Y) becomes a motoneuron (called PDA) is called transdifferentiation and can be analysed at the single cell level. The Y -to-PDA transdifferentiation is a step-wise process which englobes 1) erasure of Y rectal identity followed by 2) aquisition of a specific neuronal identity. The dramatic changes observed in markers expression suggest that this transdifferentiation process is driven by activation of key regulators which would allow the rapid switch from a cell state to another. Importantly, Y is competent for transdifferentiation, but its neighbouring rectal cells are not. From all rectal cells, only the Y cell expresses the lin-12/Notch receptor. Furthermore, this expression appears to be dynamic and restricted to the embryonic stage. Importantly, we found that a pulse of the LIN-12/Notch receptor activity is sufficient to convert another cell, into a competent Y cell that transdifferentiates into an additional PDA-like neuron. The major aim of the project is to identify the target genes that Notch uniquely activates in the Y cell and which may be involved in setting up the competence to reprogram. This work will also lead to investigate the gene regulatory network that allows a cell to transdifferentiate - but not its neighbours. We will report our latest progress at meeting.

Funding Source: ErC PlastiCell.

W-4039

THE ROLE OF ADULT HIPPOCAMPAL NEUROGENESIS IN LEARNING AND MEMORY OUTCOMES AFTER REPETITIVE MILD TRAUMATIC BRAIN INJURY IN MICE

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Mild Traumatic brain injury (mTBI) results in a broad spectrum of disabilities where the underlying etiology and long-term neuropathological ramifications are not well understood. Military personnel and athletics often accrue multiple TBIs which increase their chances of developing severe cognitive dysfunction and chronic neurodegenerative disorders. This dysfunction is amplified when stem cell-driven neurogenesis is impaired after TBI. During adult hippocampal neurogenesis, Type I and Type II neural stem/progenitor cells (NSPCs) differentiate into Type II neuroblasts and migrate radially from the subgranular zone into the granular cell layers before they differentiate into newborn neurons. To date, few studies have investigated the role of endogenous adult neurogenesis in the dentate gyrus (DG) of the hippocampus in cognitive decline after repeated mTBI. Using an established murine model of repeated mTBI, we found significant learning and memory deficits one month post-injury via Barnes Maze analysis. To determine whether these behavioral changes are the result of impaired neurogenesis, we performed cell fate analysis using BrdU labeling. While our findings show a decrease in BrdU-positive cells a month after injury, more of these cells were co-labeled with differentiated markers (NeuN/Prox1/DCX) in the DG after repeated mTBI when compared to sham, indicative of increased differentiation. Interestingly, we find increased numbers of neuroblasts and mature Prox1-positive neurons in the hilus. These findings suggest aberrant neurogenesis may be contributing to the learning and memory deficits after rmTBI. To test this, future neural stem cell ablation studies will be conducted to determine whether misguidance during neurogenesis, in part, mediates mTBI-induced cognitive dysfunction.

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W-4041

INVESTIGATION OF THE OPTIMAL HYPOXIA OPERATING CONDITIONS FOR MAMMALIAN CELL CULTURE

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The effect of hypoxia condition in mammalian cell culture has been reported for decades and has been adapted for developing and manufacturing stem cell drug products. Hypoxia condition can be induced with several methods. Especially, hypoxic CO2 incubators are widely used for

academic research and general cultivation purposes for small-scale production. However, in actual use of the equipment, the opening time of the incubator causes the hypoxia condition inside the equipment to break down, and the repetition of this would negatively affect the cell proliferation and characteristics. Therefore, we tried to find more optimal hypoxia operating conditions by measuring how the hypoxia condition changes according to the opening time of the incubator door. Basically, to measure the oxygen concentration, the incubator itself, the oxygen concentration sensor in the air inside the incubator, and the amount of dissolved oxygen in the medium were measured and compared. In addition, the difference in the oxygen concentration in the culture medium was compared between the static culture and the method of accelerating the easy gas equilibrium by stirring in medium. This provides clues as to what oxygen concentration the cultured cells are exposed to during the actual culturing process and how to proceed to expose them to a more optimal oxygen concentration. Since there are many institutions and companies that are still using traditional incubators, we hope that it will help to get better results by providing a more optimized environment to the cells.

W-4043

DEVELOPMENT OF AN AUTOMATED PLATFORM FOR PREDICTING THE TERATOGENIC POTENTIAL OF DRUGS USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Kennedy Agwamba - *New York Stem Cell Foundation, New York, U.S.*
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Bruce Sun - *New York Stem Cell Foundation, New York, U.S.*
Vignesh Nadar - *New York Stem Cell Foundation, New York, U.S.*
Reid Otto - *New York Stem Cell Foundation, New York, U.S.*
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Scott Noggle - *New York Stem Cell Foundation, New York, U.S.*
Claudia McGinnis - *Roche Innovation Center Basel, Basel, Switzerland*

The mouse embryonic stem cell test (mEST) is an in vitro assay widely used by the pharmaceutical industry to predict the teratogenic potential of new compounds. It combines a cytotoxicity study with a differentiation test to assess teratogenic potential using a statistical prediction model. Prediction inaccuracy is a major weakness of this assay, however, because it uses results obtained from a murine model to infer the teratogenic potential of compounds in humans. In addition, the mEST has important limitations such as labor-intensive cell differentiation methods and the nature of the differentiation test (which relies on a manual count of beating cardiac tissue). To address these issues, we have developed an automated embryonic stem cell test using human induced pluripotent stem (hiPS) cells and the NYSCF Global Stem Cell ArrayTM. This automated platform is being developed to facilitate rapid and efficient screening of a panel of compounds on human iPS cells and evaluate their teratogenic potential based on novel cytotoxicity and differentiation studies. Upon validation, this will provide a novel preclinical model of toxicity that will permit high throughput, automated assays to assess for potential teratogenicity in human rather than murine cells.

Funding Source: Funding provided by NYSCF (501c3) and F. Hoffmann-La-Roche Ltd.

W-4045

A 3D BIOPRINTING SYSTEM FOR ENGINEERING CELL-EMBEDDED HYDROGELS BY DIGITAL LIGHT PROCESSING

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Bioprinting has been applied to produce 3D cellular constructs for tissue engineering. Microextrusion printing is the most common used method, however, printing low viscosity bioink is a challenge for this method. Herein, we developed a new 3D printing system to fabricate cell-laden hydrogels via a DLP-based projector. The bioprinter is assembled from low-cost equipment including stepper motor, screw, LED-based DLP projector, open source computer hardware and software. The system can use low viscosity and photo-polymerized bioink to fabricate 3D tissue mimics in a layer-by-layer manner. In this study, we used gelatin methylacrylate (GelMA) as bioink for stem cell encapsulation. In order to reinforce the printed construct, surface modified hydroxyapatite has been added in the bioink. We demonstrated the silanization of hydroxyapatite can improve the crosslinking strength between the interface of hydroxyapatite and GelMA. The results showed that the incorporation of silanized hydroxyapatite into the bioink had an enhancing effect on the mechanical properties of printed hydrogel. In addition, the hydrogel had low cytotoxicity and promoted the cell differentiation of embedded human

bone marrow stem cells (hBMSCs) and retinal pigment epithelium (RPE) cells. Moreover, this bioprinting system has ability to generate microchannels inside the engineered tissues to facilitate diffusion of nutrients. We believe this 3D bioprinting system has the potential to fabricate various tissues for clinical applications and regenerative medicine in the future.

Funding Source: This work was supported by the grant from Ministry of Science and Technology, Taiwan (MOST 106-2314-B-010-031).

W-4047

KDM1A ENHANCED THE OSTEO/ DENTINOGENESISOF THE STEM CELLS FROM APICAL PAPILLA VIA BINDING WITH PLOD2

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Dental tissue-derived mesenchymal stem cells (MSCs)-mediated pulp-dentin regeneration is considered a potential approach for the regeneration of damaged teeth. Enhancing MSC-mediated pulp-dentin regeneration is based on an understanding of the molecular mechanisms underlying directed cell differentiation process. Histone demethylation enzyme, lysine demethylase 1A (KDM1A) can regulate the differentiation of some MSCs, but its role in dental tissue-derived MSCs is unclear. We obtained SCAPs from immature teeth. Alkaline phosphatase (ALP) activity assay, Alizarin red staining, quantitative calcium analysis, osteogenesis-related genes expression, and in vivo transplantation experiment were used to explore the osteo/dentinogenic differentiation. Co-immunoprecipitation (Co-IP) assay was used to investigate the binding protein. Knock-down of KDM1A reduced ALP activity and mineralization,promoted the expressions of osteo/dentinogenic differentiation markers DSPP, DMP1, BSP and key transcript factors, RUNX2, OSX,DLX2 in SCAPs, and also enhanced the osteo/dentinogenesis in vivo. In addition, KDM1A could associate with PLOD2 to form protein complex. And knock-down of PLOD2 inhibited ALP activity and mineralization, and promoted the expressions of DSPP, DMP1, BSP, RUNX2, OSX and DLX2 in SCAPs. KDM1A might have different role in different stage of osteo/dentinogenic differentiation process by binding partner with PLOD2, and final resulted in the inhibited function for the osteo/dentinogenesis in SCAPs. Our studies provided a further understanding of the regulatory mechanisms of dynamic osteo/dentinogenic differentiation process in dental tissue MSCs.

W-4049

RECOVERY OF PARALYZED LIMB MOTOR FUNCTION IN CANINE WITH COMPLETE SPINAL CORD INJURY FOLLOWING IMPLANTATION OF MSC-DERIVED NEURONAL NETWORK SCAFFOLD

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Our previous study showed that bone marrow mesenchymal stem cell (MSC)-derived neuronal network scaffold, constructed by tissue engineering approach, survived in the injury/graft site of spinal cord, integrated into the host neural circuits and served as “neuronal relay” to improve the limb motor function in a complete spinal cord injury (SCI) rat model. Whether such strategy could be capable of repairing large spinal cord tissue loss and of restoring motor function of large animal still need to be explored. In the current work we adopted the established tissue engineering method to construct canine MSC-derived neuronal network scaffold and evaluated its therapeutic efficacy in treating adult beagle dog with completely transected spinal cord injury. The results showed that after co-culturing with neurotropin-3 (NT-3) overexpressing Schwann cells in a three dimensional gelatin sponge scaffold for 14 days, TrkC (the receptor for NT-3) overexpressing canine MSCs differentiated into neuron-like cells, some of which were endowed with the capability of generating action potentials and connected each other through synaptic structure with trans-synaptic electrical activities. The complete SCI beagle dogs received the transplantation of the MSC-derived neuronal network scaffold manifested a gradual regaining of paralyzed limb motor function, along with improved eletricophysiological presentation, when compared with those only received the transplantation of gelatin sponge scaffold. MRI and DTI imaging suggested that dogs received transplantation of MSC-derived neuronal network scaffold had more robust nerve tract regeneration in the injury/graft site of spinal cord. Histological analysis showed that a portion of MSC-derived neuron-like cells survived in the injury/graft site up to 6.5 months and integrated with BDA labeled corticospinal tract nerve fibers and 5-HT positive descending nerve fibers by synapse-like structures as detected by immunoelectron microscopy. C-fos expression was observed in donor cells locating in the injury/graft site following electrical stimulations on the somatomotor cortex. Taken together, the results suggest the transplanted MSC-derived neuronal network scaffold may function as “neuronal relay” to restore paralyzed limb motor function in canine with complete SCI.

Funding Source: 1. National Natural Science Foundation of China (No.: 81330028) 2. National Key R&D Program of China (No.: 2017YFA0104701).

W-4051

SERUM DEPRIVATION ACCELERATES UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS DIFFERENTIATION TOWARDS ADIPOCYTES AND OSTEOBLASTS

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Dilafitria Fauza, – *Stem Cell and Cancer Institute, PT. Kalbe Farma, Tbk., Jakarta, Indonesia*
Windy Lois – *Stem Cell and Cancer Institute, PT. Kalbe Farma, Tbk, Jakarta, Indonesia*
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Christine Lagonda – *Stem Cell and Cancer Institute Kalbe Farma Tbk, Jakarta, Indonesia*

Serum is an essential substance in cell culture media in which commonly produced from animals. It is known that serum contains nutrition and cytokines for cell growth, as well as adhesive molecules to assist cells attachment. Furthermore, serum also provides less stress environment for the cells and could act as protease inhibitor to protect their protein stability. Fetal bovine serum (FBS) has been widely used in cell culture due to its beneficial effects and ideal for the growth of various cell types. In this study, we found that FBS deprivation in UC-MSCs culture media was able to accelerate cell differentiation towards adipocytes and osteoblasts when induced by differentiation medium compared to cells cultured in media containing serum. MSCs were isolated from umbilical cord and cultured in Minimum Essential Medium (MEM) containing 10% Fetal Bovine Serum and 1% Antibiotic-Antimycotic up to 6th passage. The cells were cultured for 9 days in two conditions: serum-free medium (starved) and serum-contained medium (non-starved) and then further checked their MSC specific surface markers (CD 105, CD 73, CD 90) and ability to differentiate into adipocytes and osteoblasts. Differentiation of the cells was observed in two time points, 7th and 14th days for adipocytes and three time points, 7th, 14th, and 21st days for osteoblasts. The differentiated cells were then continued for histological staining and quantification assay. The immunophenotype data showed that in three different batches there was an unstable result of surface markers of UC-MSC. Batch 1: CD 90 91.48%, CD 105 97.05%, CD 73 99.09%, Batch 2: CD 90 89.87%, CD 105 67.25%, CD 73 83.56%, and batch 3: CD 90 94.65%, CD 105 87.75%, CD 73 92.76%. The < 95% value of MSC surface markers could possibly be related to reduced stemness of the MSC. Starved UC-MSCs while induced by differentiation medium were differentiated into adipocytes on the 7th day, a week earlier than non-starved UC-MSC which is on the 14th day. A similar result in osteoblasts differentiation given by starved UC-MSC which differentiated in 14th days while it took 21 days for non-starved MSC. It could imply that the fate of MSC in serum-deprived condition is not only apoptosis but also promoting differentiation towards adipocytes and osteoblasts when the microenvironments of differentiation are available in situ.

POSTER SESSION I-EVEN 19:30 – 20:30

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-1002

SCALABLE BIOREACTOR PLATFORMS FOR PRODUCTION OF HUMAN AMNIOTIC EPITHELIAL CELLS-DERIVED EXOSOMES

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The human placenta harbor a variety of stem cells populations. Human amnion epithelial cells (hAECs), isolated from amniotic membrane, are currently being used in pre-clinical studies and clinical trials with reports that hAECs possess multipotent differentiation, immunosuppresive, anti-inflammatory, and anti-fibrotic activities. Their therapeutic benefits are largely attributed to their secretome, and like most stem cells, hAECs secrete large number of extracellular vesicles, particularly exosomes that carry bioactive cargo. To use amniotic exosomes as cell-free therapy, development of scalable platform for exosomes production is of critical importance. We evaluated two clinically-compatible bioreactor platforms for hAECs culture and the subsequent secretome yield and quality were compared to traditional 2D culture. hAECs were cultured under defined serum-free conditions in conventional 2D culture system, biaxial agitation bioreactor, and fixed bed bioreactor. Cell viability, pH, glucose and lactic acid levels were monitored daily. Conditioned media were sampled daily and potency assessed for immunomodulatory and pro-angiogenic activity, as has been shown in hAECs. However, only hAEC-conditioned medium from biaxial agitation bioreactor showed comparable immunomodulatory properties on T cell proliferation, HUVEC angiogenesis, and macrophage phagocytosis as expected from 2D culture. Amniotic exosomes were isolated by serial ultracentrifugation; particles size distribution was measured by NTA. Protein yield and particle numbers were significantly higher in amniotic exosomes cultured in both bioreactors compared to 2D culture. The microenvironment in bioreactor systems

altered amniotic exosomes biogenesis. The biaxial agitation bioreactor produces higher mass transfer due to its unique mixing pattern and also demonstrates better cell viability for cell suspension systems, therefore represents a robust and effective method for cGMP production of amniotic exosomes.

W-1004

MESENCHYMAL STEM CELLS FROM HUMAN DECIDUA BASALIS AS A DOUBLE-EDGED SWORD IN MODULATING THE FUNCTIONS OF HUMAN NATURAL KILLER CELLS

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Najla Alshehri - *King Abdullah International Medical Research Center, Riyadh, Saudi Arabia*
Abdulaziz Almutairi - *King Abdullah International Medical Research Center, Riyadh, Saudi Arabia*

Mesenchymal stem cells from the decidua basalis of human placentae (DBMSCs) express a compheresnive range of molecules with a modulatory functions on their targets. These properties make DBMSCs useful for cellular therapy. Here, we studied DBMSC interaction with natural killer (NK) cells, and the effects of this interactions on NK cells and DBMSCs. DBMSCs were cultured with IL-2 activated and resting unactivated NK cells isolated from healthy human peripheral blood. NK cell proliferation and cytolytic activities were then studied using functional assays. NK cell expression of receptors mediating their cytolytic activity against DBMSCs, and the mechanism underlying this effect on DBMSCs, were also studied. DBMSCs inhibited IL-2-induced proliferation of resting unactivated NK cells while increased the proliferation of activated NK cells. Moreover, DBMSCs were lysed by high number of IL-2-activated NK cells while they resisted lysis by low numbers of NK cells but their proliferation reduced. NK cell cytolytic activity against DBMSCs was mediated by a number of activating NK cell receptors. In addition, NK cell preconditioning by low number of DBMSCs did not inhibit their lysis of cancer cells but their ability to lyse cancer cells was inhibited after preconditioning them by high number of DBMSCs, and this was also associated with a reduction in cancer cell proliferation. DBMSCs also increased NK expression of molecules with anticancer activities. We conclude that DBMSCs have a dual functions on NK cells that could enhance NK cell anticancer therapeutic potential.

Funding Source: King Abdullah International Medical Research Center.

W-1006

IN VIVO IDENTIFICATION OF A NOVEL PROGENITOR IN THE MESENCHYMAL CORE OF HUMAN PLACENTAL VILLI USING A 16 COLOUR FLOW CYTOMETRY PANEL

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Anna Brooks - *University of Auckland, Auckland, New Zealand*
Larry Chamley - *University of Auckland, Auckland, New Zealand*
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During embryogenesis, the placenta initially develops at a faster rate than the embryo, giving rise to the first fetal blood vessels at around 15 days post conception, when the embryo still exists in the three germ layers. The formation of these placental blood vessels is key for the placenta to exchange nutrients between the mother and baby, but our understanding of their origins is very poor. It is thought that mesenchymal progenitors within the core of placental villi differentiate to give rise to the vascular cell lineages in the placenta. The core of placental villi is also thought to be a site of haematopoiesis, but the origins of blood cells in the early placenta prior to the establishment of the fetal circulation is unclear. Progenitors involved in these processes have not been characterised in vivo despite the important implications these cells may have for pregnancy success. Therefore, we aimed to identify novel cell populations from freshly digested placental villi using a 16 colour flow cytometry panel incorporating mesenchymal and haematopoietic stem cell markers. To do this, human first trimester (7-12 weeks) and term (37-40 weeks) placental villi (n=6) were digested with DNase, Collagenase and Dispase, first briefly to denude and discard their outer trophoblast layer, then to completely dissociate the villus core. Live (DAPI negative) cells obtained from villus core digests were simultaneously analysed for the expression of CD34, CD31, CD144, Podo, CD105, CD90, CD73, HLA-DR, CD45, VEGFR2, CD36, CD117, CD146, CD271 and CD133 using a BD FACSAria II. From the heterogeneous mix of core cells we identified a novel population that simultaneously expressed endothelial progenitor, haematopoietic and mesenchymal cell markers (CD31, CD34, CD144, CD45, CD73) in both first trimester and term tissue. This combination of markers suggests at the molecular level that this is a progenitor population that may have the capacity to differentiate to both endothelial and hematopoietic cell lineages. Future work to localise, isolate and differentiate this population will aid our understanding of how the very first human blood vessels develop, and the role that this cell may play in normal and abnormal pregnancy.

Funding Source: The University of Auckland Health Research Council (HRC) of New Zealand.

W-1008

EXOSOMES EXTRACTED FROM HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS ATTENUATED THE CARTILAGE DESTRUCTION IN THE OSTEOARTHRITIS RABBIT MODEL

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Osteoarthritis (OA) is a chronic degenerative joint disorder which is characterized by articular cartilage destruction and osteophyte formation. The major goal of this project is to developed OA rabbit model for investigating the therapeutic potential of the exosomes extracted from human umbilical cord mesenchymal stem cells (HUCMSCs). Totally 8 New Zealand White (NZW) rabbits (14 months old) were used for this study. After general anesthesia, the anterior cruciate ligament transection procedure was done for both knees of each rabbit, one was control, the other was for exosome treatment. Exosomes was purified from the 1 X 107 HUCMSCs' conditioned media. Eight weeks later, all rabbits were divided into two groups, four rabbits in each one: one dose group and two doses with an one-month interval group. All the rabbits were euthanized 12 weeks after exosome therapy. The joint surfaces were grossly examined. Histological evaluation was done. Grossly, the joints showed surface erosion both in control and exosome therapy groups. Under microscopic exam, almost all cartilage was destructed in control joints. However, the safranin O staining showed preservation of cartilage in exosome therapy groups. In conclusion, HUCMSCs exosome therapy could attenuate the cartilage destruction in osteoarthritis. Our study provided evidence for future clinical trials.

W-1010

AUGMENTATION OF ANTI-TUMOR ACTIVITIES BY CBLB KNOCKOUT IN HUMAN CD34+ CORD BLOOD-DERIVED NATURAL KILLER CELLS FOR CANCER IMMUNOTHERAPY

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CBLB is a E3 ubiquitin ligase that maintains a balance of immunity and tolerance of immune cells. It negatively regulates various signal transduction pathways for T-cell receptor, B-cell receptor and high affinity immunoglobulin epsilon receptor. In mouse models, CBLB deficiency led to increased T cell and NK cell cytolytic activities and Th1 cytokine secretion. CBLB deficient T cells were resistant to TGFβ mediated inhibition. Tumor metastasis was inhibited in CBLB knockout mouse and this inhibitory effect was regulated via NK cells specifically. Celularity has established a cultivation process to generate human placental intermediate NK (PiNK) cells from umbilical cord blood CD34+ cells with substantial cytolytic activity for cancer immunotherapy. We set out to investigate the role of CBLB in regulating anti-tumor activities of PiNK cells. Using CRISPR technology, over 90% of the wildtype CBLB alleles were modified in PiNK cells and the predominant modification was a single base-pair insertion that causes a frameshift in CBLB protein. When compared with unmodified cells, CBLB knockout PiNK (PiNK-CBLB KO) cells exhibited a similar phenotype and a higher cytotoxicity against a range of solid and liquid tumor cell lines as well as primary tumor cells. PiNK-CBLB KO cells also secreted more Th1 type cytokines in comparison to unmodified cells. In contrast to T cells lacking CBLB, PiNK-CBLB KO cells were still sensitive to TGFβ mediated inhibition. These data suggest that CBLB negatively regulates NK cell effector functions. In addition, PiNK-CBLB KO cells showed in vivo proliferation revealed by CFSE labeling and maturation evident by increased expression of CD16, KIR and NKG2A over a period of 3 weeks post infusion in NSG mice with Busulfan preconditioning and IL-15 supplementation. Moreover, PiNK-CBLB KO cells showed significantly greater anti-tumor activity in a disseminated HL60-luc mouse model compared to unmodified cells. Taken together, PiNK-CBLB KO cells exhibited higher in vitro and in vivo effector function and proliferative capacity compared to non-modified cells. These data suggest that targeting CBLB may offer therapeutic advantages via enhancing anti-tumor activities of NK cells.

ADIPOSE AND CONNECTIVE TISSUE

W-1012

JSJ37 IS A CELL-SURFACE MARKER FOR MURINE OLFACTORY MESENCHYMAL STROMAL CELLS

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Jenny Ekberg - *Griffith University, Southport, Queensland, Australia*
James St. John - *Griffith University, Southport, Queensland, Australia*

The cells of the olfactory mucosa are being widely investigated for their potential regenerative properties, especially for the treatment of spinal cord injury. However, the olfactory mucosa consists of a number of cell-types including olfactory ensheathing cells and mesenchymal stromal cells (MSCs). Characterising the specific regenerative properties of these cells has been hindered by an inability to generate pure cultures of the respective cell types. Research into the therapeutic properties of olfactory mucosal cells would be greatly enhanced by the identification of cell-surface marker proteins that could be used to specifically label these cell-types for both characterisation and purification purposes. Clinically, improvements in the purification process also ensure greater quality control over cells used to treat patients with spinal cord injuries. We have identified JSJ37 as a novel cell-surface marker protein for olfactory MSCs. JSJ37 is expressed robustly from embryonic day 12.5 through postnatal development making it an ideal cell-surface marker for specific immunolabelling of MSCs. JSJ37 is a reliable and unambiguous marker of olfactory MSCs in vivo and in vitro. In conjunction with S100-DsRed transgenic reporter mice, this further facilitates the investigation of MSC-OEC interactions in situ. Additionally, the identification of JS37 as a MSC cell-surface marker in vitro potentially enables the specific immunological selection of MSCs during olfactory biopsy purification.

W-1014

DIABETIC MICROENVIRONMENT AMELIORATES HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS REGENERATIVE CAPACITY

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Human adipose tissue-derived stem cells (ADSCs) are mesenchymal stromal cells (MSCs) that are isolated from adipose tissue. They have a strong proliferative capacity and multiple differentiation potential. ADSCs present no safety issues with respect to the genetic and epigenetic instability of the amplified cells, immune response, and tumorigenicity. Compared with MSCs from other sources, ADSCs are easily accessible and have higher proliferative capacity, making the adipose tissue a good source of autologous stem cells. Recent preclinical studies have shown beneficial effects of ADSC administration for treating diabetes in animal models. However, there is an impairment of resident and transplanted ADSC function under diabetic conditions. We hypothesize that diabetic microenvironment ameliorates the regenerative potential of ADSCs. To test this hypothesis, we cultured ADSCs in culture media supplemented with serum from either type 2 diabetic patients (with Glycated hemoglobin or HbA1c > 6.5%) or normal individuals as control (with HbA1c < 6.5%). We then examined changes in basic characteristics and regenerative potential of ADSCs cultured in diabetic or normal serum. Our results showed that ADSCs cultured in diabetic serum were highly proliferative ($p < 0.05$) compared to cells cultured in normal serum ($p < 0.05$). ADSCs cultured in diabetic serum also showed higher vasculogenesis capacity but reduced osteogenic and adipogenic differentiation capacity than those cultured in normal serum. Diabetic serum enhanced the migration of ADSCs in comparison to control ($p < 0.05$) in wound scratch assay. However, the pattern of expression of MSC specific surface markers such as CD90, CD105 and CD45 showed no significant differences between ADSCs cultured in either diabetic and normal serum ($p > 0.05$). In conclusion, these data indicate that diabetes alters ADSC intrinsic properties and impairs their function, and perhaps the efficacy of transplanted cells using autologous ADSC transplantation. Further investigations are ongoing to determine the effect of the diabetic microenvironment of the efficacy of the transplanted cells in ameliorating diabetes.

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W-1016

ADIPOSE DERIVED MESENCHYMAL STEM CELLS HAVE ANTI-FIBROTIC EFFECTS IN LUNG FIBROBLASTS FROM IDIOPATHIC PULMONARY FIBROSIS PATIENTS

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Idiopathic pulmonary fibrosis (IPF) is a progressive, lethal, and chronic respiratory disease. Though 5-year survival rate is 20-30%, we still do not have any effective therapy. So, it is required to establish a novel therapy

for IPF. Recent studies have assessed the ability of adipose derived mesenchymal stem cells (ASCs), which are expected to have anti-inflammatory and anti-fibrotic effects by secreting cytokines, chemokines, and exosomes. Here, we examined the effect of ASCs by utilizing lung fibroblasts from IPF patients. First, primary fibroblasts were obtained by culturing surgical specimens from both non-fibrotic and fibrotic lesions of IPF patients. We next investigated the expression of fibrosis-related genes, such as ACTA2 (α-smooth muscle actin) and Collagen-I, in the lung fibroblasts from IPF patients co-cultured with or without ASCs. We found that co-culture of ASCs and lung fibroblasts from IPF patients significantly down-regulated the expression of ACTA2 and Collagen-I in the lung fibroblasts compared to the ones without co-culture. Moreover, microarray analysis revealed that mRNA expression of Protein Tyrosine Phosphatase Receptor Type R (PTPRR) was significantly increased in the lung fibroblasts co-cultured with ASCs compared to the ones without co-culture. It is known that PTPRR induces dephosphorylation of ERK1/2, which plays an important role in complex cellular programs like cell proliferation and differentiation through mitogen-activated protein kinase (MAPK) signaling pathway. In addition, western blot analysis revealed that the phosphorylation of ERK1/2 was significantly inhibited in the lung fibroblasts co-cultured with ASCs. These results suggest that ASCs induce PTPRR in the lung fibroblasts, which inhibits the phosphorylation of ERK1/2 and down-regulates the expression of fibrosis related genes such as ACTA2 and collagen-I. These findings ultimately indicate the clinical applicability of ASCs for IPF therapy. We are now investigating the role of PTPRR for anti-fibrotic response in detail.

Funding Source: This work was supported in part by a grant from Rohto Pharmaceutical.

W-1018

ADIPOSE-DERIVED MESENCHYMAL STEM CELL ATTENUATES PULMONARY FIBROSIS IN BOTH BLEOMYCIN-INDUCED AND HUMANIZED MICE MODEL

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Idiopathic pulmonary fibrosis (IPF), the most common lung fibrosis, is a chronic and progressive lung interstitial disease accompanied by the pathological findings of fibrosis with a median survival of 3 years. Despite the accumulated knowledge of IPF through basic and clinical research, an effective medical therapy for IPF remains to be established. Thus, it is necessary to perform further research which provides new insights and perspectives on IPF treatment. Recently, it is reported that one of the new therapeutic candidates for IPF is adipose-derived mesenchymal stem cell (ADSC) which has several advantages such as easy accessibility and minimal morbidity compared to bone marrow-derived mesenchymal stem cell. Therefore, we investigated the possibility of ADSC as therapeutic candidate for IPF. We first demonstrated that ADSC transfer improved the pathogenesis of bleomycin-induced pulmonary fibrosis with reduced collagen deposition in histology and hydroxyproline quantification, and collagen markers such as gene expression of a-smooth muscle actin (a-SMA) and collagen-I in murine model. ADSC transfer was also investigated in humanized mice model of lung fibrosis induced by the infusion of human IPF lung fibroblasts, because the bleomycin installation model does not fully recapitulate the pathogenesis of IPF. Using this humanized mice model, we found ADSC transfer also improved fibrotic changes in the lung. Moreover, in our studies, ADSC produced many kinds of effective cytokines such as hepatocyte growth factor (HGF) against fibrosis and reduced several fibrosis-related genes, such as a-SMA and collagen-I in human IPF lung fibroblasts. Further analysis revealed that ADSC prevented the activation of ERK signaling pathway in IPF fibroblasts via upregulation of protein tyrosine phosphatase receptor-type R (PTPRR), which negatively regulates the ERK signaling pathway. These findings suggest that ADSC would be a promising therapeutic candidate for IPF.

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MUSCULOSKELETAL TISSUE

W-1020

MOLECULAR MECHANISMS REGULATING MUSCLE STEM CELLS QUIESCENCE AND EARLY ACTIVATION

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The microenvironment of a cell can have a major impact on its properties. This is especially true for stem cells, which, due to their plasticity, can radically alter their proliferation and differentiation state in response to changes in their niche. State of the art techniques have

been developed to isolate and analyze stem cells from various tissues, aiming to capture their in vivo state. However, the majority of cell isolation protocols involve lengthy mechanical and enzymatic dissociation steps followed by flow cytometry, exposing cells to stress and disrupting their physiological niche. Focusing on adult skeletal muscle stem cells (MuSC), we have developed a protocol that circumvents the impact of the isolation procedures and captures the cells in their native quiescent state. In the adult, MuSC are an indispensable stem cell population for regenerating injured or diseased muscle as well as maintaining tissue homeostasis. Adult MuSCs are G0 arrested, and their quiescent niche is largely defined by a confined anatomical location, as these cells are positioned between the membrane of the myofiber and the overlying basement membrane. Disturbance of the niche, which occurs during muscle injury or experimental extraction, triggers activation of the MuSCs. Yet, the cellular and molecular mechanisms that maintain this quiescent state remain largely undetermined. We have shown that current isolation protocols of MuSC induce major transcriptional changes accompanied by specific histone modifications, revealing previously undetected quiescence and early activation genes of potentially major biological interest. I will discuss the molecular pathways that are involved in the progressive establishment of early activation.

W-1022

THE LOSS OF EPHRINB1 BY OSTEOGENIC PROGENITOR AFFECTS MOUSE BONE HOMEOSTASIS RESULTING IN AN OSTEOPOROTIC PHENOTYPE

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Bone homeostasis, essential for maintaining skeletal integrity, is a process that tightly regulates the balance between bone formation and resorption. Bone forming osteoblasts and bone resorbing osteoclasts, derived from mesenchymal stem cells (MSC) and haematopoietic stem cells (HSC), respectively, are required for bone homeostasis. Eph receptor tyrosine kinases and their ephrin ligands, differentially expressed by MSC, HSC and their derivatives have been implicated in the processes of skeletal development and bone homeostasis. The present study investigated the importance of ephrinB1 in maintaining skeletal integrity, utilised a conditional deletion of ephrinB1 in osteoprogenitor cells (Osx:cre-ephrinB1fl/fl), under the control of the Osterix promoter (Osx:cre) in a murine model of ovariectomy-induced (OVX) osteoporosis. Micro-computed tomography and histomorphometric analyses demonstrated a significant deterioration in trabecular bone in OVX Osx:cre mice when compared to sham treated Osx:cre control mice. Importantly, Osx:cre-ephrinB1fl/fl sham mice presented similar levels of trabecular bone loss when compared to OVX Osx:cre treated mice, which did not decline further in Osx:cre-ephrinB1fl/fl OVX females. This osteoporotic phenotype observed in Osx:cre-ephrinB1fl/fl sham mice associated with an imbalance of bone homeostasis and subsequent reduction in bone formation rate. This was due to a significant reduction in osteoblast numbers and rise in TRAP+ osteoclast number and function that was analogous to OVX Osx:cre mice, as demonstrated with FACS analysis, histomorphometric analysis, and serum analysis, respectively. Subsequently, in vitro human osteoclast differentiation assays identified EphB2 as the highest expressing EphB receptor during osteoclast differentiation. Furthermore, osteoclastogenic cells cultured in the presence of ephrinB1-Fc significantly suppressed osteoclast formation when compared to the Human-Fc control. Collectively these observations suggest that ephrinB1 reverse signalling through osteogenic cells and activation of EphB forward signalling through osteoclast progenitors assists in the regulation of bone homeostasis to maintain skeletal integrity.

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W-1024

TWIST-1 REGULATED MICRORNA-376C IS A NOVEL PLAYER IN MESENCHYMAL STEM CELL GROWTH AND OSTEOGENIC DIFFERENTIATION

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The proliferation and differentiation of mesenchymal stem cells (MSC) is tightly regulated by multiple pathways that lead to the activation of specific transcription factors. Among these is the basic helix-loop-helix (bHLH) transcription factor TWIST-1. Bone marrow derived MSC (BMSC) and cranial cells (CBC) express TWIST-1, an important mediator of skeletal and head development. BMSC over-expressing TWIST-1 display decreased capacity for osteogenic differentiation. Twist-1 mutant heterozygote mice display abnormal craniofacial and skeletal structures. These abnormalities are replicated in a human childhood disorder known as Saethre-Chotzen syndrome (SCS) which is caused by a mutation in the TWIST-1 gene. Loss of TWIST-1 function in CBC results in increased osteogenic differentiation leading to premature fusion of cranial sutures and SCS. This implicates TWIST-1 as a key regulator of BMSC and CBC proliferation and osteogenic differentiation. An increasing number of studies have demonstrated the involvement of microRNAs (miRNAs) in the proliferation and or osteogenic differentiation of MSC. No BMSC and CBC specific miRNAs regulated by TWIST-1 have previously been studied. We have identified miRNA-376c, a novel TWIST-1 regulated miRNA, which is expressed in BMSC and CBC. Expression of miRNA-376c was down-regulated in BMSC and CBC during osteogenic differentiation, but was increased by TWIST-1. Furthermore, we found that loss of TWIST-1 expression in BMSC and CBC resulted in decreased levels of miRNA-376c when compared with wild type cells. Functional in vitro studies demonstrated that over-expression of miRNA- 376c reduced cellular proliferation, decreased Insulin-like growth factor 1 receptor (IGF-1R) levels and decreased the capacity for osteogenic differentiation. This study is the first to identify a TWIST-1 regulated miRNA important in BMSC and CBC osteogenic differentiation, and will help define the processes that mediate MSC growth and fate determination. Further studies may lead to the development of miRNA synthetic therapies for bone defects in the skeleton and the cranium caused by TWIST-1 mutations.

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W-1026

PHENOTYPE IDENTIFICATION AND MANIPULATION OF MOUSE HYALINAL CHONDROCYTES DURING DEDIFFERENTIATION

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Cell expansion is the first key step for autologous chondrocyte implantation, often accompanied with the chondrocyte dedifferentiation. It is also believed that chondrocytes lose their original phenotype in pathological environment, supposed as the cause of fibrosis at articular surfaces. Dedifferentiated chondrocytes exhibit a fibroblast-like phenotype and produce the mechanically inferior extracellular matrix, but the mechanisms behind are still unclear. This study aims to characterize the phenotype shifting of mouse hyalinal chondrocytes at single cell level, during the dedifferentiation process. Using FluidigmTM C1 high-throughput IFC, we captured single chondrocytes and conducted single cell RNA sequencing of 634 chondrocytes in total, containing in vitro expanded passage 0, 2, 4 and 8. We demonstrated the heterogeneity and pathological features of fibro-like chondrocytes, and uncovered an intermediate subpopulation during chondrocytes dedifferentiation, which was marked with an activation state of metabolism-related genes. By a Seahorse Extracellular Efflux Analyzer, we validated the metabolism switch during dedifferentiation. Finally, to find cocktails manipulating chondrocyte phenotypes, a high-throughput screen of 293 small molecular compounds targeting dediferentiation-related pathways, was conducted on a Col2-pd2EGFP reporter system. Chondrocytes phenotype could be easily manipulated by a candidate inhibitor targeting mitochondial function. In all, our data provides a high-resolution approach for understanding transcriptome states of chondrocyte dedidferetntiation and helps to build a molecular model for cartilage regeneration study.

W-1028

MICRORNAS GUIDE STEM CELL DRIVEN SKELETAL MUSCLE REGENERATION

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Muscular dystrophies are severe muscle diseases characterized by degeneration of skeletal and cardiac muscle. Regenerative strategies to tackle muscle degenerative disorders comprise the use of induced pluripotent stem cells (iPSCs). In this view, we have

isolated mesodermal iPSC-derived progenitors (MiPs) from murine and, more recently, human origin and we have shown that MiPs are endowed with myogenic regenerative potential, further influenced by somatic lineages retention . Here we report that human MiPs from fibroblasts and mesoangioblasts (MABs) can differentially engraft and regenerate the skeletal muscle of dystrophic mice, as MAB derived MiPs perform significantly better in vivo. We suggest that lineage retention programs of MiPs might be behind the differential myogenic capacity and we argue that such programs are driven by microRNA (miRNAs) . We further show that a selective miRNAs cocktail modulation improves the instrinsic myogenic ability of MiPs, allowing a better skeletal muscle engraftment and regeneration in vivo . Additionally, we are exploring a novel therapeutic approach for enhancing miRNAs-driven muscle regeneration. In this light exosomes have been increasingly studied and applied in regenerative medicine fields as delivery methods for tissue regeneration. Our preliminary results document the use of exosomes as miRNAs delivery systems to target muscle degenerative disorders.

CARDIAC TISSUE AND DISEASE

W-1030

INTRACELLULAR EXCITATION-CONTRACTION COUPLING IN ALIGNED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Recent advances in the cardiac field suggest that human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) may be extremely useful for drug discovery or as a therapy for repairing diseased cardiac tissue. However, hPSC-CMs are immature relative to their adult counterparts. In addition to a small round morphology, hPSC-CMs exhibit immature electrical and contractile machinery, including inferior calcium handling and unaligned myofibrils, and have a greater abundance of proteins associated with immaturity such as ssTnI, N2BA, and α-MHC. Together, these deficiencies result in poor excitation-contraction (EC) coupling: the translation of an electrical action potential to a contractile force. Here, we study the effect of artificial alignment on intracellular EC coupling in hPSC-CMs. Artificial alignment has been shown previously to improve myofibril organization and force production. We hypothesize that this myofibril organization may result in improved calcium handling by providing a more organized template for organelles and proteins involved in calcium handling to mature (sarcoplasmic reticulum, ryanodine receptors, SERCA2). We utilize microcontact printing to constrain cardiomyocyte spreading onto lines of extracellular matrix, resulting in rod-like cardiomyocytes. A modified traction force microscopy method allows for intracellular force quantification while simultaneously measuring intracellular calcium dynamics by a calcium indicator (Fluo-4). Preliminary data suggests that calcium handling is somewhat improved when cultured on patterned ECM surfaces yielding physiological aspect ratios. These results are supplemented by a 2D finite element model that accounts for myofibril organization and calcium sensitivity, allowing us to investigate in greater detail the effects alignment on maturation of EC coupling.

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W-1032

FUNCTIONAL EVALUATION OF CRISPR/CAS9 EDITED KCNQ1 MUTATIONS IN CARDIOMYOCYTES DERIVED FROM IPS CELLS OF LONG QT SYNDROME PATIENTS

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Congenital Long QT Syndrome Type-1 (LQTS-1) is a common form of cardiac arrhythmia that is characterized by a prolongation of the QT interval on an electrocardiogram. LQTS-1 is associated with mutations in the KCNQ1 gene encoding a voltage-gated potassium channel. Here, we reported to recapitulate the LQTS-1 disease phenotype in vitro by using cardiomyocytes derived from patient-specific iPSCs, to correct KCNQ1 gene mutation by CRISPR/Cas9 and to evaluate the electrophysiological phenotype following genome

editing. First, we have reprogrammed venous blood cells of a LQTS-1 patient and his healthy family member to iPSCs via Sendai virus encoding pluripotency markers. After reprogramming, iPSC identity was confirmed by expression of pluripotency genes by qRT-PCR, immunocytochemical staining and teratoma assay. To correct the heterozygous, point mutation at pore region of KCNQ1 gene, patient-specific iPSCs were co-electroporated with guide RNA, Cas9 enzyme and GFP encoding plasmid together with ssDNA carrying wild-type sequences. GFP expressing iPSCs were single-cell sorted by FACS. From the screened 476 individual iPSC colonies, 26 colonies showed CRISPR/Cas9 genome editing revealed by Sanger sequencing. While 16 edited colonies were corrected by homologous recombination to healthy allele, 10 colonies showed various ins/dels by non-homologous end joining. Using a similar strategy, the disease causing mutation was generated in *KCNQ1* gene of control iPSCs with 3% efficiency. To electrophysiologically evaluate the cardiac phenotype, cardiomyocytes generated from the healthy, patient-derived and gene-edited iPSCs were analysed for Ca^{2+} currents by Fluo-4 analysis and field potential by the multielectrode system. While we have observed a significant prolongation of QT interval in cardiomyocytes derived from LQTS-1 patient compared to healthy control, KCNQ1 gene correction shorten the QT interval and functionally alleviated the in vitro disease symptoms. Through gene-editing, we have also created new mutations for *in vitro* LQTS-1 studies, that caused prolongation of the QT phase. Collectively, we have showed a direct correlation of electrophysiological cardiac phenotype with KCNQ1 gene sequence, sheding light on the disease mechanism and the potential corrective use of gene-editing in therapy.

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W-1034

PATIENT SPECIFIC IPSC DERIVED CARDIOMYOCYTES AS A MODEL FOR DOXORUBICIN INDUCED CARDIOTOXICITY

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Doxorubicin is an anthracycline type of chemotherapy drug used to treat various types of malignancies including breast cancer, sarcoma and lymphoma, some patients however experience doxorubicin induced cardiotoxicity (DIC) which can cause heart failure and death. Genome wide association studies (GWAS) have identified genetic variation in the RARG gene that is associated with DIC. However, whether this RARG plays a causal role in DIC is currently unknown. The goal of this study was to investigate the role of RARG in DIC using patient-specific induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CMs) from patients with DIC, as well as doxorubicin-tolerant controls. We generated iPSCs from patients who experienced DIC and control patients who received doxorubicin but did not have detectable cardiac dysfunction. We performed directed differentiation to generate CMs, as compared the in vitro sensitivity of CMs from DIC cases and controls to doxorubicin. We also used CRISPR to edit the RARG gene. We assessed doxorubicin toxicity with various cellular and electrophysiological assays. iPSC-CMs from DIC cases and controls displayed expected levels of expression of cardiac markers, MYH7 and cTnT. Doxorubicin treatment caused dose-dependent cell death in iPSC-CM. iPSC-CMs from DIC cases were significantly more sensitive to the toxic of doxorubicin in vitro compared to iPSC-CMs from controls (LD50: 0.7658μM and 3.689μM respectively, $p<0.0001$). Using optical mapping, we found that doxorubicin caused an increase in calcium transient which was significantly more pronounced in iPSC-CMs from DIC cases than controls. We used CRISPR to inactivate RARG. RARG-Knock Out (KO) iPSC-CMs were protected from DIC, with less doxorubicin-induced cell death, compared to isogenic control cells with functional RARG. We observed the same results in embryonic stem cell (ESC)-derived CMs in which we deleted RARG. iPSC-CMs from DIC cases are significantly more sensitive to doxorubicin than iPSC-CMs from control patients, in vivo. Our results showing protection from DIC in cells with inactivation of RARG support a causal role of this gene in DIC.

W-1036

SUITABILITY OF IPSC DERIVED CARDIOMYOCYTES TO MODEL TISSUE SPECIFIC EFFECTS OF MOLECULAR CHAPERONES

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Fabry disease (FD) is rare defect (1 in 40000 males) caused by an X-linked deficiency of the lysosomal enzyme alpha-galactosidase A (GLA, EC 3.2.1.22). The defect leads to progressive accumulation of non-degraded substrates of the enzyme, predominantly globotriaosylceramide (Gb3. The disorder starts to manifest in adolescence typically in form of angiokeratomas, however in the third and fourth decennium of life symptoms related to renal and heart damage appear. If untreated, the patients classically succumb to the disease at age 40-60, mostly due to the end stage renal disease or heart hypertrophy. Besides supportive treatment, the therapy of Fabry disease relies primarily on regular intravenous administration of recombinant alpha-galactosidase A (ERT). However, this treatment is financially demanding, rather uncomfortable for patients and may not to be highly effective alone in treating some affected tissues e.g. cardiomyocytes or neural tissues, perhaps because large enzyme molecules are not transported efficiently to "storage" compartments. It was shown that in cases of selected mutations with residual activity, small molecules - chemical chaperons (CC) - can stabilize the protein structure of the enzyme during its synthesis, improve transport to the lysosomes and thus increase enzymatic activity. To confirm current data from in vitro and in vivo experiments we suggest generating iPSC lines from blood cells of patients with mutations found responsive to CC treatment in previous experiments. These iPSC lines can be differentiated to cellular model of functional human cardiomyocytes and the effect of different chaperones on Gb3 accumulation, enzyme stability (activity and localization) and ultrastructure phenotype can be tested in cell cultures. The iPSC derived cardiomycocytes produced from patient with mutation Q280K show that the activity of the GLA in cardiomyocytes responds well to the molecular chaperone in dossage dependent manner. The endogenous GLA activity was increased up to 76% of normal values. The non-mutant GLA activity in controls was also increased by chaperones however inhibitory effect was also apparent.

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W-1038

REDOX LIVE: GLUTATHIONE REDOX POTENTIAL SENSING IN CELLULAR COMPARTMENTS

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Redox signaling is a key player in cell metabolism and homeostasis. Rapid and sensitive assays are required to detect live redox changes within cells. To study redox events in different compartments we utilized the genetically encoded fluorescent redox sensors Grx1-roGFP2 (cyto) and its mitochondrial targeted variant, mito-Grx1-roGFP2 (mito). The sensors react with the cellular glutathione (GSH) pool and can be used to detect the GSH redox potential by ratiometric fluorescence recording. We generated stable homogenous expression of the redox sensors in HES2-cyto and HES2-mito cell lines by TALEN mediated integration. Recordings of redox sensor response to treatment with the oxidant hydrogen peroxide (H2O2) and the reductant Dithiothreitol (DTT) were performed in a 96-well plate. For cyto the mean fluorescence ratio increased to 1.28 3 0.03 fold over control when treated with 100 µM H2O2 whereas treatment with 1 mM DTT did not lead to signal decrease compared to baseline (1.00 3 0.02 vs 1.02 3 0.02, n = 4). In HES2-mito, a ratio increase of 1.40 3 0.03 fold upon maximal oxidation and a decrease to 0.84 3 0.02 compared to control was recorded (n = 4). The redox potential was calculated using the Nernst equation, cytosolic EGSH was -298 3 6 mV whereas mitochondrial EGSH was -282 3 11 mV in undifferentiated HES. Directed differentiation yielded 73% sarcomeric α-actinin positive cyto+-cardiomyocytes (CMs). Preliminary redox recordings showed an 1.70 3 0.08 fold increase in fluorescence ratio upon stimulation with 100 µM H2O2 and a 0.91 3 0.03 fold decrease with 1 mM DTT (n = 6). Similarly, differentiation of mito+ HES2 led to 87% sarcomeric α-actinin positive CMs. Full oxidation by 100 µM H2O2 led to a 1.68 3 0.10 fold increase in fluorescence ratio, maximal reduction resulted in a 0.93 3 0.04 fold decrease (n = 6). The observed EGSH in stem cell derived CMs was -296 3 6 mV in cytosol and -304 3 3 mV in mitochondria (n = 6). Our data demonstrate how redox sensors can be applied to measure redox potentials in cytosol and mitochondria in undifferentiated HES and cardiomyocyte derivatives. ROS-indicator HES will enable us to investigate ROS-mediated mechanisms controlling pluripotency and differentiation. In future experiments we plan to dissect the role of cytosolic and mitochondrial ROS signals for fate decisions and cardiomyocyte function.

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W-1040

NKX2.5+ CARDIOMYOBLAST SECRETED EXOSOMES IMPROVED METABOLIC DYSFUNCTION OF CARDIOMYOPATHIC MYOCYTES

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Exosomes secreted from cardiac precursors (CPC-exo) were found to possess cardioprotective action in post-injured hearts. The aim of the study was to assess whether CPC-exo could modulate cardiometabolism in cardiomyopathy. Using transgenic mice (2 weeks old) carrying GFP reporter driven by Nkx2.5-enhancer, postnatal Nkx2.5+ cardiomyoblast were enzymatically isolated and were purified by FACS sorter. CPC-exo was further extracted from the culture medium by ExoQuick-TC (System Biosciences LLC, Palo Alto, CA, USA) for the advanced study in human cardiomyocytes derived from iPSC of left ventricle non compaction (LVNC) patients (LVNC-hiPSC-CM). The mitochondrial function of LVNC-hiPSC-CM was examined by seahorse. The progression of cardiac mitochondrial dysfunction in association with the decrease of cell shortening was detected from day 50 through day 70 after differentiation. CPC-exo treatment (on day 61, 64, 67) prominently improved the basal and maximal mitochondrial respiration of LVNC-hiPSC-CM on day 70 in parallel with the increase of cell shortening. The exosomal miRNAs were characterized by miRNA microarray. The validation of exosomal miRNA expression by qPCR found the presence of the potential miRs, including miR-30c, -92b, -98 and -let-7a, in CPC-exo. In conclusion, the present study demonstrated the therapeutic potential of CPC-exo in the improvement of cardiometabolic dysfunction in LVNC.

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W-1042

CYTOPROTECTIVE ROLES OF A NOVEL COMPOUND, MHY-1684, AGAINST HYPERGLYCEMIA-INDUCED OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN HUMAN CARDIAC PROGENITOR CELLS

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Diabetic cardiomyopathy (DCM) is tightly linked to heart disorders and dysfunction or death of the cardiomyocytes including resident cardiac progenitor cells (CPCs) in diabetic patients. In order to restore loss-of function of resident or transplanted CPCs, much research has focused on novel therapeutic strategies including the discovery of novel function-modulating factors such as reactive oxygen species (ROS) scavengers. Here, we developed and defined a novel antioxidant, MHY-1684, for enhancing the angiogenic potential of CPCs against ROS-related DCM. Short-term treatment with MHY-1684 restored ROS-induced CPC cell death. Importantly, MHY-1684 decreased hyperglycemia-induced mitochondrial ROS generation and attenuated hyperglycemia-induced mitochondrial fragmentation. We observed that the activation process of both Drp1 (phosphorylation at site of Ser616) and Fis-1 is drastically attenuated when exposed to high concentrations of D-glucose with MHY-1684. Interestingly, phosphorylation of Drp1 at site of Ser637, which is an inhibitory signal for mitochondrial fusion, is restored by MHY-1684 treatment, suggesting that this antioxidant may affect the activation and inhibition of mitochondrial dynamics-related signaling and mitochondrial function in response to ROS stress. In conclusion, our finding of the novel compound, MHY-1684, as an ROS scavenger, might provide an effective therapeutic strategy for CPC-based cell therapy against diabetic cardiomyopathy.

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W-1044

EXTENDED IN VITRO CULTURE IS CRUCIAL FOR A ROBUST HYPERTROPHIC RESPONSE IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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There is a substantial need for improved in vitro models for studies of cardiac hypertrophy. In this study we investigated the capacity of cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) to respond to hypertrophic stimuli. The cardiomyocytes were stimulated with endothelin-1 or phenylephrine during 24 hours. These substances are known to cause hypertrophy in vivo. The cells were incubated with different concentrations of the two substances at three different time points; 25 days, 38 days, and 51 days after onset of cardiomyocyte differentiation. We performed transcriptional profiling of several genes important in the hypertrophy response (i.e., NPPB, NPPA, MEF2C, MYH7, MYH6, and ACTA1). We also quantified secreted BNP to investigate the correlation between mRNA and protein expression. Furthermore, several key genes that

are known to be upregulated in mature cardiomyocytes were also analyzed. The results show that genes involved in the hypertrophic response are upregulated at all time points when stimulated with endothelin-1. The response is dose-dependent but also dependent on the extent of in vitro culture of the cells. The most robust upregulation of hypertrophic genes was observed at the latest time point (51 days). Stimulation with phenylephrine did not show the same pronounced effects on gene expression as stimulation with endothelin-1. There was, however, a difference in the response at the different time points, similar to that observed with endothelin-1. As expected, gene expression analyses at the different time points also showed that longer time in culture leads to upregulation of genes involved in the maturation of the cardiomyocytes. Taken together, these findings show that hiPSC-derived cardiomyocytes exert a hypertrophic response when stimulated with endothelin-1 and phenylephrine. The results also show that extended in vitro culture and maturation are important aspects to take into consideration when conducting hypertrophy experiments with hiPSC-derived cardiomyocytes. These observations are also likely to extend beyond effects related to cardiac hypertrophy and should be considered when designing experiments and interpreting data from studies using hiPSC-derived cardiomyocytes.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

W-1046

MEOX1 REGULATES HEMATOPOIETIC STEM CELL INDUCTION THROUGH SOMITE DERIVED ENDOTHELIAL CELLS

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A major question in blood development is how the first haematopoietic stem cells (HSC) develop during embryogenesis. Using zebrafish we have previously identified an intra-somitic compartment, termed the endotome, which provides migratory cells that leave the somite and colonize the dorsal aorta where they regulate HSC. The homeobox transcription factor meox1 is critical to this process. Zebrafish mutants lacking meox1 exhibit two related defects in somite patterning, a reduction in the number of cells in the External Cell Layer (ECL) (a layer of muscle stem cells external to the myotome that contribute to myotome growth), and an expansion of the endotome leading to increased HSC induction (Nguyen, Hollway et al. 2014). In this study, we show that somite derived endothelial cells (i.e., endotomal cells) induce HSC formation through the deployment of chemokine signaling. Meox1 inhibits the expression of the chemokine CXCL12b, and loss of meox1 results in upregulation of CXCL12b in endotomal cells HSC induction. Using RNA sequencing and histone modification techniques in the meox1 mutant versus wild type in the early stages of development provide understanding the transcriptional network in HSC generation. We suggest the cytokine mediated pathway might play a key role in regulation of HSC induction through somite derived endothelial cells.

W-1048

PHENOTYPIC HIGH-THROUGHPUT SCREENING OF IPSC DERIVED VASCULAR CELLS COMBINED WITH BIOINFORMATIC APPROACH REVEALS NOVEL DRUG CANDIDATES FOR PULMONARY ARTERY HYPERTENSION

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Pulmonary arterial hypertension (PAH) is a progressive disorder characterized by endothelial cell (EC) apoptosis, loss of distal vessels, and occlusive vascular remodeling. Current PAH therapies only improve symptom with vasodilators, but do not reverse the pathological change underlie PAH. A previous drug screen targeting BMPR2 signaling identified FK506, however, further testing in native PA and induced pluripotent stem cell (iPSC) derived EC indicated that it was effective in improving function only in a subgroup. Given the heterogeneity of PAH with varying degrees of BMPR2 abnormality, targeting improved cell function could be a better strategy. Thus, we screened six PAH iPSC-EC lines for candidates that not only enhance BMPR2 signaling, but also improve EC function. We identified a tyrosine kinase inhibitor (TKI) that improved EC survival, angiogenesis, and reduced smooth muscle cell proliferation; it also reversed PAH in a rat model judged by reduced PA muscularization and right ventricular hypertrophy. Interestingly, we found that this TKI was superior to other TKIs, such as imatinib that had a more modest effect in reversing PAH, in activating the BMPR1-ID1 pathway and improving EC function. Using LINCS database, we confirmed that this TKI was superior in reversing PAH gene signature than other TKIs, and identified novel genes related to the improved function, such as CYCS, which reduces caspase activity, and NFATc1, which promotes VEGF mediated angiogenesis and EC survival. To further identify new compounds that reverse EC dysfunction in PAH, 4,500 bioactive compounds were tested for their ability to improve survival in six PAH iPSC-EC lines following serum withdrawal, using a luminescence assay measuring caspase 3/7 activity. We identified another

two compounds effective in all PAH lines, an antioxidant and a chloride channel inhibitor. We then compared all the agents that improved EC function in a given PAH line, and used LINCS to investigate the common pathway they were targeting. Our study suggests that using patient-specific iPSC derived vascular cells combined with a bioinformatics approach can optimize selectivity among similar drugs, and when used in large-scale drug screening can elucidate pathways that improve a common phenotypic abnormality.

Funding Source: NHLBI K99 HL135258.

HEMATOPOIESIS/IMMUNOLOGY

W-1050

ASSESSMENT OF CLONAL KINETICS REVEALS MULTIPLE TRAJECTORIES OF DENDRITIC CELL DEVELOPMENT

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A thorough understanding of cellular development is incumbent on assessing the complexities of fate and kinetics of individual clones within a population. Here, we develop a system for robust periodical assessment of lineage outputs of thousands of transient clones and establishment of bona fide cellular trajectories. We appraise the development of dendritic cells (DCs) in fms-like tyrosine kinase 3 ligand culture from barcode-labeled hematopoietic stem and progenitor cells (HSPCs) by serially measuring barcode signatures, and visualize this multidimensional data using developmental interpolated t-distributed stochastic neighborhood embedding (DiSNE) time-lapse movies. We identify multiple cellular trajectories of DC development that are characterized by distinct fate bias and expansion kinetics, and determine that these are intrinsically programmed. We demonstrate that conventional DC and plasmacytoid DC trajectories are largely separated already at the HSPC stage. This framework allows systematic evaluation of clonal dynamics and can be applied to other steady-state or perturbed developmental systems.

W-1052

GENERATION OF MATURE MEGAKARYOCYTES AND FUNCTIONAL PLATELETS IS CONTROLLED BY RNA PROCESSING

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Megakaryocytes are bone marrow cells responsible for platelet production. Their maturation involves DNA replication without cytokinesis to increase cell size and ploidy but the molecular mechanisms underlying megakaryocyte maturation are poorly understood. We have identified a distinct megakaryocyte subpopulation that is largely responsible for platelet production. Our work has also revealed that a large transcriptome shift taking place during megakaryocyte maturation depends on RNA processing. Intriguingly, we demonstrate that the RNA binding protein serine-arginine rich splicing factor 3 (SRSF3) plays a key role in the generation of platelet producing megakaryocytes. In *Srsf3*-Pf4-Cre mice, where SRSF3 expression is ablated in megakaryocytes, the platelet producing subpopulation is largely missing, resulting in severe thrombocytopenia. Both cell surface marker expression and ultrastructural analysis using transmission electron microscopy supports the specific loss of platelet producing megakaryocytes when SRSF3 is depleted. RNA-sequencing of different subpopulations of SRSF3 deficient and wildtype megakaryocytes demonstrated that RNA processing mediated by SRSF3 is essential in tuning the megakaryocyte transcriptome. The large shift in RNA repertoire during maturation identified in wildtype cells was absent in Srsf3-null megakaryocytes. Moreover, platelets derived from *Srsf3*-Pf4-Cre mice were morphologically and functionally abnormal. Platelet RNA-sequencing showed that the SRSF3 deficient platelets had greatly altered RNA composition that only partially reflected changes in the megakaryocyte RNA repertoire, suggesting that SRSF3 helps defining platelet 'RNAome' that is critical for platelet function. Our work reveals post-transcriptional RNA processing as a major mechanism regulating megakaryopoiesis and platelet biogenesis, and may reveal new avenues in tackling thrombogenic and haematopoietic disorders.

W-1054

CIS-DIMERIZED JAM-A IS CRITICAL FOR HSC HOMING AND ENGRAFTMENT POST-TRANSPLANT

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The success of hematopoietic stem cell (HSC) transplants relies on the innate ability of HSC to home to bone marrow (BM), engraft and ultimately sustain long-term hematopoiesis. Although it is well appreciated that stem cell engraftment and hematopoietic recovery in humans is highly dependent on the number of infused HSC, it is known not all transplanted HSC and progenitors home to BM. Thus, greater understanding of homing mechanisms should lead to improved transplantation strategies that are not solely reliant on HSC infusion number but focus on the quality of the HSC and their ability to home to BM. Herein, we identified the cis-dimerized form of junctional adhesion molecule A (cdJAM-A) to be highly expressed on murine and human HSC. Notably, blocking JAM-A significantly impairs homing efficiency and engraftment in murine transplant models and was demonstrated to be a critical regulator of HSC quiescence in the niche: injection of anti-cdJAM-A blocking antibody induced preferential expansion of murine BM HSC. Furthermore, treatment of mice using the clinical mobilization agent granulocyte-colony stimulating factor (G-CSF) significantly reduced cdJAM-A expression on HSC as a result of JAM-A cleavage by tumor necrosis factor α-converting enzyme (TACE), which we demonstrated to be upregulated. G-CSF treatment was also associated with reduced expression of the key adhesion receptors CXCR4 as well as α4β1 and α9β1 integrins, which together contributes to stem and progenitor mobilization. Analysis of G-CSF mobilized peripheral blood progenitors also revealed significantly lower expression of cdJAM-A, α4β1, α9β1 and CXCR4, which resulted in reduced homing efficiency post-transplant when compared to progenitors mobilized using the α4β1/α9β1 integrin and CXCR4 antagonists, BOP and AMD3100, respectively. Consequently, we show that a single injection of BOP plus AMD3100 for 1 hour mobilized HSC and progenitors that had significantly greater long-term multi-lineage engraftment potential compared to a 4-day G-CSF approach. Our results identify cdJAM-A as a critical regulator of HSC trafficking and maintenance in the niche and suggests the identification of G-CSF independent mobilization strategies that do not abrogate cdJAM-A expression or function should result in improved clinical HSC transplants.

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W-1056

MAPPING THE TRANSCRIPTOME OF THE HUMAN HAEMATOPOIETIC STEM CELL DEVELOPMENTAL NICHE

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The first human haematopoietic stem cells (HSCs) with long-term, multilineage repopulating capacity first develop in the aorta-gonad-mesonephros (AGM) region between days 32 and 40 post-fertilisation. The molecular mechanisms driving HSC development are not well characterised in humans and efforts to mimic this process ex vivo have been unsuccessful. Intriguingly, within the AGM the HSCs are localised to the ventral portion of the dorsal aorta, co-incident with the appearance of haematopoietic cell clusters which adhere to the ventral aortic endothelial lining. In this study we have mapped the spatially resolved transcriptome for the human embryonic AGM region in order to elucidate molecular signals promoting HSC generation. To achieve this, we have optimised laser-capture-microdissection which allows fine dissection of the aortic endothelium and underlying mesenchyme into discrete domains along the dorsal-ventral axis. RNA-sequencing and bioinformatics analysis of cDNA libraries generated from these dissectates has brought to light genes and pathways polarised along this axis both proximally localised to the clusters and in the perivascular and outer mesenchymal layers. Notably, genes with potent roles in vasoconstriction such as renin and endothelin were ventrally polarised in the perivascular layer indicating a potential role for blood pressure regulation in the HSC developmental niche. Such insights from this data will be functionally validated for roles inherent to HSC generation and allow future refinement of differentiation protocols for the in vitro production of human HSCs for clinical transplantation.

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W-1060

GENETICALLY MODIFIED HESC-DERIVED NK CELLS FOR TREATMENT OF PERSISTENT HIV RESERVOIRS

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This project is evaluating whether genetically modified natural killer cells (NKs), that express an anti-HIV chimeric antigen receptor (CAR), could be used to eradicate persistently replicating HIV in vivo, in the context of anti-retroviral therapy (ART). ART effectively suppresses viral loads in HIV infected individuals. However, the pharmacokinetics and biodistribution of the drugs are uneven throughout the body, and consequently result in creation of pharmacologic sanctuaries that permit low levels of persistent HIV replication over long periods of ART, which could lead to development of drug resistance. Pharmacological approaches to solving this problem have had only limited success so far. Here we propose to use an NK cell therapy based approach to directly target and remove persistent HIV producing cells. We opted to use human embryonic stem cells as the source of genetically modified NKs for several reasons. Peripheral blood derived NK cells arerefractory to genetic manipulation and the use of hematopoietic stem cells, which could later be differentiated into NKs, could also be challenging given their limited life span and propensity to differentiate in vitro. On the other hand, hESC can be maintained in culture in an undifferentiated state indefinitely, and as such they can be extensively genetically manipulated, characterized for potential genotoxic events at the nucleotide level, and expanded to clinically relevant quantities. Also, due to the extended length of their telomere ends, hESC-derived NK cells are less likely than their normal counterparts to undergo cellular senescence and immunological exhaustion upon expansion. We have generated hESC-derived NKs that express an anti-HIV CD4/zeta CAR, that can bind to the HIV gp120 envelope protein on the surface of infected cells, and thereby facilitate NKs recognition of target cells and provide additional activation stimuli for their effector functions. We have shown that the CD4-zeta CAR-expressing NKs kill HIV-infected cells at a significantly higher level than the control NKs, even at a very low effector to target ratios. We are now initiating in vivo experiments in the recently published humanized mouse model of HIV latency (Marsden et al., PLoS Pathog. 2017) to evaluate the potential of these cells to eradicate HIV reservoirs in the context of ART.

W-1062

HIGH PLOIDY LARGE CYTOPLASMIC MEGAKARYOCYTES ARE RESPONSIBLE FOR PLATELET PRODUCTION

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Megakaryocyte (MK) maturation involves DNA replication without cytokinesis, resulting in large (≤65μm) polyploid cells (8N, 16N, 32N and 64N) within the bone marrow. It has long been accepted that large, high ploidy MKs are responsible for the production of platelets; the cells required for blood clotting and wound repair. We now report MKs of single ploidies can be further sub-fractionated by the expression of antigens, including CD41, into large cytoplasmic MKs (LCM, CD41bright) and small cytoplasmic MKs (SCM, CD41dim). We demonstrate LCM prospectively isolated from RFP mice and transplanted into C57Bl/6 mice released significantly more (19-fold) RFP+ platelets than SCM. Following a challenge with anti-platelet serum, which depletes mice of circulating platelets, platelet recovery was accompanied by both a significant increase in high ploidy MK as well as LCM. Furthermore, in a mouse model with normal total MK numbers, but significantly diminished LCM (Pf4-Cre-Srsf3 knockout), severe thrombocytopenia resulted with ~10% the number of platelets. We hypothesis LCM are mature, platelet producing MKs and have undertaken single cell RNAseq and TEM studies to further elucidate the differences between these sub-populations within individual ploidies. Importantly, we identified LCM and SCM in normal human samples and together with our murine findings that ploidy alone is not sufficient for accurately predicting platelet generation, the data suggests attempts to clinically increase platelet production must not only drive high ploidy MK development, but specifically target the formation of LCM.

W-1064

FOXO1 PROMOTES THE MIGRATORY RESPONSE IN POLY(I:C)-STIMULATED HUMAN MSCS

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Mesenchymal stromal cells (MSCs) can potently regulate the functions of immune cells and are being investigated for the management of inflammatory diseases. Toll-like receptor 3 (TLR3)-stimulated human MSCs (hMSCs) exhibit increased migration and chemotaxis within and toward damaged tissues. However, the regulatory mechanisms underlying these migratory activities are unclear. Therefore, we analyzed the migration capability and gene expression profiles of TLR3-stimulated hMSCs using RNA sequencing, wound healing, and transwell cell migration assay. Along with increased cell migration, the TLR3 stimulation also increased the expression of cytokines, chemokines, and cell migration-related genes. The promoter regions of the latter showed an enrichment of putative motifs for binding the transcription factors forkhead box O1 (FOXO1), FOXO3, nuclear factor kappa B (NF-κB1), and RELA proto-oncogene, NF-κB subunit. Of note, FOXO1 inhibition by FOXO1-selective inhibitor AS1842856 significantly reduced both migration and the expression of migration-related genes. In summary, our results indicate that TLR3 stimulation induces hMSC migration through the expression of FOXO1-activated genes.

PANCREAS, LIVER, KIDNEY

W-1066

MOUSE RENAL SUBCAPSULAR TRANSPLANTATION OF HUMAN PSC-DERIVED KIDNEY ORGANOIDS INDUCES NEO-VASCULOGENESIS AND SIGNIFICANT GLOMERULAR AND TUBULAR MATURATION IN VIVO

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Human pluripotent stem cells (PSCs) have the unique ability to differentiate into all lineages that may facilitate approaches for regenerative medicine, such as the generation of tissues for renal replacement. Long term application, however, will require transferability between PSC lines and significant improvements in organ maturation. A key question is whether time or a patent vasculature is required for ongoing morphogenesis. We generated kidney organoids by applying the temporospatial mechanisms that regulate the induction of renal structures during development. The 3-dimensional structures, derived in fully defined medium conditions, contain structures derived from both ureteric epithelium and metanephric mesenchyme progenitor populations. Here we show that the kidney organoids remain disorganized and immature upon prolonged culture. We therefore transplanted the kidney organoids under the renal capsule in immunodeficient mice and observed host-derived vascularisation of the organoid in the absence of any exogenous stimuli. We employed the combinatorial approach of organoid transplantation and an abdominal imaging window to serially image the transplanted organoids in vivo. Imaging of organoids under the renal capsule confirms functional glomerular perfusion as well as connection to pre-existing vascular networks in the organoids. Wide-field electron microscopy demonstrates that transplantation results in formation of a glomerular basement membrane, fenestrated endothelial cells and podocyte foot processes. Furthermore, compared to non-transplanted organoids, polarisation and segmental specialisation of tubular epithelium is observed. These data demonstrate that functional vascularisation is required for progressive morphogenesis of human kidney organoids. The matured mini-kidneys are an important step forward for future applications in the development of a bioengineered kidney.

W-1068

ABCG2 DEFICIENCY IN HEPATOCYTES DISRUPTS MITOCHONDRIAL FUSION/FISSION BALANCE AND IMPAIRS REPROGRAMMING-MEDIATED MOUSE LIVER REPAIR

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The ATP binding cassette transporter ABCG2 are found to express in a wide variety of stem cells as well as several somatic tissues including the liver. Here, we utilized ABCG2 knockout mice and found that ABCG2-deficient hepatocytes had increased amounts of fragmental mitochondria accompanied by disruption of mitochondrial dynamic and function. We demonstrated that this disruption was due to elevated levels of intracellular and mitochondrial protoporphyrin IX content, which further led to increased reactive oxygen species and up-regulated DRP-1-mediated mitochondria fission via p53 pathway activation. Correspondingly, we hypothesized that impairment in mitochondrial dynamics can affect hepatocyte reprogramming. By treating mice with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), a porphyrinogenic agent, we confirmed that periportal hepatocytes can be reprogrammed into Sox9-expressing progenitor cells capable of replenishing damaged hepatocytes. Moreover, we were able to determine that ABCG2 contributed to this process by transporting N-methyl-PPIX, which is produced during the breakdown of Cytochrome P450. Therefore, ABCG2 deficiency could not only disrupt the balance of p53-regulated mitochondrial fusion/fission, but also restrict N-methyl-PPIX transportation thus inhibiting Sox9 induction and hepatocyte reprogramming. In contrast, this inhibition could be reversed by p53 deletion. Collectively, our findings suggest ABCG2 transporter protects hepatocytes from PPIX accumulation and links mitochondrial dynamics with hepatocyte reprogramming during liver injury.

W-1070

KIDNEY EXPRESSION OF KLK4 IN HUMAN BIOPSIES IN LUPUS NEPHRITIS

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hKLK4 is one of the fifteen proteases of the Kallikrein-Kinin system (KLKs). Their broad functions include regulation of inflammation and blood pressure, among others. Recently, it was found that hKLK4 decreases in Lupus Nephritis (LN) kidneys, favouring fibrosis and disfunction, and although miR-422a increase was reported as the responsible, the molecular mechanisms underlying these processes require to be elucidated. The main purpose of this research is to evaluate in a LN cellular model the molecular pathway by which human Mesenchymal Stem Cells from Bone Marrow (hBM-MSC)-mediated hKLK4 exerts its protective function, which was recently described in a LN animal model. At this preliminary stage, we aimed to determine the protein expression of KLK4 in renal tissue from patients with LN (1) compared with patients with nephritis in other autoimmune disease (2) and nephritis no autoimmune (3). In five kidney biopsies from each of the three groups, we measured hKLK4 expression by immunohistochemistry along with infiltration of lymphocytes T (CD3) and monocytes/macrophages (CD68). Expression intensity was obtained with imageJ software. Statistical analysis for comparing the media of the intensities included ANOVA test for KLK4 and a Wilconson test for CD68 and CD3. Histological findings were markedly altered in (1) versus the controls. We found hKLK4 differentially expressed between the groups significantly as follows: (1) < (2) < (3) (p-value < 0.0000). Similarly, as it was observed in vivo, LN is related with monocyte/macrophage (p-value < 0.004) but not with lymphocytes infiltration (p-value < 0.22). These preliminary findings, allowed us to confirm the affectation of hKLK4 regulation in LN with a probable inflammatory mechanism. Thus, in further experiments we will test other antibodies commercially available as limited publications do exist, establish its use as a biomarker of disease progression and explore the role of increased hKLK4 as a potential biological therapeutic in conjunction with the immunomodulatory properties of a cell therapy based on hBM-MSC. Alternatively, we will explore the use of anti-sense therapy against miR-422a using functionalized nanoparticles. All this together as the initial approach for the foundation of a program in regenerative medicine for LN.

Funding Source: Clínica de la Costa, Barranquilla, Colombia.

W-1072

MODELING MODY1 AND MODY4 USING PLURIPOTENT STEM CELLS

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Maturity onset diabetes of the young (MOD¬Y) is a monogenic, autosomal dominant inherited disease that constitutes up to 5% of all diabetic cases. It presents at childhood or adolescence, and eventually leads to pancreatic β-cells dysfunction. 14 genes are known to cause different types of MODY, many of them

encode transcription factors essential for pancreatic development and function. MODY disorders are driven by haploinsufficiency, where loss-of-function mutations in a single allele of one of the MODY causing genes leads to the diabetic phenotype in heterozygous patients. Two important transcription factors that regulate early stages of pancreas development are hepatocyte nuclear factor 4 alpha (HNF4α) and pancreatic and duodenal homebox 1 (PDX1), and mutations in these genes leads to MODY1 (5-10% of MODY cases) and MODY4 (1-2% of MODY cases) disorders, respectively. In order to model MODY1 and MODY4 we generated induced pluripotent stem cells (iPSCs) harboring HNF4α and PDX1 mutations either by reprogramming of patient's somatic cells or by CRISPR/CAS9 gene editing. Utilizing a step- wise protocol to differentiate the pluripotent cells towards endodermal and pancreatic progenitor cells enables exploring the role of the transcription factors in their relevant stage of pancreas development. Thus, we differentiated MODY1 iPSCs towards primitive gut tube cells, and analyzed their gene expression profile. We found a group of HNF4α targets with a downregulated expression in MODY1 cells and characterized their molecular features. Our molecular analysis suggests that the number of HNF4α binding sites, their distance from the transcription start site, and the number of other transcription factor binding sites affect the expression levels of HNF4α target genes. These features may help explain the molecular manifestations of haploinsufficiency in MODY1 disease. Additionally, we created both homozygous and heterozygous PDX1 mutant lines, with different genetic background, aimed to model MODY4 disease. Differentiation of these cells towards pancreatic progenitors cells and analyzing their expression profile, together with the previous analysis of MODY1 cells, will contribute to revealing the outcome and mechanism of haploinsufficiency in key regulators of pancreas development.

W-1074

SENESCENCE SUPPRESSED PROLIFERATION OF HOST HEPATOCYTES AND INDUCED EXPRESSION OF INSULIN-LIKE GROWTH FACTOR 2 FOR LIVER REPOPULATION

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Hepatocyte transplantation holds great promise as an alternative to orthotopic organ transplantation in the treatment of liver diseases. However, to obtain the expected rate of liver repopulation was still hard because of unknown mechanisms during the process of hepatocytes proliferation in the recipient livers, which should be studied widely and deeply. In fumarylacetoacetate hydrolase deficient ($Fah^{-/-}$) mice model, we explored cellular destiny under liver injury after NTBC withdrawal. Next, we investigated the potential mitogens as "driver" for promoting proliferation in recipient $Fah^{-/-}$ mice. Gene knockdown assays were performed to determine the regulating mechanism of insulin-like growth factor 2 (IGF2) mitogens on enhancing proliferation. Finally, evaluation on effects of the applying IGF2 on promoting liver repopulation was performed in several animal models. Hepatocytes became senescence in $Fah^{-/-}$ mice when exposed to liver injury, resulting in precondition for proliferation of transplanted hepatocytes. Moreover, senescent host hepatocytes with cell arrest presented high-expression of IGF2, which directly enhanced proliferative capacity of transplanted hepatocytes. Decreased IGF2 expression of $Fah^{-/-}$ mice reduced liver repopulation after hepatocyte transplantation, regulated by inhibiting the activation of PI3K/Akt and MAPK pathways. Furthermore, supplementation of IGF2 could improve liver repopulation in several models with liver injury. Similarly, in the liver of Tyrosinemia Type I patients, hepatocytes also underwent senescence and induced IGF2 expression. These findings underscore the underlying mechanisms of completed liver repopulation in $Fah^{-/-}$ mice and indicate that IGF2 could be studied as a hepatocyte mitogen for liver cell transplantation therapies.

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W-1076

FUNCTIONAL AND TRANSCRIPTOMIC EVALUATION OF HUMAN PLURIPOTENT STEM CELL DERIVED PANCREATIC PROGENITORS USING MULTIPLE DIFFERENTIATION PROTOCOLS

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The differentiation of human pluripotent stem cells (hPSC) to fully functional insulin-producing beta cells is likely to be achieved most efficiently by recapitulating embryonic development of the pancreas. A critical stage of pancreas development is the establishment of multipotent pancreatic progenitors (PP) that can give rise to all lineage of the pancreas, including endocrine beta cells. Here we present a thorough characterization of three differentiation protocols adapted from recent publications for deriving PP from hPSC. All three protocols efficiently generated PP expressing the canonical transcription factors PDX1, SOX9 and NKX6.1. hPSC from multiple donors displayed varying propensities to generate PP using the individual differentiation protocols and we found that early modulation of BMP signalling could alleviate the efficiency of poorly differentiating hPSC lines. We differentiated an NKX6.1-GFP hPSC reporter line side-by-side to the PP stage of the three protocols and FACS isolated GFP+ and GFP- cells for whole transcriptome analysis. We observed significant differences in the PP transcriptional profile from the different protocols, with the largest diversity present in the GFP- populations. Despite this heterogeneity, we identified a common PP gene expression signature including NKX6.1, SOX9, NOTCH1 and PTF1A and enriched for pancreas multipotent progenitor signature genes known to be expressed in human foetal pancreas tissue. Interestingly, we noted that expression of several monogenic and neonatal diabetes genes; e.g. GCK and HHEX differed significantly between protocols both in terms of expression levels and specificity to the GFP population. Finally, we observed differences in the ability of the PP derived from the different protocols to differentiate further towards the endocrine lineage, including beta cells. The distinct transcriptomic signatures of the PP derived with the three protocols may provide clues for further development and refinement of protocols for deriving fully functional pancreatic beta cells. Moreover, the observed protocol diversity could have important implications for present and future interpretations of disease-modelling using hPSC.

Funding Source: This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (StemBANCC grantn° 115439).

W-1078

COMPARATIVE STUDY OF NORMOXIC CULTURED AND HYPOXIC PRECONDITIONED HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELL THERAPY IN A RAT MODEL OF RENAL ISCHEMIA-REPERFUSION INJURY

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The purpose of this study was to determine the therapeutic effects of hypoxic preconditioned human bone marrow-derived mesenchymal stromal cell (hBMSC) and compare with normoxic cultured hBMSC in a rat model of renal ischemia-reperfusion injury (IRI). Fifty male Sprague-Dawley rats were randomly divided into 5 groups (10 animals per group): sham, nephrectomy control, IRI control, normoxic cultured hBMSC injection, hypoxic preconditioned hBMSC injection. To induce renal IRI, the left renal artery was clamped with a vascular clamp for 40 minutes, and the right kidney was removed. 4 x 106 hBMSCs were injected via left renal artery shortly before IRI. Serum creatinine, BUN and glomerular filtration rate were evaluated 4 days prior to IRI, and 1, 2, 3, 4, 7 and 14 days after IRI. For histological studies, the kidney was removed 14 days after IRI. Normoxic cultured hBMSC injection significantly reduced the extent of elevation in serum creatinine compared with the IRI control group 3 days after IRI. Normoxic cultured hBMSC injection significantly reduced the extent of decrease in glomerular filtration rate compared with the IRI control group 14 days after IRI. Hypoxic preconditioned hBMSC injection significantly reduced the extent of elevation in serum creatinine compared with the IRI control group 3, 7, 14 days after IRI. Hypoxic preconditioned hBMSC injection significantly reduced the extent of decrease in glomerular filtration rate compared with the IRI control group 7, 14 days after IRI. Sirius red stain for the degree of fibrosis showed that renal cortex of both hBMSC injection groups were significantly less fibrotic than that observed in the IRI control group. TUNEL assay showed significantly decreased apoptosis in renal cotex of hypoxic preconditioned hBMSC injection compared to the IRI control group. A greater increase in glutathione reductase and glutathione peroxidase was observed in renal cotex of hypoxic preconditioned hBMSC injection than in the IRI control group. These findings further suggest that anti-oxidative responses were elicited by IRI and hBMSC treatment contributed to further anti-inflammatory and anti-oxidative effects after IRI in this study. Renal function is effectively rescued from renal IRI through renal arterial injection of both normoxic cultured and hypoxic preconditioned hBMSC.

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W-1080

OPTIMIZATION OF THE GENERATION OF RENAL COLLECTING DUCT EPITHELIUM FROM HIPSCS

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The ureteric bud (UB) develops as a side branch of the nephric duct (ND) forming a branched tree-like structure that will ultimately form the ducts of the urinary collecting system (CD). Here we report the development of a protocol to recreate the ND/UB cell lineage via the directed differentiation of human pluripotent stem cells (hiPSCs). Differentiation was performed using fluorescently-tagged human iPSC lines (GATA3: m-Cherry and RET (tdTOMATO+) as a readout of collecting duct differentiation with the protocol an adaptation of our previously described protocol for patterning to anterior intermediate mesoderm (Takasato et al, 2015). Cells subjected to this differentiation protocol initiated the expression of key genes thought to be of ND/UB lineage including PAX2, PAX8, GATA3, WNT9B, GFRα1, and RET. The cellular composition and epithelial organization within organoids and extended monolayer cultures were evaluated using immunofluorescence. This revealed robust epithelial structures which were GATA3+PAX2+ECAD+KRT8+RET+ETV5+, which is consistent with an enriched nephric duct/ureteric bud epithelium. At day 7+5 17.8% of cells became RET/tdTomato+. Subsequently, RNA was extracted from the sorted populations to evaluate expression levels of a subset of genes distinguishably expressed in ureteric tip+ and stalk+ segments. Strikingly, the expression levels of genes associated with cells of the ND/UB tip+ were significantly increased, including RET, GFRα1, and ETV4. Conversely, evaluation of the gene set of UB/stalk markers (WNT7B, WNT9B, and TACSTD2) did not show either increase or equivalence of RNA levels when compared to human foetal kidney expression (HFK). The establishment of this methodology provides a valuable platform to now investigate the capacity of this epithelium to respond to a metanephric mesenchyme. We also anticipate that the use of this methodology will ultimately facilitate three-dimensional bio-printing and allow the recreation of larger kidney structures.

EPITHELIAL TISSUES

W-1082

REGENERATION OF THE ENTIRE HUMAN EPIDERMIS USING TRANSGENIC STEM CELLS

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Junctional epidermolysis bullosa (JEB) is a severe and often lethal genetic disease caused by mutations in genes encoding the basement membrane component laminin-332. Surviving patients with JEB develop chronic wounds to the skin and mucosa, which impair their quality of life and lead to skin cancer. Here we show that autologous transgenic keratinocyte cultures regenerated an entire, fully functional epidermis on a seven-year-old child suffering from a devastating, life-threatening form of JEB. The proviral integration pattern was maintained in vivo and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained not by equipotent progenitors, butby a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew in vitro and in vivoand produce progenitors that replenish terminally differentiated keratinocytes. This study provides a blueprint that can be applied to other stem cell-mediated combined ex vivo cell and gene therapies.

W-1084

EFFECT OF PRO-REGENERATING COMPOUNDS ON THE HUMAN SKIN-RELEASED SOLUBLE FACTORS DURING THE RE-EPITHELIALIZATION PHASE OF WOUND HEALING

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In wound-healing, during re-epithelialization phase, epithelial precursor cells are highly requested and migrate gradually from the edges of the wound. The process is stimulated and controlled by growth factors produced by fibroblasts and keratinocytes. We previously developed a human 3D reconstructed skin model, "migration model", that introduces a step of keratinocyte migration such as the one observed in wound-healing re-epithelialization phase. Understanding the growth factor, cytokine, and chemokine communication network between keratinocytes and fibroblasts during the wound healing re-epithelialization phase is crucial to improve the efficacy of pro-regenerative compounds. In this study, we first characterized the effects on the migration model of four pro-epithelializing compounds: a plant polyphenol (Ellagic Acid), an essential oil of flower, a Centella Asiatica extract and Ascorbic Acid. The effects were evaluated on i) keratinocyte migration kinetics and ii) reconstructed epidermis thickness and quality. Second, the conditioned media collected during the 3D reconstruction process was analyzed using a protein multiplex approach and, for each tested compound, a specific signature of soluble factors released during the re-epithelialization process was identified. A comparison of these signatures allowed key factors (MCP-1, MCP-3, GM-CSF) involved in the re-epithelialization process to being determined, thus contributing to a better understanding of the mechanisms involved in cutaneous regeneration.

W-1086

COMBINING TALEN, CRISPR AND HOMOLOGOUS RECOMBINATION TECHNOLOGIES TO PRODUCE HUMAN DISEASE-IN-A-DISH MODELS OF CYSTIC FIBROSIS (CF).

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Nowadays, modeling human genetic disorders using induced pluripotent cells has become a common strategy thanks to a combination of patient-derived cell lines and genome editing technologies. Sometimes, however, the best *in vitro* model for the dissection of complex cellular phenotypes might arise from long established laboratory cell lines. In this communication we will discuss the production and characterization of a collection of Calu-3 cell lines in which the most common Cystic Fibrosis mutations have been introduced. Cystic Fibrosis is a hereditary disease produced by the absence or malfunctioning of the Cystic Fibrosis Transmembrane Conductase Regulator (CFTR) gene. To date over 1,200 alterations in the DNA composition of the CFTR gene have been detected. CF is a degenerative disease, which can be considered as the main genetic cause of death in Caucasian children. Its first manifestations occur in early childhood, generally affecting pancreas, intestine and respiratory tract, later extending to other organs. Calu-3, a human airway epithelial cell line derived from a lung adenocarcinoma, has many characteristics that made it the most used model for the study of Cystic Fibrosis. Calu-3 cells show high levels of CFTR expression, CAMP-dependent Cl- secretion and form polarized epithelia. However, a limiting factor in the applicability of Calu-3 cells is the lack of genetic variants that could mimic the phenotypes associated with the most common human CFTR genotypes. A combination of CRISPR and TALEN, together with ssODN-mediated knock-in and co-selection strategies have been used to generate the classical p.F508del and G551D mutants, as well as a series of new null and deletion alleles. In addition, the resulting lines have been equipped with a genetically encoded Iodine Yellow Fluorescent Protein sensor, that detects physiological changes inside the cells. By providing a collection of different CFTR genotypes on

the same genetic background, this new cell platform will allow the careful dissection of the molecular and physiological mechanisms affecting CFTR expressing cells. This data together with the information obtained from more complex structures, like epithelial organoids, will provide a comprehensive approach to the study of cystic fibrosis.

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W-1088

TRANSIENT SCUTE ACTIVATION VIA A SELF-STIMULATORY LOOP DIRECTS ENTEROENDOCRINE CELL PAIR SPECIFICATION FROM SELF-RENEWING INTESTINAL STEM CELLS

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The process through which multiple types of cell lineage-restricted progenitor cells are specified from multipotent stem cells is unclear. Here we show that, in intestinal stem cell (ISC) lineages in adult Drosophila, in which the Delta-Notch-signaling-guided progenitor cell differentiation into enterocyte is the default mode, the specification of enteroendocrine cells (EEs) is initiated by transient Scute activation in ISCs that is driven by transcriptional self-stimulation combined with a negative feedback regulation between Scute and Notch targets. Scute activation induces asymmetric ISC divisions that generate EE progenitor cells (EEP). Moreover, the mitosis-inducing and fate-inducing activities of Scute guide each EEP to divide exactly once prior to its terminal differentiation, yielding a pair of EEs. Therefore, the transient expression of a fate inducer specifies both the type and the number of committed progenitor cells from stem cells, which could represent a general mechanism used for diversifying committed progenitor cells from multipotent stem cells.

W-1090

GRAINYHEAD ISOFORMS FUNCTION IN DROSOPHILA INTESTINAL STEM CELL MAINTENANCE AND DIFFERENTIATION

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The constant renewal of the intestinal epithelium is achieved through the activity of a population of intestinal stem cells (ISC). These cells maintain tissue homeostasis by ensuring balance between cell division and the production of the differentiated cell types of the intestine. This process is under strict molecular control with ISC maintenance and function controlled by the expression of a particular subset of genes and their production of multiple protein isoforms. However, the mechanism by which different protein isoforms are able to regulate intestinal regeneration remains poorly understood. Using the adult intestine of Drosophila melanogaster, we identified grainyhead (grh) as a candidate gene that is able to influence regeneration using different protein isoforms. grh produces two protein isoforms, GRH.N and GRH.O via alternative splicing. Transcripts spliced with exons 4 and 5 produce GRH.O protein isoform while those without produce GRH.N. Expression analysis performed using droplet digital PCR shows that transcripts for GRH.N and GRH.O, the neural specific isoform are expressed in the intestine, albeit at low cellular levels. Further studies using cell lineage tracing techniques and GRH null mutants result in a reduction of progeny arising from a single ISC. Interestingly, cell lineage tracing using a GRH.O specific mutant resulted in a more severe reduction in progeny number and the loss of GRH.O mutant ISCs over time. In the opposite experiment, GRH.O was ectopically expressed in ISCs and its immediate daughter, the enteroblast (EB). Over expression in ISCs did not lead to a change in ISC number while over expression in EBs led to a block in differentiation and an increase in EB number. On the other hand, ectopic expression of GRH.N in ISC and EBs led to a loss of ISC and EBs through forced differentiation. We therefore hypothesize that GRH.O is required for ISC maintenance while GRH.N is needed for differentiation.

EYE AND RETINA

W-1092

COMPARATIVE STUDY OF HUMAN ADIPOCYTE-DERIVED STEM CELLS FROM ORBIT AND ABDOMEN

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Adipose tissue contains abundant multipotent stem cells with strong proliferative and differentiating into adipocytes, osteocytes and chondrocytes. However, adipocyte-derived stem cells showed variable characteristics based on the tissue-harvesting site. This study aimed to compare human adipocyte-derived stem cell from orbit (Orbital-ASCs) and abdomen (Abdominal-ASCs). Orbital and abdominal ASCs were isolated by fat debulking procedure during an upper or lower blepharoplasty operation and liposuction respectively. We performed Oil Red O staining for Adipogenesis, Alzarin Red for osteogenesis and Alcian blue for chondrogenesis. Real time PCR was used to measure mRNA levels of genes involved in this tri-lineage differentiation. CD surface antigens were analyzed by flow cytometry and cytokine profiles (Eotaxin, Fractalkine, GRO, IL-6, IL-8, IP-10, MCP-1, and RANTES) by Luminex assay kit. Our results showed Orbital-ASCs has potent tendency of differentiation into adipocytes and osteocytes than compared to Abdominal-ASCs, whereas as chondogeneis was potent in Abdominal-ASCs, which was further illustrated by comparing the genes involved in tri-lineage differentiations. Also, Orbital-ASCs and Abdominal-ASCs have many cell surface markers in common. However, CD34, CD73 and CD105 are expressed higher in Orbital than abdominal-ASCs. Unlikely, Orbital-ASC expressed CD 31, CD45, CD90, CD146 and HLA-DR lesser than abdominal-ASCs. Furthermore, Orbital-ASCs express higher levels of Eotaxin, Fractalkine, GRO, IL-6, IL-8, IP-10, MCP-1, and RANTES than Abdominal-ASCs. In conclusion, tissue-harvesting site is a strong determinant for characterisation of adipocyte-derived stem cells. Understanding defining phenotypes of such cells is useful for making suitable choices in different clinical indications.

W-1094

THE APPLICATION OF STEM CELL TISSUE-ENGINEERED BIO-POLYMER BASED BIOMATERIAL SCAFFOLDS FOR RETINAL PROGENITOR CELLS CULTIVATION

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The use of tissue-engineered scaffolds as physical supports for cell delivery represent a promising approach for treatment of retinal degenerative disorders. In this study, polymer scaffolds for cell retinal progenitor cells (RPC) were fabricated using electrospinning method employing basic polymers such as poly-(L-lactic acid) (PLLA), poly (lactic-co-glycolic acid) (PLGA), poly-caprolactone (PCL) and a blend of poly-caprolactone (PCL) with poly (glycerol-sebacate) (PGS). Following the fabrication, the RPCs were seeded onto the scaffold samples for up to 7 days. Image analysis methods were applied on stained image of the seeded scaffolds. The extracted features reveal that RPCs grown on aligned PCL/PGS polymer blend nanofibers exhibited expanded morphology, whereas the cells did not display such characteristic morphology across the other three types of scaffolds (i.e. PLLA, PGS and PCL). These results demonstrate that the PCL/PGS polymer scaffolds could offer a carrier modality for stem cell-derived RPCs for the restoration of vision in subjects who have lost vision from retinal degenerative disorders.

W-1096

MONOLAYER CULTURE OF HUMAN PLURIPOTENT STEM CELL DERIVED PHOTORECEPTORS FROM THREE-DIMENSIONAL OPTIC CUP ORGANOIDS.

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Retinal dystrophies and macular degeneration lead to degradation of photoreceptors, resulting in permanent vision loss and placing an increasing burden on global health care. To understand the underlying pathogenic events, it is important to consider how photoreceptors are affected. Due to the difficulty in obtaining ocular tissue from living donors, patient induced pluripotent stem cells (iPSCs) provides a powerful tool to understand fundamental disease processes and subsequent compound screening to rescue retinal function. Further, organoids offer a powerful model to study both development and disease pathology. Retinal organoids/ neural retinas can be generated from human iPSCs. These organoids generally recapitulate early events in human development, and show organisation of cells into structures of the human neural retina. Here, we used neural retina as a source of photoreceptors, with the aim of obtaining cultures of human photoreceptors in sufficient amount and purity for experimental work. Neural retinas/optic cups were generated from human PSCs using protocols developed by Nakano et al (2012) and Reichman et al, (2017). Photoreceptors were then isolated from hPSC-derived whole optic cups by magnetic activated cell sorting (MACS) with selection by the photoreceptor marker and cell surface antigen, CD73. CD73 positive cells were sorted from 100-day-old and >200 day-old-derived optic cups. CD73+ cells were plated onto Matrigel and subsequent immunohistochemistry, qPCR and functional assays were performed to assess maturity and functionality

of the isolated cells. Our data suggest that selection of photoreceptors using CD73 is an effective method for isolating photoreceptors from three dimensional optic cups, to obtain a pure photoreceptor population, thus indicating a powerful tool for culturing mature photoreceptor cells in vitro.

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W-1098

FUNCTIONAL CORNEAL ENDOTHELIAL REGENERATION FROM HUMAN EMBRYONIC STEM CELLS

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The corneal endothelium is a hexagonal monolayer forming a boundary between the Descemet's membrane and the anterior chamber. This layer of cells plays a crucial function in the maintenance of the dehydration and transparency of cornea. Once corneal endothelial cells (CECs) are damaged or malfunctions, corneal edema and vision loss ensues. In order to improve the sight, corneal transplantation is the goal standard treatment for the management of CECs injury or dysfunction at present. However, shortage of cornea donors is the main difficulty in transplantation. Replacement of the damaged corneal endothelium by CECs directly is more applicable. Unlike corneal epithelium can regeneration from limbal stem cells, CECs are terminally differentiated with barely cell division capabilities after well development. Therefore, the acquisition of suitable and sufficient cell resource for CECs transplantation is extremely important in the field of corneal regenerative medicine. Here we elucidate an applicable differentiation procedure to derive human CECs from human embryonic stem cells (hESCs) under defined culture conditions. After differentiation of hESCs into CEC-like cells, the corneal endothelium differentiation marker N-cadherin, tight junctional protein ZO-1, functional pump protein sodium-potassium ATPase, and the main component of the corneal stroma collagenVIIIA1 were detectable. By this study, we can obtain quantity of human CECs in hope of bringing these cells into tissue engineering and clinical use in the future.

STEM CELL NICHES

W-1100

A METHOD TO ISOLATE AND TRANSPLANT MOUSE HEMATOPOIETIC STEM CELLS ALONG WITH THEIR NICHE ALLOWING FUNCTIONAL HEMATOPOIETIC STEM CELL ENGRAFTMENT WITHOUT MYELOABLATION

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Hematopoietic stem cell (HSC) transplants provide the best chance of 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 donor HSCs and the toxicity of myeloablative regiments necessary to enable engraftment. Attempts at expanding HSCs ex vivo prior to transplantation have generated mixed results and may not maintain the functional hematopoietic repopulating cells. We investigated the survival and engraftment potential of HSCs from Luciferase-GFP mice isolated with their native niche microenvironments as intact units. We devised a new micro-fluidics-based method to isolate intact niches containing phenotypic CD150+CD48-Sca+cKit+Lin- HSCs surrounded by Thy+, 6C3+, VECadherin+ stromal cells and tested the ability of HSC-Niche units to support HSC survival in vitro and HSC engraftment into non-myeloablated immunodeficient mice. For the in vitro experiments, HSCs were cultured (1 x 105/10cm3 well) for 2 weeks: a) within their cellular niches, or b) alone. Cultured HSCs were then dispersed from niches/culture by mechanical dissociation and transplanted into myeloablated mice by intravenous injection to assay HSC mediated hematopoietic reconstitution. Results indicated HSC-Niches cultured for 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 by bioluminescence

in vivo imaging (IVIS) of the left femur. Our results indicated that isolating and transplanting HSC-Niches facilitates ex vivo HSC survival and non-myeloablative HSC engraftment. Our approach also suggests a new paradigm for stem cell therapy using high-throughput microfluidic-based FACS isolation of intact stem cells-niche units for ex-vivo culture/modification and site-specific transplantation.

W-1102

INFUSED BONE MARROW DERIVED CELLS HAVE TWO FUNCTIONS THAT PHAGOCYTOSIS OF DAMAGED CELLS AND REPAIR OF FIBROSIS

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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 2004). In this study, we estimated characterization of infused BMC in liver cirrhosis using Electron Microscopy (EM) and the therapy of environment in recipient liver. C57BL/6 mice were injected with CCl4 twice a week to make the liver cirrhosis. GFP-positive BMC were infused from tail vein and sacrificed at 4 weeks after BMC infusion. The liver sample was fixed using both paraformaldehyde+glutaraldehyde and made epon section. We analyzed the characterization of the infused GFP-postive BMC using both EM and Immune EM (IEM). We analyzed the image of IEM, comparing with the character of positive cells by immunohistochemistry and double fluorescent staining (Antibody:GFP,MMP9,hepatoblastmarker-Liv2,Liv8-CD44,A6,EpCAM,CXCR4,transcription regulator-maternal of inhibitor of differentiation -Maid). We analyzed some kind of gene by Real-Time PCR(Gene: p21,Sirt1,Sirt6,P16, AK4,H-mox,Ncam,AFP etc). We found two kinds of GFP positive BMCs in recipient cirrhosis liver using IEM method. One 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 were circular forms and 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 Kupffper cell in size.The other group of GFP positive BMCs was similar to hepatocyte in size(15-30um) and located around fiber. MMP9 positive cells, Maid positive cells, CXCR4 positive cells,Liv8 positive cells were same. These cells were round forms and different from Kupffer cell or stellate cell in feature and had the increase of lysosome structure in cytoplasm. These cells were located on fiber in hepatic cord and repaired fibrosis. We detected two kind of infused BMCs. The BMCs repaird liver fibrosis and worked the phagocytized damaged hepatocyte and maintenance of liver.

W-1104

INVESTIGATING LGR5 AS AN EPITHELIAL STEM/PROGENITOR MARKER IN THE ADULT MOUSE UTERUS

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Leucine-rich repeat containing G protein-coupled receptor 5 (Lgr5) is a Wnt-regulated marker of proliferative epithelial stem cells in the murine intestine, stomach and hair follicle. It has been proposed Lgr5 may also mark hormonally responsive stem/progenitor cells in the endometrium, the highly regenerative lining of the uterus. Our aim was to examine whether the widely used Lgr5-GFP mouse can be used to identify endometrial stem cell populations during cyclic endometrial regeneration associated with the estrous cycle. GFP expression was investigated in adult (5-6 months of age) mouse endometrium of Lgr5-GFP mice in combination with immunofluorescence detection of the epithelial marker EpCAM. Uteri at various stages of the hormonally regulated estrous cycle (metestrus, proestrus and dietestrus) where identified by vaginal smear cytology. Lgr5-GFP was detected by confocal microscopy using its endogenous fluorescence in formaldehyde-fixed frozen sections, and by immunodetection with an antibody recognising GFP. Lgr5-GFP small intestine and ovary were used as positive controls. As previously reported, Lgr5-GFP cells were readily detected in the intestinal crypt and also in the ovarian surface epithelium, verifying our immunolabelling and imaging techniques. Lgr5-GFP+ cells were not observed in the adult mouse endometrium at any of the estrous cycle stages examined, by either endogenous GFP fluorescence or antibody-based detection. We conclude that, in spite of fact that Lgr5-GFP identifies stem cells in several other organs, it does not mark a stem/progenitor population in the adult mouse endometrium. A lack of Lgr5-GFP expressing epithelial cells in the adult endometrium may indicate that this tissue is maintained by a stem/progenitor population that is regulated by mechanisms distinct from the Wnt/Lgr5 signalling that operates in the intestine and other organs.

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W-1106

REMODELING OF TYPE I COLLAGEN BY MMP13 SECRETION OF MESENCHYMAL STEM CELLS IN BONE HEALING

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Matrix metalloproteases (MMPs) are essential for the intra- and extra-cellular biology of stem cells, such as proliferation, survival, morphogenesis, and differentiation through extracellular matrix remodeling. Type I collagen (Col I) is a major component of bone tissues, which is known to promote osteogenic differentiation of human mesenchymal stem cells (hMSCs). However, the mechanism of the effects of Col I on osteogenesis or bone healing is still not clear. In this study, we found that MMP13, among other MMPs, is highly expressed in hMSCs grown on a Col I matrix during osteogenic differentiation. The MMP13 knock-down lead to diminished osteogenic differentiation and MMP13 overexpression increased osteogenic differentiation. Moreover, rhMMP13 treated Col I matrix stimulated the capacity of calcium deposition of hMSCs. In order to characterization of Col I treated with rhMMP13, the results shown that unwind Col I without cleavage (upto 10 ng per 1 μg of Col I). In addition, Col I triggered integrin α3 expression and RUNX2 translocation into nucleus from cytosol. It is known that RUNX2 is transcription factor which bind to MMP13 promoter. We suggest that MMP13 initiated and enhanced osteogenic differentiation of hMSCs through unwind Col I and the activation of focal adhesion molecules including integrin α3. Consistent with in vitro experiment, rhMMP13 treated Col I sponge improved bone regeneration of the calvarial defect in mouse cranium compared with control Col I sponge. Taken together, we suggest that unwind Col I by MMP13 promote osteogenic differentiation of hMSCs with Col I/MMP13/RUNX2 positive feedback loop.

W-1108

DEXRAS1 IS A HOMEOSTATIC REGULATOR OF EXERCISE-DEPENDENT PROLIFERATION AND CELL SURVIVAL IN THE HIPPOCAMPAL NEUROGENIC NICHE

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Adult hippocampal neurogenesis is highly responsive to exercise, which promotes the proliferation of neural progenitor cells and the integration of newborn granule neurons in the dentate gyrus. Here we show that genetic ablation of the small GTPase, Dexras1, suppresses exercise-induced proliferation of neural progenitors, alters survival of mitotic and post-mitotic cells in a stage-specific manner, and increases the number of mature newborn granule neurons. Dexras1 is required for exercise-triggered recruitment of quiescent neural progenitors into the cell cycle. Pharmacological inhibition of NMDA receptors enhances SGZ cell proliferation in wild-type but not dexras1-deficient mice, suggesting that NMDA receptor-mediated signaling is dependent on Dexras1. At the molecular level, the absence of Dexras1 abolishes exercise-dependent activation of ERK/MAPK and CREB, and inhibits the upregulation of NMDA receptor subunit NR2A, bdnf, trkB and vegf-a expression in the dentate gyrus. Our study reveals Dexras1 as an important stage-specific regulator of exercise-induced neurogenesis in the adult hippocampus by enhancing pro-mitogenic signaling to neural progenitor cells and modulating cell survival.

W-1110

STEM/PROGENITOR CELLS CONTRIBUTE TO EPITHELIAL REPAIR FOLLOWING ENDOMETRIAL BREAKDOWN IN A MENSTRUATING MOUSE MODEL.

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Endometrial regeneration is a highly complex, tightly controlled process. Stem/progenitor cells have been implicated in the regeneration of the tissue. Using the stem cell marker telomerase reverse transcriptase (Tert), we have shown mouse Tert (mTert) promoter activity in epithelial, endothelial and leukocyte populations in cycling mouse endometrium1. We hypothesised that cells expressing mTert may be involved in endometrial repair and remodelling in a mouse model of menses, and contribute to repair of the luminal epithelium. mTert-GFP mice were subjected to a previously published mouse model of menses2. Briefly, mice were ovariectomised, treated with oestradiol and progesterone, artificially decidualised and progesterone removed to induce a menses-like event. Tissues were collected for histochemical and flow cytometry analysis at 0hrs, 8hrs, 24hrs and 48hrs after progesterone withdrawal. mTert reporter activity was identified in rare

cells in the residual (unshed) luminal epithelium during breakdown (8hrs), repair (24hrs) and remodelling (48hrs). Histological analysis of mTert reporter activity in 17,902 epithelial cells revealed that only 1.1% of epithelial cells were mTert+. There was a significant increase in the percentage of mTert+ luminal epithelial cells at the repair ($p<0.001$) and remodelling ($p<0.05$) time-points compared with prior to tissue breakdown (0hrs). Triple immunofluorescence staining for mTert-GFP, epithelial marker EpCAM and proliferation marker Ki67 revealed extensive proliferation of residual luminal epithelial cells during repair and remodelling. mTert-GFP+ cells were typically observed as clusters, interspersed between Ki67+ proliferating cells. Proliferating epithelial cells were rarely mTert+ (0.72%). These findings are the first to show putative epithelial progenitors present in repairing luminal epithelium of endometrium. The clusters of epithelial mTert-GFP+ cells suggests the endometrium prepares for cyclical re-epithelialisation by distributing stem/progenitor cells along its luminal surface. We propose that epithelial mTert activity is activated to support the rapid re-epithelialisation of the endometrium, by undergoing asymmetrical division to form a transit amplifying cell that then rapidly divides.

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W-1112

HUMAN DENTAL PULP STEM CELLS: CHARACTERIZATION AND NEURONAL DIFFERENTIATION

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Mesenchymal stem cells (MSC) represent a population of multipotent cells that have a wide distribution in adult tissues. Dental pulp mesenchymal stem cells (DPSC) have been the target of numerous researchers because of the easy extraction and the elevated potential for therapy. Furthermore, due to its common ectodermal origin with neurons, it can be used as treatment of neurodegenerative diseases. This study aimed to characterize DPSC phenotypically, functionally and analyze the expression of the neuronal markers βtubulinIII and nestin after the induction of neuronal differentiation. This study had the approval of the local Research Ethics Committee (CAAE:42751615.8.0000.0020). Dental pulps were extracted, sectioned into small pieces and dissociated with collagenase type I. Immunophenotypic characterization and differentiation of the cells was carried out between P3 and P5. For the immunophenotypic characterization, a panel of antibodies suggested by the International Society for Cellular Therapy for the definition of MSC and co-stimulatory molecules CD40, CD80 and CD86 were used. The DPSC were differentiated into four lineages (adipogenic, osteogenic, chondrogenic and neuronal) for 21 days. For neuronal differentiation, the cells were analyzed by immunofluorescence technique with anti-βtubulinIII antibody and by qRT-PCR with nestin gene. The DPSC had plastic adherence, fibroblast-like morphology, immunophenotypic profile positive for CD29, CD73, CD90, CD105, CD166 and reduced expression for CD14, CD19, CD34, CD45, HLADR and the co-stimulatory molecules CD40, CD80 and CD86. In the DPSC, it was possible to observe the osteogenic and chondrogenic differentiation but not the adipogenic differentiation. After neuronal differentiation, it was possible to observe the expression of βtubulinIII and an increase in nestin expression. This study demonstrates that DPSC are easy to obtain and expand in culture. In addition, they have characteristics in common to MSC obtained from other tissues and can be used as an alternative source of stem cells. Due to their great potential for neuronal differentiation, DPSC may be an excellent option for studies to treat neurological diseases or injuries in the future.

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W-1114

DROSOPHILA MUSASHI IS REQUIRED IN THE OVARY TO REGULATE FOLLICLE STEM CELL FATE DECISIONS

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Recent studies have shown that the Musashi (Msi) family of RNA-binding translational repressors are expressed in proliferative cells of the mammalian central nervous system, intestine, skin, blood, mammary gland and stomach, leading to the proposal that Msi proteins are stem cell markers and regulate stem and progenitor cell biology. Using the Drosophila testis as a model system, we previously demonstrated that Drosophila Msi is required intrinsically in germline stem cells (GSC's) for

maintenance of the GSC fate. In this study, we further investigate whether loss of Msi function affects stem cell regulation in the ovary. We found that GSC regulation in the Drosophila ovary was not dependent upon Msi function, since GSC clones lacking Msi function were maintained at the same frequency as control clones over a period of 3 weeks. However, we found that follicle stem cell (FSC) clones lacking Msi function were slowly lost over this same period. In addition, lineage tracing experiments and clonal studies revealed that Msi mutant FSCs preferentially contributed to the population of escort cells over follicle cells. Cell cycle dynamics were also altered in the population of msi mutant FSCs, with an increase in G2 cells observed using the fly FUCCI system. These results lead us to conclude that Msi plays an integral role in co-ordinating the cell fate decisions of FSCs.

NEURAL DEVELOPMENT AND REGENERATION

W-2002

THREE-DIMENSIONAL INDUCTION OF DORSAL, INTERMEDIATE AND VENTRAL SPINAL CORD TISSIES FROM HUMAN PLURIPOTENT STEM CELLS

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The spinal cord contains more than twenty distinct subclasses of neurons, which form well-organized neural circuits that are capable of sensing the environment and generating motor behavior. Recent studies have described the efficient in vitro generation of single neuronal population in the spinal cord, including spinal motor neurons and V2a interneurons, however, the formation of a three-dimensional structure of spinal cord tissues has not been described. In the present study, we first demonstrate a new protocol for dorsal spinal cord-like tissue induction from human pluripotent stem cells. Our protocol enabled the efficient induction of continuous neuroepithelial structures that expressed several dorsal progenitor domain markers, and the expression pattern was similar to developing embryonic dorsal spinal cord. Furthermore, these in-vitro dorsal spinal cord-like tissues could generate four different types of dorsal interneurons. By the activation of Shh singling, intermediate and ventral spinal cord-like tissues were successfully induced. After dissociation of these tissues, somatosensory neurons and spinal motor neurons were detected and expressed neurotransmitters in the same manner as in vivo. Our induction method recapitulates the in vivo developmental process of spinal cord formation, and thus will contribute to the research on the organization of human spinal cord and application towards regenerative medicine.

W-2004

THERAPEUTIC POTENTIALS OF HUMAN NEURAL STEM CELL PRECONDITIONED WITH BAICALEIN-ENRICHED FRACTION FOR ISCHEMIC STROKE

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Ischemic stroke is triggered by occlusion of blood circulation to brain, resulting in an area of ischemia where the brain tissue becomes irreversibly damaged. Neural stem cell (NSC) is a promising cell source for brain tissue regeneration. Nonetheless, the damage caused by ischemic stroke is impossible to be completely repaired or replaced by endogenous NSCs due to low neuronal turnover that could integrate into functional neuron network. Therefore, it is imperative to develop new strategies to improve the therapeutic potential of NSC for stroke recovery. Recently, many studies are focusing on neuroprotective properties of medicinal herbs as complementary treatment for neuroregenerative treatment. This study aimed to extract baicalein-enriched fraction (BF) from the leaves of a local plant - Oroxylum indicum and use the extract to precondition human NSC line (H9-hNSC) prior to transplantation into in vitro ischemic stroke (IVIS) model developed using differentiated SH-SY5Y cells exposed to oxygen-glucose-deprivation insult for 2h, following by reperfusion for 20h. Baicalein was eluted in its highest content (~29%) in 100% methanol as solvent, indicated by TLC and HPLC screening assays. Preconditioning of H9-hNSCs using 1.56 µg/mL of BF for 24 hours significantly increased proliferation, cell viability and lineage specific differentiation, compared to non-treated cells. Besides, BF-preconditioning also activated various target genes involved in neuroprotective signaling including the antioxidant markers Nrf2 (1.92-fold) and SOD1 (2.16-fold) which play role to protect the transplanted H9-hNSC against ischemic-induced oxidative damages, as well as the angiogenic markers VEGFA (4.13-fold) and ANGPT1 (3.96-fold) which play important role in blood cell maturation and blood vessel stabilization. Interestingly, BF-preconditioned H9-hNSCs also showed significant higher migration rate towards IVIS model compared to non-treated cells. More importantly, these transplanted cells remain viable and started to form neurite outgrowth

inside the ischemic IVIS model for up to 72 hours. This study suggests that pharmacological preconditioning of NSCs using BF could be a promising therapeutic tool for ischemic stroke.

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W-2006

ROOF AND FLOOR PLATE CELLS ORCHESTRATE STEM CELL IDENTITY AND REGENERATION OF THE ZEBRAFISH SPINAL CORD

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Zebrafish have a remarkable capacity to regenerate entire CNS tissue including the spinal cord following an injury. Ependymal cells lining the central canal are neural stem cells (NSCs) in the zebrafish spinal cord .They are activated by the injury and regenerate lost tissue. However, spinal cord contains different types of cells and axonal tracts which are distinctly patterned through the dorsal-ventral (D-V) axis. Therefore, how regenerated cells and tracts can acquire the correct final positional identity within the injured spinal cord is an important question that needs to be answered. During embryonic development, positional identity within the spinal cord is determined by ventralising and dorsalising signalling molecules such as Shh and members of the TGF-β family which are secreted by floor and roof plate cells. By using in vivo imaging and reporter lines we indicated that NSCs in the zebrafish spinal cord keep their embryonic D-V patterning profile outside the embryonic development. Remarkably, the floor and roof plate like-cells in the ependymal layer of the spinal cord actively influence the identity of NSCs. Suppression of ventralising and dorsalising signals from the floor and roof plate like-cells resulted in shifting of D-V marker expression in the NSCs. Interestingly, retention experiments of Histone 2B (H2B)-mcherry fusion protein in embryo showed that floor and roof plate like-cells are the first cells that become quiescent in the ependymal layer. The quiescent cells of the floor and roof plate are maintained until adulthood as specialised radial glia population defining the ventral and dorsal counter points of the ependymal layer. Following injury, these cells are able to produce their own lineage and keep their position during the ependymal layer regeneration, suggesting that they play a pivotal role in orchestrating patterning during repair process. A lack of these signalling centres may be a significant factor that hinders spinal cord regeneration in mammals. Restoring the lost signals and cell types could dramatically improve neural regeneration in mammals.

W-2008

NEURAL REGENERATION MECHANISMS IN TRANSPLANTATION OF NEURONAL CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS WITH THE EFFECT OF THEIR PARACRINE SECRETION

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Brain damage causes temporary or permanent disabilities of the organism because of deprivation of oxygen and nutrients. 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 in paracrine manners would be associated with motor function recovery in hemiplegic mice. In order to obtain suitable cell types for graft recipients with stroke and brain damage, we have modified a protocol for differentiating human induced pluripotent stem (hiPS) cells to cells phenotypically related to cortical motor neurons. Moreover, we applied cell sheet technology to neural cell transplantation due to the idea that retaining cell-cell communications made in cell sheet was important to repair host brain architecture. We cultured hiPS cells and stimulated them to become motor neurons. To make a neural cell sheet, cells were cultured on temperature-responsive gelation polymer coated plate (Upcell®) for average 14 days depending on the neuronal maturation, where they extended axon-like processes. Hemiplegic mice were generated by cryoinjury of the motor cortex and then the neuronal cells were injected into the periventricular area or we placed the sheets on the brain surface through the burr hole. The grafted neurons migrated toward and distributed over the injured motor cortex passing through the corpus callosum from the periventricular area where the neurons were initially injected. Neurons derived in the cell sheet also migrated into the damaged cortex. The migrating neurons expressed motor neuron markers. The transplanted cells secreted various molecules contributing to functional improvement. The motor functions were significantly improved in the beam walking test and rotarod test. Our results indicated that transplantation of neuronal cells or the novel neuronal cell sheet, the latter of which retained

cell-cell interactions after transplantation, may become applicable to restoring the motor functions of patients suffering from hemiplegia. To that effect paracrine factors may contribute.

W-2010

RETINAL GROWTH FACTORS INSTRUCT ROD PHOTORECEPTOR PROGENITOR FATE OR PERMIT CONE PHOTORECEPTOR PROGENITOR FATE FROM MAMMALIAN RETINAL PRECURSORS

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During mammalian retinal development, seven classes of cells are produced in a temporally stereotyped manner from retinal progenitor cells (RPCs). Despite this highly conserved order of retinogenesis, it is not clear whether all retinal progenitor cells (RPCs) are inherently multipotent, or if there are distinct molecularly identifiable subclasses of RPCs with more restricted lineage potential. Here, we use adult murine and human retinal stem cells (RSCs), as well as RPCs derived from the embryonic retina to functionally and molecularly model the lineage specification of retinal photoreceptors, indicating that external cues are able to dictate a photoreceptor restricted output to all RPCs. Normally quiescent (in vivo) adult RSCs readily proliferate in vitro to produce downstream RPCs. When single murine RSC-derived RPCs were exposed to taurine and retinoic acid (T/RA) for 28 days, clones arose consisting solely of rod photoreceptors. In contrast, when single RSC-derived RPCs were exposed to COCO (a TFGB, WNT, and BMP inhibitor) for a similar time period, the resultant clones contained an almost pure population of cone photoreceptors. To further confirm that adult RSCs were accurately able to model these developmental lineages, RPCs derived from the embryonic murine retina were exposed to the two differentiation paradigms. T/RA or COCO application to embryonic RPCs produced nearly pure populations of rod or cone photoreceptors, respectively. When RSCs were derived from adult human tissue, their downstream progenitors produced similar percentages of cone or rod photoreceptors when exposed to the above mentioned external cues, suggesting that the mechanism of photoreceptor specification may be conserved across human and murine development. This functional data indicates that T/RA is able to instruct all RPCs to acquire a rod photoreceptor restricted potential, while cone-specific progenitor output may be revealed by a default mechanism in the absence of major morphogen signalling pathways. In order to uncover the molecular identity of photoreceptor restricted progenitors, we have carried out RNA-seq across time-points of T/RA or COCO exposed murine embryonic RPCs. Subsequently identified signature genes should then allow us to trace the identity of these photoreceptor restricted progenitors in vivo.

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W-2012

HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS WITH CHROMOSOME 1Q21.1 DELETIONS DISPLAY ALTERED CORTICAL PATTERNING AND SYNAPTIC DEREGULATION

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1q21.1 deletions and duplications are highly penetrant copy number variants (CNVs) which are characterized by variable phenotypes such as developmental delay, schizophrenia and autism spectrum disorders. The 1q21.1 critical region spans approximately 1.35 Mb (from 145 to 146.35 Mb) and includes at least 12 genes, among which PRKAB2, FMO5, CHD1L, BCL9, ACP6, GJA5, GJA8, GPR89B are particularly significant. Although the clinical features of this CNV have been defined, there is little evidence connecting these features to dysfunction in cells of the neuronal lineages. To investigate the effect of 1q21.1 deletion on the functionality and morphology of neurons, we have generated induced pluripotent stem cell (iPSC) lines from patients carrying 1q21.1 deletions. Using two discrete developmental patterning paradigms these iPSCs were differentiated into cortical neurons. In both paradigms the 1q21.1 deletion had a significant effect on cortical layer patterning. Furthermore, this phenotype led to an increase in the number of synapses associated with these neurons. Critically, these cellular phenotypes were associated with functional phenotypes as measured by multiple electrode arrays. Finally, using RNA sequencing from these samples we are now examining the link between the cellular phenotype identified and the underlying genetic aberration to provide clear links between this phenotype and the underlying genotype.

W-2014

NEURAL STEM CELLS PROMOTE MOUSE NERVE REGENERATION VIA SCHWANN CELL DIFFERENTIATION INDUCED BY HUMAN AND MOUSE IL12

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Regeneration of injured peripheral nerves is a slow and complicated process that could be improved by implantation of neural stem cells (NSCs) or nerve conduit. Implantation of NSCs along with nerve conduits promotes the regeneration of damaged nerve, likely because (i) conduit supports and guides axonal growth from one nerve stump to the other, while preventing fibrous tissue ingrowth and retaining neurotrophic factors; and (ii) implanted NSCs differentiate into Schwann cells and maintain a growth factor enriched microenvironment, which promotes nerve regeneration. Here, we identified IL12p80 (homodimer of IL12p40) in the cell extracts of implanted nerve conduit combined with NSCs by using protein antibody array and Western blotting. Levels of IL12p80 in these conduits are 1.6-fold higher than those in conduits without NSCs. In the sciatic nerve injury mouse model, implantation of NSCs combined with nerve conduit and mouse IL12p80 not only increases the regenerated nerve diameter up to 4.5-fold but also improves motor recovery in behavioral analyses. *In vitro* studies further revealed that mouse IL12p80 stimulates the Schwann cell differentiation of mouse NSCs through the phosphorylation of signal transducer and activator of transcription 3 (Stat3). We have also produced and purified human IL12p80 from CHO-S cells. Human IL12p80 recapitulated the results obtained when mouse IL12p80 was used. These results suggest that both human and mouse IL12p80 can trigger Schwann cell differentiation of mouse NSCs through Stat3 phosphorylation and enhance the functional recovery and the diameter of regenerated nerves in a mouse sciatic nerve injury model.

Funding Source: Ministry of Health and Welfare, Taiwan; Ministry of Science and Technology, Taiwan

W-2016

INHIBITION OF GSK BETA INCREASES NEUROGENESIS IN NEURAL PROGENITORS DERIVED FROM STEM CELLS AND IN VIVO AFTER SPINAL CORD INJURY

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Spinal cord injury (SCI) results in neural loss and consequently motor and sensory deficit below the injury. Recently, we have reported the regenerative effects and significant improvement of locomotor function in complete transection rat model of SCI following transplantation of oligodendrocyte progenitors cells (OPC) and motoneuron progenitors (MP) derived from hESC. Transplantation of these progenitors promote astrogliosis, thorough activation of jagged1-dependent Notch and Jak/STAT signalling supporting axonal survival. Induction of astrogliosis and neurogenesis can be achieved by inhibition of glycogen synthase kinase-3 (GSK3) well known molecule involved in several signalling pathways. In the present study we assess the in vitro effects of GSK-3 inhibitor Ro3303544 (Ro) using ependymal stem cells as well as hESC and hiPSC-derived neural progenitors. Our result show significant increase of neurogenesis (Tuj1 and MAP2) in the cells treated with Ro compared to untreated cells during 3 days of treatment. Neurogenesis and functional recovery was observed in complete transection mouse model of spinal cord injury after treatment with Ro. This study will contribute to the discovery of new combined therapies including transplantation of pluripotent stem cells derivates in combination with pharmacological strategies for axonal growth.

W-2018

GENERATION AND CHARACTERIZATION OF TRANSPLANTABLE ESC-DERIVED MOTOR NEURONS

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Motor neurons (MNs) are highly specialized neurons located in the central nervous system (CNS) that finely control the activity of muscles in the body through complex neuronal circuitry. They can be broadly divided into two types; upper MNs, located in cerebral cortex and lower MNs located in the brainstem and spinal cord. Lower MNs (LMNs) are found in the ventral horn of the spinal cord and are anatomically organized in columns characterized by specific gene expression profiles and muscle targets. Degeneration of LMNs is implicated in a number of devastating neurological diseases including Amyotrophic Lateral Sclerosis (ALS). More recently, cell replacement therapy using derived MNs derived from human embryonic stem cells (ESCs) has been proposed as treatment for replacing the lost cells and/or protecting the host cells. However, this approach is hindered by the low efficiency of MN differentiation and the high heterogeneity of the transplanted cell population. In this study, we derived human LMNs from two different human reporter ESC lines using a small molecule-based 2D adherent differentiation protocol and antibody signatures for characterizing our cell population in vitro. Secondly, these PSC-derived MN progenitors were stereotaxically injected into the ventral horn of cervical or lumbar region of spinal cord of athymic rats. We show that, using this protocol, we are able to specifically modulate the generation of different LMNs subtypes in accordance with gene and protein expression profiles. Furthermore, transplantation of human derived MNs using our reporter cell lines allow us to better characterize, track and study the relationship between our cells and the host environment after injection into the spinal cord. In conclusion, this study will be a valuable new source of knowledge about the development of MNs and their possible application in cell replacement therapy for motor neuron diseases.

W-2020

GDNF ENHANCES THE FUNCTIONAL INTEGRATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED DOPAMINE GRAFTS IN PARKINSONIAN RATS

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The derivation of neurotransmitter and region specific neuronal populations from human pluripotent stem cells (hPSC) provides impetus for cell therapies. In Parkinson's disease (PD), the degeneration of midbrain dopamine (DA) neurons underlies basal ganglia dysfunction and motor symptoms. The direct replacement of these neurons using foetal tissue, as well as neurotrophic support of both host- and graft-derived neurons, have provided proof-of-principle that disease modification and functional recovery are possible. However, our understanding of the in vivo properties of hPSC-derived neurons and their response to developmentally relevant neurotrophic cues is lacking. Here we adopt a combined approach utilising LMX1A- and PITX3-GFP hPSC reporter lines and targeted viral overexpression of glial derived neurotrophic factor (GDNF) to track and modulate transplanted DA neurons. Transplantation of DA progenitors into GDNF-rich striatal tissue promoted DA neuron survival, leading to enhanced motor recovery in PD rats. Strikingly, delayed overexpression of GDNF, 3 weeks after DA progenitor transplantation, also promoted motor recovery by mechanisms including enhancing graft plasticity, elevated DA metabolism and cFos activation of striatal neurons. These results highlight the potential of targeted neurotrophic support strategies to improve graft outcome following transplantation.

W-2022

IDENTIFICATION OF HUMAN-SPECIFIC GENE REGULATORY ELEMENTS THROUGH THE USE OF HUMAN AND CHIMPANZEE IPS CELLS

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The human brain has undergone a rapid evolution that has given us a cognitive advantage compared to other primates. On a genetic level, humans and chimpanzees share about 98.8% similarity, and yet there are clear differences in brain volume, cell number, and complexity. In this study, we are testing the hypothesis that transposons may constitute human-specific regulatory elements in neural progenitor cells. To this end, we use iPS cells from humans and chimpanzees, which we differentiate into forebrain progenitors in vitro using extrinsic factors. We then investigate transcriptional differences between the species, as well as human-specific binding of the co-repressor protein TRIM28 and the histone mark H3K9me3 to identify transposons involved in transcriptional repression.

W-2024

ACCELERATED MATURATION AND IMPROVED FUNCTIONALITY OF HUMAN IPSC-DERIVED NEURONS WITH THE B-27TM PLUS NEURONAL CULTURE SYSTEM

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Human iPSC-derived neurons have increasingly become a valuable system for the study of neurological disorders. Robust cell reprogramming and improved differentiation protocols enable scientists to generate patient-specific, disease in a dish models for disorders such as Parkinsons, Alzheimers, and Autism, among others. These human models tend to be flexible, scalable and maintain many of the characteristics of found in these disorders, which are key requirements for their use in mechanistic and drug discovery studies. Further, the development of gene editing technology has spawned intense interest in the use of gene-edited, patient-specific iPSC-derived neurons in cell therapy applications for the treatment of neuro-degenerative disorders. A critical step in generating useful iPSC-derived neurons is neuronal maturation. During maturation neurons extend neurites to form highly connected networks, express synaptic markers, and become electrically active. Typical maturation conditions are inefficient, generating poorly matured neurons with low levels of functionality over extended periods of time. Recently we developed a new neural maintenance/differentiation system, (B-27TM Plus and Neurobasal Plus) and showed significantly improved neuronal survival, maturation, and functionality of primary rodent neurons compared to other culture systems. Here we expand our studies to PSC-derived neurons, utilizing multiple human lines PSC and different approaches for neural stems cell derivation. Diverse endpoints were used to interrogate maturation; neurite outgrowth, neuronal maturation marker expression (through quantitative imaging), and functionality through Multi-Electrode Array (MEA) analysis. We found that human PSC-derived neurons matured in the new "Plus" system showed both accelerated neurite outgrowth and improved activity as compared to other approaches. Additionally our studies highlight the importance of optimizing several key parameters, including extracellular matrix coating concentrations and delivery conditions for improved reproducibility and quality of stem cell derived neural cultures.

NEURAL DISEASE AND DEGENERATION

W-2026

SCREENING FOR ANTI-EPILEPTIC COMPOUNDS WITH AN IN VITRO EPILEPSY MODEL BASED ON HUMAN IPSC-DERIVED 3D NEUROSPHERES

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With over 50 million sufferers worldwide, epilepsy is one of the most prevalent neurological disorders. All current anti-epileptic dugs (AEDs) only treat symptoms, with little to no effect on the underlying disease mechanisms or co-morbidities, and carry significant risk for adverse effects. Moreover, up to one-third of all epileptic patients are refractory to AEDs. As such, there is an urgent demand and significant unmet medical need for more effective and safer AEDs. Towards that end, we set out to develop an *in vitro* epilepsy model based on human induced pluripotent stem cell (iPSC)-derived 3D neurospheres formatted in high throughput screening 384-well plates. This platform comprises a physiologically relevant co-culture of astrocytes and cortical neurons from a single human donor source of iPSCs. The neurospheres show high morphological homogeneity, as well as large, consistent, spontaneous and synchronous calcium oscillations, and are responsive to a variety of pharmacological neuromodulators. For epilepsy drug screening, the platform was first characterized with known neuromodulators and AEDs alone. Next, a collection of 120 targeted proprietary compounds was tested. These compounds were selected based on their demonstrated ability to modulate *in vitro* the activity of ion channels implicated in epilepsy. The library also comprised compounds with different mechanism of action, such as $GABA_A$ channel activators, and glutamate channel inhibitors. This 120 drug panel was screened against the neurosphere platform at 3 different concentrations (0.1, 1, 10 µM). Synchronous, large calcium oscillations were inhibited over 40% by 72 of the 120 compounds, consistent with their known ion channel activity and potential as novel AEDs. The assay also showed a large dynamic range, with 36 compounds reaching over 99% inhibition in a concentration-dependent fashion. None of the test compounds demonstrated toxicity as neuronal activity recovered upon washout and there was no change

in the fine structure of the spheroids. Finally, the neuropsphere platform generated remarkably consistent and reproducible results, displaying minimal variability across wells and plate replicates. These results suggest that the this platform is a useful and robust cell-based platform for high-throughput drug screening.

W-2028

HIGH-CONTENT IMAGING OF IPSC-DERIVED HUMAN NEURONS FOR TOXICITY SCREENING

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There is a significant need for in vitro systems that more closely model the human nervous system and its response to environmental toxins. Such a platform would have greater predictive power to indicate which compounds pose a risk. Toward this goal, we have developed a platform centered on the use of iPSC-derived human neurons. First, iPSCs were gene-edited to ubiquitously express eGFP. We then patterned these iPSCs to a neuroepithelial fate and next to neuronal progenitors before finally differentiating them into neurons. Spinal motor neurons were generated in this manner and used for this proof-of-concept project. They were plated in 384-well format for high-content imaging. Optimizing imaging in this manner required attention to the source of cells, plate surface coating, medium composition, staining protocol, imaging parameters, and the timeline for neuron maturation and neurite outgrowth. Optimization of all parameters yielded a sensitive and robust system with a Z-prime value greater than 0.5.

W-2030

HUMAN IPSC-DERIVED CORTICAL NEURONS WITH A NOVEL FRAMESHIFT PSEN2 MUTATION INCREASE THE RATIO OF AGGREGATE PRONE AMYLOID BETA

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Heterozygous mutations in Presenilin 2 (PSEN2) cause nearly fully penetrant autosomal dominant Alzheimer Disease (AD). PSEN2, an essential component of the gamma-secretase complex of proteins, catalyzes a crucial step in the amyloidogenic amyloid precursor protein (APP) cleavage cascade to generate amyloid-beta (Aβ) peptides. Accumulation of aberrantly-produced insoluble Aβ isoforms is an important component of AD pathogenesis. While most driver PSEN2 mutations are missense, we have discovered a novel heterozygous PSEN2 two-basepair deletion frameshift mutation (PSEN2K115fsX) in two unrelated individuals with AD. To determine whether this mutation exhibits molecular hallmarks of AD, we generated induced pluripotent stem cells (iPSCs) from patient fibroblasts to study cell type-specific mutational effects. Following differentiation of control and mutant iPSCs into cortical neurons, we measured secreted Aβ isoforms in conditioned media and found that the ratio of insoluble to soluble Aβ is increased from mutant cells. To validate this PSEN2 mutation as a molecular driver of these observations, we are testing whether correction of the mutation in genome-edited isogenic iPSC lines rescues the Aβ phenotype. Finally, this frameshift mutation offers a unique way to probe the impact of major biochemical changes to the expression and function of one PSEN2 allele. We are analyzing the impact of mutation on PSEN2 RNA splice isoforms as well as exploring the greater role of PSEN2 AD mutations in human microglia through iPSC differentiation.

W-2032

MODELLING MICROGLIAL DYSFUNCTION IN HUNTINGTON'S DISEASE USING HUMAN iPS CELL-DERIVED MICROGLIA

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Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the huntingtin gene, leading to the translation of an aberrant and pathogenic mutant Htt (mHtt). It is increasingly evident that immune activation within the CNS, primarily driven by microglia, is a key feature of HD. In addition, inflammation caused by a gain of toxicity of mHtt in the CNS and the periphery represents an important mechanism in the pathogenesis of HD, with current evidence suggesting that efficacy of immune

system function may act as a disease modifier (Rocha et al., 2016). Studies in mouse models and post-mortem human brains have shown that HD microglia acquire an inflammatory and hyper-reactive phenotype. Here, we have used an established microglial differentiation protocol to derive microglia (MG) from human HD iPSC lines containing 109 CAG repeats (HD109) with the aim of characterising their phenotype. Expression of canonical MG genes such as TMEM119, GPR34 and GAS6 was confirmed by qRT-PCR. The HD MG cells were functionally validated by stimulation with LPS and IL-4 and were found to exhibit a lower basal respiration rate, indicative of mitochondrial dysfunction. An investigation of the bioenergetics phenotype of the cells is ongoing. We then performed RNA sequencing to explore microglial-specific transcriptional changes associated with mHtt. Correlation and principal component analysis revealed a similar but distinct profile of HD109 MG cells compared to WT MG cells, with gene set enrichment analysis confirming an enrichment for microglial sensome and immune pathways. We have also carried out pathway analysis to predict downstream processes affected in HD109 MG cells and have identified antigen processing and presentation (FDR p= 0.001), chemokine signalling (FDR p= 0.029) and phagocytosis (FDR p= 0.035) as being impaired or dysregulated. Overall, these findings suggest that mHtt expression in iPSC-derived microglia alters several intrinsic immune-related functions. Current studies are confirming these observations in isogenic cell lines. This work will further our understanding of microglial involvement in HD pathogenesis by enabling in-depth mechanistic studies bridging the gap between HD clinical and animal models.

W-2034

RESTORING CENTRAL INHIBITION RESOLVES NEUROPATHIC PAIN

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Nerve injury can lead to devastating pain and for the majority of patients there are no effective therapies. We and others demonstrated in Drosophila and rodent that peripheral injury triggers a coordinated response from primary afferents resulting in loss of central inhibition, and culminating in a permanently sensitized nociception circuit. Loss of central GABAergic tone is necessary for neuropathic pain to develop, disrupting inhibitory signaling is sufficient to trigger pain sensitization without injury. Previous studies have shown that transplantation of rodent inhibitory interneuron precursors from the medial ganglionic eminence (MGE) enhances GABAergic signaling in the brain and spinal cord. To test the therapeutic potential of restoring central GABAergic inhibition, we generated and transplanted hiPSC-derived GABAergic inhibitory neurons into the spinal cord of mice suffering from neuropathic pain. Remarkably, hiPSC-derived inhibitory neurons promoted lasting pain relief without side effects. We show that transplanted hiPSC-GABAergic neurons integrate into the adult mouse spinal cord and completely reverse the mechanical hypersensitivity produced by peripheral nerve injury. For the first time we describe a hiPSC transplant therapy that is an effective and long-lasting treatment for neuropathic pain.

W-2036

GLUCOCORTICOID INDUCES CELL DEATH VIA DYSFUNCTION OF MICROTUBULE INDUCED BY IMBALANCE OF MITOCHONDRIAL CALCIUM LEVEL AND SUBSEQUENT AUTOPHAGY INHIBITION IN HUMAN NEURONAL CELLS

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Glucocorticoid has been widely accepted to induce Alzheimer's disease (AD) through increasing two hallmarks, extracellular accumulation of amyloid β (Aβ) and tau hyperphosphorylation. However, the mechanism of cell death or memory impairment induced after microtubule dysfunction still remains elusive. Therefore, we investigated the glucocorticoid effect on the microtubule destabilization as well as dysfunction of vesicle trafficking, both of which subsequently induce the cell apoptosis. Upon cortisol treatment, Hsp70 bound glucocorticoid receptor (GR) was translocated into mitochondria and GR-bcl-2 interaction was induced. Subsequently, bcl-2 bound GR induced ER-mitochondria coupling increasing calcium influx into the mitochondria which was blocked by knockdown of bcl-2. This phenomenon resulted in decrease in cytosolic calcium level and the phosphorylation of AMPK. The decrease in p-AMPK activated mTOR pathway, which eventually caused autophagy inhibition through downregulation of autophagy induction related genes. The dysfunction of autophagy failed to degrade SCG10 through ubiquitination, resulting in microtubule destabilization. The microtubule dysfunction decreased the binding of kinesin and transport of mitochondria and memory related receptors, AMPA receptors. Thus, the perinuclear clumping of mitochondria and decreased movement of AMPA receptors into synapse were shown upon cortisol treatment, reversed by autophagy induction or microtubule stabilizer. In conclusion,

glucocorticoid regulates mitochondrial calcium system via ER-mitochondria coupling which triggers the memory impairment and cell death obstructing autophagy and microtubule stabilization.

W-2038

WHEN YOUNG IS OLD ENOUGH: ALZHEIMER'S DISEASE-RELEVANT PATHOLOGY IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS AFTER 4 WEEKS OF MATURATION

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Alzheimer's disease (AD) is a complex neurodegenerative disorder, characterized by extracellular β-amyloid plaques and intracellular tangles consisting primarily of tau. Recent brain imaging studies show that AD-characteristic pathology occurs prior to the onset of behavioural dysfunction. Basal forebrain structures contain the earliest affected cells in the progression of AD, suggesting that in vitro differentiation of these populations would provide a model for early AD pathology studies. Here we assessed whether AD-relevant pathological hallmarks are present in basal forebrain neuronal cultures of individuals with sporadic AD (SAD) in comparison to cultures from healthy individuals after as little as nine weeks differentiation. Induced pluripotent stem cell-derived neuroectoderm cells were guided towards ventral forebrain fate via high sonic hedgehog and fibroblast growth factor 8. The resulting progenitors were matured through electroporation-introduced expression of LIM homeobox 8 (LHX8) and gastrulation brain homeobox 1 (GBX1), followed by supplementation with fibroblast growth factor 2 and nerve growth factor for 4 weeks. After confirming the presence of cholinergic and dopaminergic neurons, we quantified extracellular β-amyloid concentrations and the expression of tau (total, misfolded, phosphorylated). Neuronal cultures from SAD individuals showed a higher β-amyloid 42/40 expression ratio, increased presence of misfolded tau, and higher tau phosphorylation. The diagnosis-related difference in AD-relevant proteins present in basal forebrain cultures indicates the induction of genetically driven pathological processes. Our results show that human iPSC-derived basal forebrain cultures provide a suitable system to study cell type specific AD mechanisms without the need for long-term maturation.

W-2040

MODELLING ALZHEIMER'S DISEASE USING SINGLE CELL RNA SEQUENCING ANALYSIS FROM HUMAN PLURIPOTENT STEM CELL-DERIVED CEREBRAL ORGANOIDS

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Late-onset Alzheimer´s Disease (AD) is the most common type of dementia and it appears to be caused by defective clearance of amyloid β in the brain. Strong evidence suggests that polymorphisms in the APOE gene are the major genetic risk factor. However, the mechanisms of APOE in AD are still poorly understood. One of the major limitations for the study of AD has been the lack of a human disease model. Here, we have generated patient-specific induced pluripotent stem cell (iPSC) lines with different APOE genotypes (homozygous APOE alleles 3 and 4) and differentiated them into human cerebral organoids. After three months in culture, cerebral organoids showed some of the hallmark pathological features of AD, including amyloid β deposits and phosphorylation of Tau. To further investigate the role of APOE isoforms, we used single cell RNA-sequencing to characterize the transcriptomes of 8,809 and 7,672 cells from organoids with homozygous genotypes of APOE alleles 3 and 4 respectively. The resulting analyses allow us to identify differences in cell type population of the organoids, and the activation of signaling pathways involved in amyloid clearance and Tau phosphorylation that may play a role in the progression of AD. We have demonstrated the feasibility of recapitulating key pathological events observed in AD using human cerebral organoids derived from patients-specific iPSCs. The usage of patient specific iPSC-derived organoids might also help clarify ambiguous results obtained in different AD models.

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W-2042

LONG-TERM ADDITION OF PROSTAGLANDIN E2 UPREGULATES PITX3 EXPRESSION IN HESC-DERIVED MIDBRAIN DOPAMINERGIC NEURONS

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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by motor and non-motor symptoms. The motor symptoms of disease caused by loss of midbrain dopaminergic (mDA) neurons in the substantia nigra. In most cases the aetiology of is unknown, although it is thought that neuroinflammatory mediators such as prostaglandin E2 (PGE2) and tumor necrosis factor (TNF) play a role in disease progression. Studies in mice have shown that PGE2 can exert either neuroprotective (EP2/EP4 receptor) or neurotoxic (EP1 receptor) effects, while ablation of TNF activity is neuroprotective in models of PD. While the TNF effect is likely to be mediated indirectly it is unclear whether PGE2 effects are mediated directly at the neuron or indirectly via other cell types. We assessed expression of two markers of midbrain identity; tyrosine hydroxylase (TH, the rate limiting enzyme in the synthesis of dopamine) and Paired-Like Homeodomain 3 (PITX3, a key transcription factor involved in the long-term survival and maintenance of midbrain dopaminergic neurons), in floor-plate derived neurons enriched by flow cytometry at day 25 of differentiation (LMX1AeGFP/wt) and cultivated until day 70. From days 40 to 70 neurons were incubated with either 300nM PGE2, 20ng/ml TNF or vehicles. At day 70 neurons were harvested for qPCR analysis (ΔΔct). TNF had no effect on either PITX3 or TH transcript (120+34 and 135+27% of control, respectively, n=6). In contrast PGE2 significantly upregulated PITX3, but not TH expression in cultures (199+27* and 92+18% of control, respectively, *p<0.05, n=3). This finding is particularly intriguing as the transcriptional regulation of TH is thought to be co-regulated by PITX3. Overall, this suggests that long-term addition of PGE2 upregulates PITX3 expression in midbrain cultures via a mechanism independent of the PITX3-TH regulatory transcriptional network. Studies in our lab are currently underway to investigate the specific pathway that PGE2 utilises to regulate PITX3.

W-2044

AMYOTROPHIC LATERAL SCLEROSIS MOTOR NEURONS EXHIBIT STRESS GRANULE DYSFUNCTION DUE TO LOSS OF SMN FUNCTION

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Motor neurons disorders (MNDs) are characterized by progressive degeneration of motor neurons in both familial and sporadic cases. The two widely studied MNDs are 1) Spinal Muscular Atrophy (SMA), the most common MND that affects children and 2) Amyotrophic Lateral Sclerosis (ALS), an adult-onset motor neuron degenerative disease. Despite SMA and ALS being genetically unrelated, studies have shown that the loss of SMN-rich gems was observed in ALS and overexpression of SMN improves motor neuron survival in ALS. Multiple studies have also provided evidence suggesting a common pathological pathway in SMA and ALS, where SMN played a vital role in motor neuron survival. In this study, we found that motor neurons derived from patient-derived induced pluripotent stem cells (iPSCs) show loss of SMN function despite having normal SMN protein levels. SMN plays a critical role in stress granule assembly, enhancing motor neuron survival and the loss of SMN function leads to stress granule dysfunction and death of ALS motor neurons.

W-2046

EFFECTS OF INFLAMMASOMES ON DIFFERENTIATION OF NEURAL PROGENITOR CELLS INTO NEURAL CELLS

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Previous studies have suggested that neuroinflammation is an important factor in the pathogenesis of neurodegenerative diseases such as Alzheimer's diseases or Parkinson's diseases. Activation of cytosolic protein complexes known as inflammasomes leads to the activation of pro-inflammtory cytokines such as IL-1beta or IL-18. Enhanced production of pro-inflammatory cytokines by sustained stimulation of inflammasomes in glial cells during neuroinflammation has been shown to result in severe neurodegeneration. Although adult neural regeneration (neurogenesis) after neural damage is carried out by proliferation and differentiation of neural progenitor cells (NPC), impairment of neurogenesis has been shown to contribute to cognitive decline in neurodegenerative diseases. In addition, it is recently found that stimulation with alpha-synuclein which is one

of the etiologic factors in Parkinson's diseases activates inflammasomes in adult NPC. Thus, in the present study, we determined involvement of inflammasome activation on proliferation or differentiation of NPC. The NPC were generated from mouse induced pluripotent stem cells (iPS cells) by the protocol published previously (Lee et al., Nat Biotechnol 2000). Activation of inflammasomes in NPC was examined by the expression of inflammasome components (NLRP3, ASC, or caspase-1), IL-1beta, or IL-18. Proliferation of NPC was examined by MTT assay. The differentiation potential of NPC into neural cells was evaluated by NeuN expression using immunofluorescence staining or western blot analysis. Treatment of mouse NPC with TNF (100 U/ml) significantly induced the expression of caspase-1, IL-1beta, and IL-18. The treatment with TNF (100 U/ml) also significantly inhibited NeuN expression in differentiated NPC. In addition, the simultaneous treatment with TNF and IL-1beta (0.3 ng/ml) significantly enhanced the inhibitory effect. Pretreatment with BAY11-7082 (an inhibitor of inflammasome activation; 10 microM) significantly reversed the effect of TNF. These results suggest that the activation of inflammasomes in mouse NPC inhibits the differentiation into neural cells.

CANCERS

W-2048

THE M(6)A METHYLTRANSFERASE METTL13 DRIVES LEUKEMIA STEM CELL SELF-RENEWAL IN HUMAN ACUTE LYMPHOBLASTIC LEUKEMIA

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Normal hematopoietic stem cells (HSCs) accumulate genetic (DNA) and epitranscriptomic (RNA) changes that promote the emergence of pre-leukemic clones that have gained survival and proliferative advantages. Epitrancriptomic modifications include several important RNA processing events, including RNA editing, methylation (N6-methyladenosine (m^6A) and alternative mRNA splicing. These events add additional layers of dynamic regulation that can all contribute to both cancer initiation and cancer progression. Detection and selective inhibition of CSC methylation, therefore, represents a promising new human stem cell research derived technology that could be translated to improve care for patients with a broad array of CSC driven malignancies that are resistant to therapy. RNA methylation and editing are both crucial events implicated in the oncogenic transformation of pre-malignant progenitors that harbor clonal self-renewal and survival-altering mutations. However, whether m^6A mRNA methylation driven editing is involved in the malignant progression of Acute Lymphoblastic Leukemia (T-ALL) has not been examined. Whether ADAR1-mediated RNA editing is involved in lymphoid malignancies including T-ALL has not been examined. Our preliminary data suggest ADAR1 expression is increased in $CD34^+$ T-ALL compared with $CD34^+$ cord blood controls, suggesting ADAR1 activation may provide an unexplored therapeutic target in T-ALL. In this context, our central hypothesis is that epitranscriptomic events such as m^6A methylation signals induce aberrant RNA editing driven by ADAR1 activation in T-ALL-initiating cells that accentuated by enhanced survival and self-renewal capacity. We have identified an upregulation gene expression of both ADAR1 and METTL13 in T-ALL primary CD34+ cells using RT-qPCR. To understand the RNA editing dependent mechanisms governing ADAR1-mediated self-renewal, we performed RNA-sequencing of cord blood $CD34^+$ cells transduced with ADAR1 and pCDH backbone. Our sequencing data revealed that ADAR1 overexpressing cells harbored elevated levels of METTL13, expression compared to pCDH backbone. METTL13 was thus the only methyltransferase significantly upregulated upon ADAR1 activation. Together these data indicate a cross-talk between RNA editing and RNA methylation.

W-2052

NOVEL DRUG SCREENING METHOD USING MICROSCOPIC IMAGING OF EPIGENETIC LANDSCAPE (MIEL)

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Epigenetic alterations have been previously implicated in cancer initiation, recurrence and chemotherapy resistance. DNA sequencing has revealed that mutations in genes associated with regulation of chromatin organization are prevalent in human cancers highlighting the importance of developing drugs which target them. However, the screening paradigms aimed at identifying such drugs are mostly cell-free assays that are non-

indicative in properties ranging from cytotoxicity to cell proliferation and metabolic rates. Using primary glioma stem cells (GSC) we have developed a novel cell based assay to identify unknown compounds that inhibit epigenetic regulators such as DNA methyl transferase (DNMT) and histone deacetylase (HDAC). Microscopic Imaging of Epigenetic Landscape (MIEL) captures the patterns of nuclear staining for histone modifications by deriving statistical texture features which are then used for multivariate analysis to compare the epigenetic landscape of different cell populations. In a preliminary pilot experiment using MIEL, we have analyzed the alteration in epigenetic landscape induced by 22 drugs to find that the proximity of cells treated with functionally similar drugs suggests that they may induce similar patterns of epigenetic changes. Evaluation for quality of these assays show that functional classes were efficiently distinguished on a 2D map using MIEL which illustrates the unique advantage of this approach compared to threshold-based analysis. Results show that cells treated with Sirt1 inhibitor positioned proximal to the DNMTi cluster and was confirmed to have DNMTi functions. This demonstrated MIEL's capability to identify new epigenetic function of already established drugs. We are currently utilizing MIEL to develop robust reference drugs to screen in parallel with 224 compounds containing structures similar those currently being used in clinical trials in search for desirable compounds.

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W-2054

H3K27 AND H3K9 HISTONE LYSINE DEMETHYLASES ARE INVOLVED IN HUMAN HSPC AGING AND MYELOID LEUKEMIA

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Aging of hematopoietic stem cells (HSCs) is marked by functional decline of the hematopoietic system, inducing an increased incidence of myeloid malignancies. Previous studies have demonstrated total histone expression to regulate Saccharomyces lifespan, as well as H3K9me3 level alteration during mammalian HSC aging. We hypothesized that histone demethylases may contribute to human HSC aging and acute myeloid leukemia (AML). Gene expression analysis revealed reduction in multiple H3K9 demethylases, among them KDM3B, KDM4A and KDM4B in aged CD34+ hematopoietic stem and progenitor cells (HSPCs) compared to cord blood HSPCs. This was associated with increase in corresponding H3K9me3 level in aged HSPCs as well as more committed CMP and GMP populations. However there was no alteration in H3K9me3 in AML blasts (median age 50 years) compared to age matched normal hematopoietic cells, suggesting differential histone regulation in aging and leukemia. Unlike aged HSPCs, where KDM6 family, which target H3K27me3/2, was downregulated, AML blasts showed increased expression of KDM6A and KDM6B. High KDM6 together with low EZH1/EZH2 led to reduced H3K27me3 in AML blasts. This prompted us to investigate the anti-leukemic potential of KDM6 inhibition. GSKJ4, a small molecule inhibitor targeting KDM6A and KDM6B selectively inhibited growth of multiple AML cell lines. It restored H3K27me3 and induced apoptosis, as well as reduced the survival and ex vivo clonogenic potential of primary AML blasts. KDM6 silencing in vitro further confirmed AML cell addiction to KDM6 signalling. KDM4B and KDM4C were also upregulated in AML. TCGA database analysis showed significant positive correlation of KDM6 with KDM4 demethylases in AML patients. Combining GSKJ4 with KDM4B or KDM4C inhibition caused additive AML cell death. ChIP-seq and transcriptome analysis revealed KDM6A-dependent DNA repair pathway to be significantly upregulated in primary AML blasts. Additionally genome wide binding analysis of KDM6A and KDM6B in corresponding knockdown AML cells, along with transcriptome profile of paired samples revealed homolog specific regulation of target genes involved in AML maintenance. Together these data indicate that H3K9 and H3K27 demethylases may have non-redundant roles in HSC aging and myeloid leukemia.

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W-2056

IPS TECHNOLOGY REVEALED THE GENETIC AND FUNCTIONAL DIVERSITY PRESENT IN A MYELODYSPLASTIC SYNDROME PATIENT

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Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell diseases characterized by inefficient hematopoiesis and risk of progression to acute myeloid leukemia (AML) with poor prognosis. The relationships between genetic architecture and clonal evolution in MDS remain poorly understood. We used reprogramming technology and transplantation into immunodeficient mice to functionally dissect the genetically defined subclones of secondary AML evolving from MDS (MDS/sAML) with normal karyotype

and multiple FLT3-internal tandem duplication (ITD) mutations. We successfully established more than 30 iPSC lines from sAML clones (sAML-iPSC) and 4 normal iPSC lines from normal clones at the same time, utilizing episomal methods. sAML-iPSC lines displayed characteristic morphology and expressed pluripotent stem cell markers at the levels comparable to those in isogenic normal iPSC lines. Most of the sAML-iPSC lines retained normal karyotype. About half of the sAML-iPSC lines harbored one out of four types of FLT3-ITDs identical to the primary sAML cells, reflecting the mosaicism of the FLT3-ITDs in the patient's sAML cells. SNP-CGH analysis revealed that all sAML-iPSC lines shared the chromosome 11p uniparental disomy (UPD) identical to those of the primary sAML cells. Hematopoietic differentiation efficiency of sAML-iPSC lines was comparable to that of isogenic normal iPSC lines. However, hematopoietic progenitor cells (HPCs) from sAML-iPSC lines displayed two to three times enhanced colony formation ability in myeloid-conditioning semifluid medium. Colonies formed by sAML-iPSC-derived HPCs were predominantly composed of immature myeloblasts and could be replated for at least five rounds. To functionally evaluate the subclonal diversity in vivo, we performed xenotransplantation of HPCs from several sAML-iPSC lines into immunodeficient mice. HPCs from FLT3-ITD-positive sAML-iPSC lines displayed a higher ability to engraft and induced more severe sAML-like disease in recipient mice than those from FLT3-wild-type sAML-iPSC lines. Our data suggest that this iPSC-based system could be useful for analysing clonal architecture and biological feature of subclones of MDS to identify candidate genes responsible for initiation and progression of MDS.

W-2058

EXPLORE THE HUMAN OVARIAN CARCINOGENESIS USING A SPONTANEOUS TRANSFORMED SYNGENEIC MODEL

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Improving screening and treatment options for epithelial ovarian cancer patients has been a major challenge in cancer research. Development of novel diagnostic and therapeutic approaches for high-grade serous ovarian cancer (HGSC) has been hampered by controversies over the origin to the disease and a lack of spontaneous HGSC models to resolve this controversy. Over long-term culture in our laboratory, a fallopian tube epithelial (FE25) cell line spontaneously transformed after propagating more than 100 passages (FE25L). The objective of this study was to determine if the FE25L cell line is a good model of HGSC. FE25L cells grow faster than early passage parental FE25 cells in a doubling time of 64 and 160 h, respectively. FE25L cells form colonies in soft agar after follicular fluid (FF) treatment, an activity for which FE25 cells have negligible capacity. Aberrant Wnt/β-catenin and Nf-κB signaling were also noted. Upregulation of Ccnd1 and loss of Cdkn2a in FE25L tumors is consistent with changes identified in human ovarian cancers by The Cancer Genome Atlas. Intraperitoneal injection of FE25+FF cells into severe combined immunodeficient mice produced cytokeratin+, WT1+, and PAX8+ tumors, a histology resembling human HGSC. This study has identified the FE25L cells as the first spontaneous human cell model of HGSC and provides evidence for the fallopian tube epithelium as a possible origin of HGSC. Wnt/β-catenin and Nf-κB signaling may play an important role in carcinogenesis.

W-2060

CAR-T CELLS WITH MULTIPLE ANTI-CANCER SPECIFICITIES - NEXT GENERATION IMMUNOTHERAPY FOR ADENOCARCINOMA

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Chimeric antigen receptor (CAR-) T cells exploit the intrinsic cytotoxic function of T cells whilst manipulating specificity by expressing a nominal antigen-specific receptor containing a cytoplasmic activation domain. This technology is dramatically improving anti-cancer therapy but to mitigate against tumour escape via mutation, we have developed multi-specificity CAR-T cells. The primary target is TAG-72, a glycosylation mutant on adenocarcinomas, with secondary targeting via the T cell receptor (TCR) or other CARs. We have compared second generation CAR constructs with either CD28 or 4-1-BB signalling. The relative cytotoxic efficacies of these variant CAR-T cells were evaluated using the real time impedance-based xCELLigence assay. CAR-T cells were isolated by flow cytometry and maintained in basal media supplemented with IL-2, IL-7, IL-15 or IL-21 3 serum supplements for 24-48h. Cell activation was determined by flow cytometry for CD137 and HLA-DR expression. For cytolytic function both TAG-72hi and TAG-72low/neg target cell lines were exposed to CAR-T for at least 20h. Parallel studies were performed with non-transduced T cells from the same donors. In addition to the CAR-specific killing, CD3/CD28 cross-linking and high IL-2 levels resulted in polyclonal TCR hyper-stimulation with potent elimination of multiple target cells induced by both CAR-T and non-transduced T cells. This non-specific target cell killing was ameliorated by removing the CD3/CD28 stimulation and reducing IL-2 levels in the culture system. In contrast, IL-7 maintained low level CAR-T activation as determined by expression

of HLA-DR/CD137 comparable to baseline. IL-7 also restored antigen specificity with complete elimination of TAG-72hi but not TAG-72low/neg cells in vitro; non-transduced "resting" T cells showed no killing. Through real-time cell monitoring we have identified culture conditions with the ability to augment CAR-T function in vitro. Importantly, manipulation of exogenous growth factors and cytokines significantly enhances target cell elimination to the detriment of target-antigen specificity. Taken together, these studies highlight the importance of the production process in achieving the fine balance between highly antigen-specific but lethal CAR-T cells for the eradication of cancer.

W-2062

USING MOUSE GASTRIC STEM CELL MODELS TO INVESTIGATE HOW NFKB1 TRANSCRIPTION FACTOR FUNCTION PREVENTS THE DEVELOPMENT OF GASTRIC CANCER

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Gastric cancer (GC) is a significant cause of cancer-related deaths especially since the early phase of the disease is typically asymptomatic, with diagnosis usually occurring at later stages of the disease that coincides with poor patient outcomes. Intestinal type gastric cancer (IGC), the most common type of gastric adenocarcinoma, is intimately linked to chronic inflammation resulting from Helicobacter pylori infection. The sequential phenotypic changes catalogued during IGC development are thought to arise from genetic changes driven in large part by chronic inflammation. While inflammation associated changes within both the immune system and gastric epithelium cooperate to drive GC development, current thinking indicates that genetic changes in the adult gastric epithelial stem cells normally sustaining gastric epithelial homeostasis are responsible for cancer development. My host laboratory has recently identified the NF-B1 transcription factor as a gastric cancer tumour suppressor. Mice lacking NF-B1 present with a 'sterile' inflammatory phenotype that triggers chronic gastric inflammation, which over time develops into IGC after progressing through the same steps in disease development seen in H. pylori induced IGC. In this project, the use of the NF-B1 deficient mouse model will be employed to examine the hypothesis that inflammation induced damage and changes to gastric stem cells are a key step in the initial stages of GC development. I will be employing a combination of flow cytometry, 'organoid' culture methodology and gene expression analysis to understand how inflammation alters the properties of gastric epithelial stem cells. It is envisaged that this work will provide a better understanding of the relationship between gastric cancer stem cells and the inflammation process that in the longer term will result in improved therapeutic options.

CHROMATIN AND EPIGENETICS

W-2064

HETEROCHROMATIN CONDENSATION IS MEDIATED BY JMJD1A AND JMJD2C DURING PHYSILOGICAL STEM CELL AGING

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Previous work has demonstrated that cellular senescence is accompanied by an extensive spatial rearrangement of heterochromatin. However, the question of whether heterochromatin reorganization underlies MSC aging and the mechanisms involved in initiating and maintaining this unique epigenetic state remain unclear. Here, we show that MSC senescence is accompanied by a heterochromatin dynamics involving both heterochromatin remodeling and heterochromatin decomposition. We have identified two conserved histone H3 Lys 9 demethylases JMJD1A and JMJD2C, which cooperatively mediate the heterochromatin condensation and heterochromatin destruction at the different stages of MSC senescence. Upon senescence entry, JMJD1A and JMJD2C transcriptionally activate chromosome condensation genes, such as CENPA, NCAPG2 and NCAPD2, to promote heterochromatin condensation. Deficiency of JMJD1A or JMJD2C dramatically increases DNA damage response and accelerates cellular senescence, whereas overexpression of JMJD1A/JMJD2C or NCAPD2/NCAPG2 rescues Doxorubucin-induced DNA damage and MSC senescence. In addition, MSCs and bone tissues derived from jmjd1a knockout mice exhibit reduced heterochromatin condensation machinery and exacerbated DNA damage response. Overexpression of JMJD1A or NCAPD2 rescues Dox-induced DNA damage in jmjd1a-/- MSCs. Importantly, a marked downregulation of JMJD1A and JMJD2C associated with a decrease in H3K9me3/2, HP1g and heterochromatin condensation genes is found in various stem cells derived from old human individuals. In contrast, excessive expression levels of JMJD1A or JMJD2C has been found in progeria patient stem cell samples. Mechanistic studies demonstrate that excessive JMJD1A or JMJD2C leads to an extensive loss of H3K9 me3/2 mountains at the chromosome level and heterochromatin destruction. In conclusion, our study has revealed a previously undefined role of histone demethylase in modulating heterochromatin condensation, which functions as a protective mechanism against DNA

damage during stem cell aging. Moreover, our study also provides a direct evidence that epigenetic machinery is differentially regulated and might play distinctive roles in physiological and pathological aging.

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W-2066

DISSECTING CORE REGULATORY CIRCUITRIES OF HUMAN PANCREATIC DIFFERENTIATION THROUGH EPIGENETIC PROFILING

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Chromatin transitions guide cell differentiation and development, yet how they coordinate gene control circuits is poorly understood. Using pancreatic development from human pluripotent stem cells as a model, we studied epigenetic remodeling through defined stages of the β-cell lineage. Integrating analyses of DNA methylation, chromatin accessibility, and chromatin modification elucidated lineage- and stage-specific regulatory domains and their dynamics during differentiation, revealing the pioneer factors and epigenetic priming that steer endocrine cell specification and maturation. We then focused on super-enhancer domains (SE), inferring core regulators by finding SE-driven transcription factors in interconnected auto-regulatory loops at each developmental stage. These core regulatory circuits comprise previously known pancreatic master regulators and novel candidates. The latter include LIM homeobox transcription factor 1-β, which we validate as critical for pancreatic endocrine progenitors, and the circadian regulators Basic helix-loop-helix family member e40 and e41, which we characterize as markers of β-cell maturation. The approach and results show how regulatory chromatin transitions establish transcriptional circuits that orchestrate human pancreatic development, and provide a general framework for dissecting regulatory circuits of differentiation.

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W-2068

LOSS OF POLYCOMB REPRESSIVE COMPLEX 2 IMPAIRS LUNG BRONCHIOLAR CELL GROWTH AND UNDERLIES CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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EZH2, the functional enzymatic component of the Polycomb Repressive Complex 2 (PRC2), is a histone methyl transferase that tri-methylates histone H3 at lysine 27 (H3K27me3). This protein plays a significant role in maintaining tissue specificity by silencing gene programs that control lineage fate. We hypothesized that PRC2 controls cell fate of the lung stem cells. In order to test this hypothesis, we generated mice in which we can conditionally delete Ezh2 upon ingestion of doxycycline. To examine growth, differentiation and self-renewal potentials of different lung epithelial cells, we FACS-isolated distal lung bronchioalveolar stem cells (BASCs) and alveolar type two cells. We cultured these cells in air-liquid-interface Matrigel cultures. We observed a 2-fold decrease in both bronchiolar and alveolar lung organoids derived from BASCs, while there was no difference in Ezh2 knock-out alveolar cells compared to control. To test in vivo potential of EZH2 depleted epithelium to repair, we performed naphthalene injury, which specifically injures the bronchiolar club cells. We observed that 7 days post naphthalene injury; Ezh2 knock-out lungs had significantly less repair, as measured by repopulation of bronchiolar lung cells. Given that we observed that lung stem cells were impaired when PRC2-mediated gene repression was perturbed, we sought to learn if PRC2 is also dysregulated in Chronic Obstructive Pulmonary Disease (COPD). We first performed Gene Set Enrichment Analysis and found that gene up-regulated in COPD patients relative to healthy smokers were also up-regulated in Ezh2 knock-out adenocarcinoma cells. We next obtained lung tissue from healthy patients and patients with COPD and performed immunohistochemistry for the PRC2 mark H3K27me3 and immunofluorescence for basal and club cells. There was a significant decrease in H3K27me3, coupled with an increase in goblet cells that contained high expressions of club cell markers in COPD lung compared to healthy controls. Together our results indicate that PRC2 plays an important role in distal lung stem cell function, which may be perturbed in COPD patients. ChIP-seq and RNA-seq studies to understand the transcriptional and epigenetic changes leading to these phenotypes are underway.

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W-2070

CHANGES IN CHROMATIN ARCHITECTURE AND GENE EXPRESSION OF HUMAN EMSCS IN PROLONGED CULTURE

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The dynamic remodelling of human endometrium mainly depends on resident adult stem cells, including perivascular mesenchymal stem/stromal cells (MSCs). Endometrial MSCs (eMSCs) purified using the SUSD2 marker are clonogenic and have typical MSC properties. Their therapeutic potential and ease of accessibility make eMSCs attractive candidates for clinical purposes but spontaneous differentiation and senescence of MSC during culture expansion required to generate large scale cell manufacture hampers clinical utility. We reported that blockade of transforming growth factor β receptor (TGF-β-R) signaling by a small molecule, A83-01, attenuates loss of stemness in prolonged culture. The aim of this study was to identify the transcriptional regulators and downstream gene pathways responsible for A83-01-induced maintenance of the eMSC state. SUSD2+ eMSCs cultured with or without 1 μM A83-01 in serum-free medium and 5%O2 were subjected to RNAseq and ATACseq. Integrated transcriptomic and genomic analysis revealed that A83-01 altered the expression of 1,463 genes (DEG) and there were dynamic changes in the chromatin architecture, exemplified by opening of 3,555 and closing of 2,412 genomic regions (Bonferoni adjusted $p < 0.05$). Gene ontology analysis showed an up-regulation of DEG involved in cell growth and down-regulation of cell fate commitment DEG. Mining of the RNA-seq data revealed that TGF-β-R negatively regulates extracellular matrix (ECM) genes, e.g, COL1A1, COL1A2 and SPARC. Cross-referencing the transcriptomic profile and ATAC-seq data identified a linear correlation between DEG and differential chromatin opening ($p = 1.0 \times 10\text{-}6$, t-test). Binding motif discovery analysis using HOMER revealed enrichment of a high affinity binding site for nuclear receptor subfamily 4 group A member 1 (NR4A1, previously NUR77, $p = 1.0 \times 10\text{-}36$) in cis-regulatory regions of eMSCs expanded in A83-01 medium. NUR77 is an endogenous inhibitor of TGF-β signalling and may mediate down-regulation of ECM genes. Taken together, integrated global analysis of genome architecture and gene expression represents a step forward in elucidating the molecular mechanisms underpinning the maintenance of a less differentiate state of expanded eMSCs by A83-01 that ultimately may accelerate their clinical translation.

W-2072

RNA BINDING PROTEINS WORK COOPERATIVELY TO REGULATE MYOGENESIS THROUGH MICRORNA

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RNA binding proteins are important players in regulating stem cell differentiation. We studied the functions of RNA binding protein Musashi 2 in myogenesis. Myogenesis is a complex process orchestrated by many factors to precisely regulate the proliferation and differentiation of muscle stem cells. Musashi proteins play important roles in supporting HSC stemness maintenance and promoting hematologic malignances. In contrast, Musashi protein is pro-differentiation and facilitating muscle stem cells exiting the self-renewal stage and entering differentiation. We found that RNA binding protein HuR and Msi2 work coordinately to regulate the processing of microRNAs. HuR forms the basic scaffold to recruit another RNA binding protein Msi2. The amount of Msi2 serves as a trigger for the processing of pri-miR7. Sufficient amount of Msi2 will prevent the processing of pri-miR7 and facilitate muscle stem cell differentiation. To initiate this process, more HuR protein was exported to cytoplasm. The cytoplasmic HuR can bind the 3' UTR of Msi2 and facilitate its translation. More Msi2 protein was produced and imported to the nuclei. Therefore, more Msi2 was recruited to the Msi2-HuR complex to prevent miR7 processing and further enhance differentiation of muscle stem cells. The positive feedback circuit mediated by HuR and Msi2 facilitate the efficient differentiation of muscle stem cells. How the same protein assumes different functions in hematopoietic cells and muscle cells is under further investigation.

ORGANOIDS

W-2074

SCALABLE PRODUCTION OF REPRODUCIBLE CEREBRAL ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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In recent years, various human organoid models have drawn a great deal of attention due to the seemingly limitless potential to model complex human diseases in a non-invasive manner. Cerebral organoids (CO) are of particular interest as it is now possible to model aspects of human neural development in a more physiologically relevant three-dimensional setting. This platform, partnered with advancements in both single cell analysis and gene editing techniques has laid the foundation for studying neural development at greater depth. Though this model will undoubtedly offer a plethora of data pertaining to both neural development and disease, a current challenge is the substantial variation in the overall CO morphology and cellular composition, as well as efficiency in generating healthy, mature organoids. Thus, to utilize this system to the fullest degree, it is critical to address this variability within the organoid differentiation platform to provide a more robust model for experimental manipulation. Here we build upon the platform developed by Lancaster et al. Nat Protoc. (2014) 9: 2329-40 and outline a method to generate whole brain cerebral organoids with increased efficiency that each share similar overall cellular composition, both phenotypically and at the single cell level. This optimized platform allows for an increase in both the number of CO's generated in a single batch, with increased structural and functional uniformity between each organoid. Using this platform, it is now possible to generate uniform CO's with >85% efficiency across multiple batches.

W-2076

GENERATION OF ARTIFICIAL MATERIAL-FREE AND OXYGEN/NUTRIENT EXCHANGE FEASIBLE BIO-MIMETIC TISSUE MODULE USING ADIPOSE MESENCHYMAL STEM CELLS

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Many researchers are trying to produce bio-mimetic organoid for regenerative medicine and disease models for now. However, these trials have limitations such as neglected biological activity, artificial materials engaging, necrosis and degeneration of organoid core. Therefore, we developed two step self-assembly technique platform that can generated several millimeter sized bio-mimetic 'tissue module (TM)' using mesenchymal stem cell, which is not involved any kind of artificial-material and formed unique three dimensional structure. Furthermore, we differentiated the TM into cartilage like large-scale tissues successfully. Human mesenchymal stem cell was isolated and purified from human thigh fat. Firstly, we produced 'microblock (MB)' from the cells by G-forces and standardized the condition of various sized-MBs from 50 to 500 μm. Secondly, we rearranged the various sized-MBs in the limited space. Then we optimized the condition of the rearranged MB in artificial material-free condition generated 3~4 mm sized TM that formed oxygen and nutrient exchange feasible three dimensional structure. In these serial processes, we demonstrated the successive self-organization of TM by analyzing total rate, size, number, occupied space, remaining space and velocity of generated TM using time-lapse live imaging as well as morphologic changes. We categorized serial procedures during TM self-assembly as follows; initiation, margination, condensation, lifting and folding. Furthermore, we differentiated TM for chondrogenic lineage, which showed chondrogenic characters and adhered with human cartilage successfully by ex vivo experiment. Whole process were analyzed and proved by histological, biochemical analyses, 3D tissue clearing and imaging and bio-kinematics analyses. In this study, we suggest a novel platform for organoid production technology, which can overcome the limitations of bio-mimetic organoid production. And it can be applied for many kind organoids generation for regenerative medicine.

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W-2078

'NEXTGEN' HUMAN BRAIN ORGANOIDS USING 3D PRINTED GELATIN METHACRYLATE

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The generation of brain organoids derived from human pluripotent stem cells (PSCs) is a significant step towards better in vitro modelling of neurodevelopment and disease. Brain organoids are discerned by their cellular and structural complexity, with characteristics of developing embryonic brains. Conventional methods of organoid formation are limited to small-scale operation and require multiple handling steps following stem cell aggregation, coating with expensive and undefined tumour-derived Matrigel™ basement membrane preparation, and arduous bioreactor based differentiation and expansion methods. We have initially demonstrated gelatin methacrylate (GelMA) to be a cell growth substrate for rapid and novel induction of brain organoids from human induced PSCs (iPSCs). GelMA is a versatile, 3D printable semisynthetic matrix that incorporates the intrinsic bioactivity of natural matrices with the fidelity of synthetic biomaterials for more defined and clinically-compliant cell support. Towards scaling up organoid production we have now 3D printed GelMA-based microwell arrays to generate large numbers of organoids for higher throughput R&D. Constructs consist of densely packed cell soma with regional divisions resembling cortical plate or rudimentary grey matter tissue with underlying white matter-like tissue, as well as hollow neural tube-like structures. With larger numbers of organoids we are progressing our understanding through immunofluorescent-based histochemistry of cortical lamination using layer-specific markers of cerebral neocortex and early progenitor regions; markers including RELN, CTIP2, TBR1, SATB2, PAX6, NES, and SOX2, as well as the forebrain specific marker, FOXG1. Moreover, we have demonstrated coordinated glutamate-responsive neural network activity of formed neurons by extracellular recordings using multi-electrode arrays (MEAs). The optimised "NextGen" method provides a defined, simplified and higher throughput platform for "brain on a bench" research and translation of iPSCs, neural derivatives and neural organoids, including in vitro modelling of brain development and disease, tissue engineering and regenerative medicine.

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W-2080

ORGANOID MODELING OF REPRODUCTION-CRITICAL ORGANS: DEVELOPMENT AND CHARACTERIZATION OF ORGANOIDS FROM MOUSE PITUITARY AND FROM MOUSE AND HUMAN ENDOMETRIUM

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The pituitary gland and endometrium are essential for mammalian reproduction. The pituitary controls the menstrual cycle through secretion of LH and FSH which regulate estrogen and progesterone production that drive the endometrium's remodeling to make it blastocyst-receptive. Dysfunction of these organs is dismal for reproductive success. Research of their biology and disease is hampered by the lack of reliable and robust study models. Therefore, we seeked to develop organoids from both organs as new powerful in vitro study tools. First, we succeeded in establishing long-term expandable and stable organoid models from mouse and human endometrium. The organoids recapitulate typical endometrial cell characteristics and biological responses, in particular to the reproductive hormones thereby mimicking the menstrual cycle in a dish. Moreover, organoids were developed from pathological samples including endometriosis and endometrial cancer. These organoids reproduce disease-specific features which are stable during long-term expansion. Second, we achieved the development of robustly expandable organoids from mouse pituitary. The organoids originate from the gland's stem cells and display an immature phenotype. Particular hormonal differentiation occurs after subrenal implantation. Recently, we discovered that the mouse pituitary possesses regenerative capacity, and that the local stem cells are activated during regeneration. Application of

the organoid protocol to this pituitary damage-and-regeneration model yielded different organoid types from damaged and undamaged pituitary, dissimilar in morphology, expansion capacity and (stemness) gene expression, suggesting the possibility of different stem cell populations or states in the pituitary. Taken together, we developed organoid models from the reproduction-critical pituitary and endometrium which provide novel in vitro tools to study biology, remodeling/regeneration and disease, and the involvement of stem cells in these processes.

W-2082

INTERROGATION OF CONGENITAL DEGLYCOSYLATION DISORDER-INDUCED NEURAL ABNORMALITIES IN NGLY1-DEFICIENT HUMAN PLURIPOTENT STEM CELLS AND CEREBRAL ORGANOIDS

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Although NGLY1 gene mutations that lead to NGLY1 deficiency and neurological symptoms have been recently identified as the cause of a congenital deglycosylation disorder in pediatric patients, how NGLY1 deficiency disturbs normal cerebral development and causes neurological abnormalities remains unknown. We are probing how the malfunction of NGLY1 glycosidase causes abnormal neurodevelopment and cerebral phenotypes. Through gene editing and cell reprogramming, we obtained NGLY1-deficient human pluripotent stem cells (hPSCs) from normal human embryonic stem cells (hESCs) and patient samples. To investigate the effects of NGLY1 deficiency on the early neural development, we streamlined two-dimensional and three-dimensional differentiation protocols for neurogenesis in hPSCs. NGLY1 knockout appeared to have a negligible impact on the viability and cellular pluripotency of undifferentiated hPSCs. Neuroepithelial spheres were obtained in both control and NGLY1-deficient hPSCs, suggesting that the commitment of hPSCs to the neural lineage is not profoundly hindered by compromised NGLY1 activity. Compared with control cells, the neural derivatives of NGLY1-deficient hPSCs showed a high propensity of apoptosis, suggesting that NGLY1 activity may be critical for the viability of neural progenitor cells and the success of their subsequent differentiation. Using high-content imaging approaches, we characterized the hPSCs that underwent two-dimensional differentiation and cerebral organoid development, enabling our discoveries of cellular and structural defects of upper- and lower-layer neurons with NGLY1 malfunction. Array-based transcriptomic analysis revealed stage-specific abnormalities in the NGLY-deficient cerebral organoids. Using single-cell RNA sequencing, we are examining the potential alterations of cellular heterogeneity among control, NGLY1-deficient and NGLY1-overexpressing cerebral organoids. Representing a comprehensive interrogation on the NGLY1 deficiency-induced neural abnormalities at cellular and molecular levels, our study has indicated that NGLY1 deficiency may bias cell fates during neural differentiation and lead to the abnormal development and functional defect of the human brain.

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W-2084

ELUCIDATION OF UNIFORMED MECHANISMS INVOLVED IN 3D SPHEROID FORMATION OF DIVERSE HUMAN & MOUSE PLURIPOTENT STEM CELLS & NON-STEM CELLS

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3-dimensional (3D) cell culture systems for spheroid formation increasingly appear to better mimic physiological states than standard 2D culture. However, the biological consequence of spheroid formation differs for different cell types: for pluripotent embryonic stem cells (ESCs), 3D-spheroid formation mimics embryo development and results in loss of pluripotency/stemness with a concomitant occurrence of differentiation and lineage commitment; in contrast, spheroid formation in somatic cell types may allow for selection of higher 'stemness' possessing cells, i.e. selection of somatic stem cell (SSC) populations such as neural and mammary stem cells, as well as tumor-initiating cells (TICs) in abnormal cancer cells. Despite

these diverse biological consequences, however, there may be a core process governing the ability for 3D spheroid formation across diverse cell types. To elucidate such processes, we performed transcriptome analysis on conventionally 2D- and 3D-cultured mouse ESCs, normal somatic cells, and cancer cells, with subsequent functional validation. As expected, pluripotency markers such as Oct4, Sox2, Nanog, & Klf4 were significantly down-regulated in ESCs after 3D spheroid formation; in contrast, these four markers were not significantly upregulated in mesenchymal stromal cells (MSCs) or cancer cells after spheroid formation. Consistently downregulated pathways for all cell types include the cell cycle and related proliferative processes, which was validated with functional assays. Unexpectedly, a number of metabolic pathways are uniformly regulated in 3D spheroid formation for all 3 cell types. These murine cell-generated data were validated in the human system, using human induced pluripotent stem cells (iPSCs), MSCs, and cancer cells. These findings reveal the profound cell-specific changes brought about by 3D spheroid formation, and contribute to further understanding on the physiological relevance of 3D *in vitro* culture systems.

W-2086

ESTABLISHMENT OF HUMAN PRIMARY HEPATOCELLULAR CARCINOMA-DERIVED ORGANOID CULTURES FOR DISEASE MODELING AND DRUG SCREENING

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Hepatocellular carcinoma (HCC) is a leading cause of cancer fatalities globally, with a particularly high prevalence in Southeast Asia and Hong Kong. Recent next generation sequencing studies revealed that a majority of HCC harbors recurrent mutations, many of which are targets of current approved drugs. However, further translational research of these findings into novel HCC therapies has been precluded by the lack of reliable experimental models. The value of 2-dimensional (2D) cancer cell culture in predicting clinical efficacy has been continuously challenged, while the establishment rates of patient-derived xenografts (PDX) are low, time-consuming and even if successful, limited by their scalability. Recent reports have found liver cancer-derived 3-dimensional (3D) organoid cultures to highly recapitulate features of the most common subtypes of human primary liver tumors. Based on this protocol, we have successfully established a pilot bio-bank of HCC organoids specific to Asian ethnicity. Our HCC organoids fully resemble histological architecture of their corresponding original tumor tissue, with HCC phenotype validated by the presence of both hepatocellular markers AFP and GPC3, as well as the absence of ductal/cholangiocarcinoma markers CK19 and EpCAM. Whole exome sequencing revealed that these HCC organoids and their corresponding tumor tissues concordantly harbor driver mutations in tumor suppressor genes including the more frequently mutated TSC2 and AXIN1, which have both previously been documented in large-scale HCC studies. In addition, our HCC organoid samples also retain the hepatitis B virus (HBV) genome, closely mimicking one of the common features of Asian HCC. The successful establishment of these patient-derived HCC organoids will allow for a wide spectrum of biological processes including drug sensitivity screening as well as disease modeling. Applications of these new pre-clinical HCC models will shed insight into drug resistance mechanisms of HCC and facilitate the development and translation of HCC targeted therapies.

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TISSUE ENGINEERING

W-2088

APPLICATION OF A HUMAN VENTRICULAR CARDIAC ORGANOID CHAMBER FOR PRECLINICAL DRUG SCREENING EXPERIMENT

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Differentiation of the human embryonic stem cells (hESC) into ventricular cardiomyocytes (vCMs) provides an unlimited source of cells for in vitro model in drug screening. However, such 2D cell cultures are not physiological and limited information on cardiac physiology can be obtained. On the other hand, 3D construct of human ventricular cardiac organoid chamber (hvCOC) offers a contractible chamber,

which allows evaluations on the relationship between developed pressure and volume that better characterize cardiac function. This study aims to develop hvCOC fabricated from human embryonic stem (HES2) cells as a drug screening model. Direct cardiac differentiation was performed to differentiate HES2 into vCMs. The vCMs were then fabricated into hvCOC. For drug screening, compounds with positive (dobutamine, isoproterenol, levosimendan and digoxin) and negative inotropic effects (disopyramide, verapamil and nifedipine) were added to the hvCOC 10 days after their fabrication. Parameters of mechanical functions of the hvCOCs were analyzed by the measurement of developed pressure and stroke volume during spontaneous contraction and upon electrical pacing. When paced at 1 Hz, the hvCOC beat at 60 bpm with measurable developed pressure and stroke volume. Upon treatment, hvCOC responded to the inotropes with significant increases in cardiac output and stroke work with positive inotropic drugs and decreases in cardiac output and stroke work with negative inotropic drugs. These results suggested that the hvCOC functioned as a 3D construct of human cardiac tissue for drug screening experiments.In conclusion, hvCOC provides a physiological platform for ex-vivo drug screening and discovery. Together with hiPSC reprogramming technology, this model can be developed into a patient-specific platform for personalized precision medicine

W-2090

TOPOGRAPHICAL CUES ENHANCE THE FUNCTIONAL MATURITY OF PLURIPOTENT STEM CELL DERIVED ENDOTHELIAL CELLS

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Pluripotent stem cell derived endothelial cells (PSCEC) are an attractive alternative endothelial cell (EC) source for various clinical applications. However, current differentiation protocols yield a heterogeneous PSCEC population, with uncontrollable specification into arterial or venous subtypes. Arterial and venous EC are phenotypically and functionally different thus, inapt matching of exogenous EC to host site can possibly affect clinical outcomes, exemplified by venous graft mismatch in an arterial environment, which necessitates specific maturation of PSCEC into a more homogeneous arterial or venous population. Since arterial and venous EC reside on distinctively different basement membrane architectures and substrate topography has been shown to regulate mature EC phenotypes, we postulate that substrate topography can modulate PSCEC maturation into the two subtypes. We conducted comprehensive characterisation of PSCEC, verifying that they were phenotypically and functionally immature compared to primary coronary arterial EC (HCAEC) and saphenous venous EC (HSaVEC) but did not exhibit any subtype bias. We then employed a Multi Architectural Chip (MARC), consisting of 16 unique topographies on a polydimethylsiloxane (PDMS) substrate, to screen topographies that can differentially modulate the expression of arterial (Notch) and venous (COUP) specific markers in PSCECs. 5 topographies, including different gratings and convex micro lenses, were screened out based on Notch and COUP cellular expression, which were validated to distinguish between HCAEC and HSaVEC. These topographies were further tested for their functional effects on PSCEC, including low density lipoprotein (LDL) uptake, Nitric Oxide synthesis, angiogenic potential and immunophenotypes. We discovered that dominant effect of all substrate topographies investigated, was on the functional maturation of PSCEC into an arterial phenotype, indicated by enhanced Notch expression, high LDL uptake and augmented angiogenic potential. This denoted that the default PSCEC maturation pathway mediated by substrate topography may be towards an arterial phenotype, further can be combined with other environmental cues, like soluble factors and shear stress, to obtain purer arterial cell source for clinical applications.

W-2092

OPTIMISING PAEDIATRIC AUTOLOGOUS STEM CELL ISOLATION, GROWTH AND CHONDROGENIC DIFFERENTIATION FOR CARTILAGE BIOENGINEERING

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In Europe, over 800 children/year are born with missing or malformed ears (anotia/microtia), which require multi-step surgery to reconstruct cartilage to improve appearance and function. Currently, the gold standard

for ear reconstruction is achieved by harvesting autologous rib cartilage. While generally successful, the end-results may vary and repeated surgeries can adversely affect children's physical and psychological well-being. Our end goal is to bioengineer cartilage tissue using autologous paediatric patient-derived stem cells. As an abundant, easily accessible, highly expandable and safe source of autologous cells, adipose tissue provides an attractive stem cell source (pADSCs). We have shown that chondroblasts (CHs) can be derived from paediatric microtic ears and are capable of chondrogenic differentiation. Our aim is to optimise and standardize protocols for isolation, growth and differentiation of pADSCs and CHs complying with GMP standards. In addition, we hypothesize that microtic ear chondroblasts may be of therapeutic use either by potentiating pADSC chondrogenic capability, or as an alternative autologous cell source. pADSCs were isolated using dissociation and explant methods. The efficiency of each method was compared for number of cells isolated, time to passage and amount of tissue needed. Chondroblasts were isolated using an explant method. Cells were isolated and expanded either in 5% Human Platelet Lysate (HPL) or 10% Foetal Bovine Serum (FBS) for 5 passages. Cell growth, morphology and metabolic activity were compared at each passage. The differentiation potential of pADSCs towards chondrogenic, adipogenic and osteogenic lineages was also compared. pADSC isolation by the explant method generated a larger number of cells, more rapidly, and from a smaller tissue sample. Both pADSCs and CHs grown in HPL proliferated faster than cells grown in FBS, while maintaining their chondrogenic potential. In co-culture experiments, age and sex matched pADSCs and CHs were mixed (5%, 10% and 20% CH), and chondrogenic differentiation assessed by cell morphology, histology and immunolabelling. Addition of 20% CHs appeared to enhance pADSCs chondrogenesis. Hence combining autologous pADSCs and microtic ear CHs could provide an effective strategy for auricular reconstruction.

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W-2094

A NEW METHOD FOR HEPATIC-SPECIFIC-HIPSC-DERIVED CELLS RECELLULARIZATION

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Liver transplantation is currently the main approach for treatment of irreversible hepatic injuries but the availability of this organ for transplantation as well as immunological compatibility between donors and recipients are still public health issues. Recently, new technologies have been developed to produce decellularized organ matrices to be used as biological scaffolds aiming the reconstruction of new organs with hepatic specific cell lines. However, current decellularization methodologies result in decreased quality of original extracellular matrix (ECM). In order to enhance scaffold recellularization with hiPSC-hepatic-derived cells, the aim of the present work was to improve the quality of the liver-ECM following the decellularization process, by performing a pre-coating with hepatic cells-conditioned medium (CM). Livers from male Wistar rats (300g) were decellularized after 12h of perfusion using 1% Triton-X solution with 0.05% NaOH (pH=11) and then, were submitted to histology, immunohistochemistry, scanning electronic microscopy and DNA-content analysis. Livers-ECM were pre-coated with HepG2-CM and then, recellularized up to 5 weeks with hepatoblasts and mesenchymal stem cells (MSC), both differentiated from human-iPSCs and HUVEC. hiPSCs were differentiated towards hepatocytes-like cells, which were well characterized. The integrity and organization of the pre-coated and non-coated livers-ECM were kept after decellularization as well as coating procedure. Absence of nuclei and cellular residues were confirmed by optical and electronic microscopy. Residual DNA-content was under the immunogenic limits (10ng/mg; $p< 0.001$). Proteomic analyses showed enrichment of the pre-coated liver-ECM quality, when compared with non-coated livers-ECM and with the HepG2-CM. Moreover, the recellularization of pre-coated livers-ECM were significantly improved. The generation of new bioengineering technologies for liver reconstruction are important to improve organ transplantation.

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W-2096

HUMAN MESENCHYMAL STROMAL CELL SECRETOME AS CELL-FREE THERAPEUTIC APPROACH FOR REGENERATIVE MEDICINE

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Multipotent mesenchymal stromal cells (MSC) are considered as a promising tool for regenerative medicine. Recent studies revealed the poor local engrafting and survival of implanted cells and indicated that the benefits of MSC therapy was mediated mostly by producing multiple bioactive components, including soluble factors (SF) and extracellular vesicles (EV), stimulating angiogenesis, neurogenesis, activating tissue-resident stem cells and modulating immune reactions. It highlighted the role of MSC as key regulators of reparation and regeneration processes in damaged tissues. MSC secretome, as conditioned media (MSC-CM) or its fractions, may have considerable advantages over cell therapy for biosafety, manufacturing and storage with comparable regenerative potential. We have developed the optimized MSC-CM production protocol based on dynamics of key factors secreted by human adipose-derived MSC during long-term conditioning in different xeno-free, chemically defined media. Computational prediction modelling was used to overcome the high donor-dependent variability of MSC-CM. We showed that MSC-CM stimulated survival and migration of endothelial cells and human fibroblasts in vitro. Both SF and EV of MSC-CM exhibited antifibrotic effects preventing and even reversing TGFb-induced fibroblast differentiation into myofibroblasts. Regenerative effects of MSC-CM in vivo were analyzed by their ability to restore spermatogonial stem cell niche and functionalize the tissue engineering constructs like collagen membrane for bladder wall defect reconstruction in rabbits. Using the rat cryptorchidism model of spermatogenesis failure, we revealed that MSC-CM injected under tunica albuginea recovered testes hypotrophy, prevent seminiferous tubules fibrosis, normalized spermatogonial stem cell differentiation and function of Sertoli and Leydig cells and resulted in increase of total and moving spermatozoa count. These effects were comparable to cell therapy with MSC from the same donors. Collagen membrane functionalized by MSC-CM significantly better restored injured bladder function by engaging smooth muscle cells, stimulating angiogenesis and modulating immune reactions. Our findings provide strong basis to develop cell-free therapeutic approaches for regenerative medicine.

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W-2098

BIOCOMPATIBILITY OF POLYAMIDE/GELATIN MESH WITH OR WITHOUT ENDOMETRIAL MSC IN AN OVINE MODEL OF PELVIC ORGAN PROLAPSE.

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Pelvic Organ Prolapse (POP) is a clinical burden affecting 25% of women. We are developing a novel tissue engineering approach to regenerate vaginal tissue damaged by childbirth injury. We hypothesise that autologous ovine endometrial MSC (eMSC) improves the biocompatibility of gelatin-coated polyamide mesh (PA/G) implanted into the vaginal wall by switching pro-inflammatory M1 macrophages to a wound healing M2 phenotype, regenerating tissue and improving the biomechanical properties of vaginal tissue. eMSC were isolated from ovine hysterectomy tissue by FACS as CD271+CD49f- cells, cultured and labelled with FITC-Iodex-paramagnetic nanoparticles and seeded onto PA/G mesh. Parous BLM ewes (n=36) with objectively measured vaginal wall weakness (POP) were divided into 3 groups: incision control, PA/G mesh, autologous eMSC seeded onto PA/G (n=6/gp). Tissue was harvested at 7 and 30 days. Immunohistochemistry was used to quantify M1 (CD86) and M2 (CD163, C206) macrophages around mesh filaments. Smooth muscle was quantified in Mason Trichrome-stained tissue. Multiaxial biomechanical properties were tested by ball burst test. FITC-labelled eMSC were detected in vaginal tissue at 7 and 30 days. Similar numbers of M1 and M2 macrophages were found in PA/G and eMSC/PA/G groups and the M1 and M2 macrophage response was significantly greater than the incision control. Greater disruption of the smooth muscle was observed in the vaginal wall in PA/G and eMSC/PA/G explants compared to controls. Mesh erosion into the vagina occurred in 23% of day 7 and 38% of Day 30 meshes. Biomechanical testing showed no differences between groups. Implanted autologous eMSC persisted in vaginal

tissue for 30 days, but transvaginal insertion of PA/G and eMSC/PA/G mesh disrupted vaginal wall integrity and may have masked beneficial effects of eMSC on macrophage polarisation. Our data suggests that non-degradable biomaterials delivered by transvaginal surgery has significant adverse effects in an ovine model of POP and alternate materials should be considered for POP.

Funding Source: NHMRC

W-2100

ULTRASOUND AND MESENCHYMAL STEM CELLS IN THE TREATMENT OF DSS-INDUCED ULCERATIVE COLITIS

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Ulcerative Colitis (UC) is an Inflammatory Bowel Disease (IBD) that presents with symptoms of diarrhea, weight loss, abdominal pain and blood in the stool. Dextran Sulfate Sodium (DSS)-induced colitis is a chemical model that closely mimics human UC. DSS triggers an immune response due to colonic epithelial damage. Despite available treatments, the sustained remission of UC is still difficult to maintain. Mesenchymal stem cells (MSCs) have been extensively studied due to their immunosuppressive properties and assistance in tissue repair. Ultrasound (US) therapy was shown to prevent kidney ischemia-reperfusion injury (IRI) in mice through the nicotinic cholinergic anti-inflammatory pathway (CAIP), which is involved in IBD. The aim of this study was to determine the efficacy of MSCs and/or US treatment in DSS-induced colitis. UC was induced by administration of DSS 2% in drinking water for 7 days. Human bone marrow-derived MSCs were administrated intraperitoneally at days 5, 7, 9 and 11. US treatment was applied using clinical parameters from days 4 to 10, 7 min/day 1Mhz, 2 w/cm^2, 10% duty cycle with a 5 cm^2 transducer. C56Bl/6 mice were monitored daily for weight loss, stool consistency and blood in the stool. Colon and spleen samples were collected at day 14 for further analysis. The groups in which MSCs and US were administered alone showed improvement in clinical scores ($p<0.05$), but no difference was seen when the treatments were combined. All groups presented histological improvement in colon scores compared to DSS 2% alone. US alone treatment was comparable to controls ($p<0.05$) and improved colon length ($p<0.05$). Spleens were enlarged with MSCs/US and MSCs alone ($p<0.05$) as compared to the other groups. MSCs/US and MSCs treatments decreased the percentage of CD3+CD8+ T cells, while increasing F4/80+CD206+ anti-inflammatory macrophages (M2) in the spleen ($p<0.05$). These results suggest that US with IP MSCs may have a therapeutic role in IBD.

Funding Source: NIH and CAPES

W-2102

REDEFINING BONE MARROW STROMAL CELL (BMSC) CHONDROGENIC INDUCTION AND INDUCTION KINETICS

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Osteoarthritis (OA) is the leading cause of pain and disability worldwide. The capacity to repair cartilage defects with bone marrow stromal cells (BMSC - sometimes referred to as mesenchymal stem cells) would have a profound impact on patient health and productivity. Despite immense promise, and decades of research, the field struggles to produce stable articular chondrocytes (AC) from BMSC. To date, protocols using BMSC-derived chondrocytes are not capable of forming hyaline cartilage and the resulting tissues tend to undergo hypertrophy (forming bone-like tissue), particularly in vivo. The field's inability to form stable hyaline cartilage from BMSC is a major obstacle preventing the widespread development of effective BMSC-mediated cartilage defect repair strategies. We used our novel 3D cell culture platform, the Microwell-mesh, to produce spheroid array cultures. We evaluated the specific differences between human BMSC and normal human AC at pre-induction and during early and late stages of induction using RNA-Seq, qRT-PCR, biochemical and histological analysis. Our RNA-Seq data show the gene expression divergence between BMSC and AC during pre-induction and during early and late stages of induction. Gene pathway analysis identified potential targets for directing BMSC toward a more AC-like phenotype that may be applied at different stages of the induction protocol to improve cartilage tissue production and prevent hypertrophy. Through this project, we aim to optimise the induction of BMSC toward an AC-phenotype and ultimately use these tissues to rebuild damaged cartilage and prevent the development and progression of OA.

W-2104

NOVEL AMNION-BASED SCAFFOLD MADE BY DECELLULARIZATION-DRY FREEZING APPROACH EXHIBITS ENHANCED CELL ENGRAFTMENT AND GROWTH, INCREASED STRENGTH AND IN VIVO BIOCOMPATIBILITY

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Current vascular replacement grafts used in congenital heart defect corrective surgery have poor longevity and growth potential. Recipient patients often require multiple reoperations. Tissue engineering has the promise to produce a graft with the potential to grow, remodel and repair. Here we aimed at developing an autologous graft made of amnion-based scaffold seeded with thymus-derived mesenchymal stem cells (MSC). The developed human amnion-based scaffold was made by an enzymatic decellularization process followed by freeze-drying as a single or multi-layered structure. These structures were compared to native amnion for seeded cell viability and biomechanical properties then were tested for their in vivo biocompatibility. Our results demonstrated that while native amnion tissue supported no cell growth, the decellularized-amnion allowed cell engraftment and growth. Additionally, preservation of the scaffold by freeze-drying it as a single layer, further improved engraftment and cell growth. Multi-layering the freeze-dried amnion-scaffolds resulted in similar cell growth potential of the single layered construct but superior mechanical strength. The multi-layered construct showed in vitro biocompatibility with endothelial cells, smooth muscle cells, cardiac myocytes, and cord-blood-derived MSCs. When implanted in a piglet model of left pulmonary artery grafting, the multi-layered construct showed in vivo biocompatibility as demonstrated by the development of endothelial cell layer in the inner side of the graft and a smooth muscle layer in the outer side. In conclusion, our developed amnion-derived scaffold represents an off-the-shelf biocompatible structure that can be seeded with the patient's own MSCs to produce an autologous vascular graft.

Funding Source: Sir Jules Thorn Trust, Enid Linder Foundation

W-2106

SPATIALLY PATTERNED SCAFFOLDS MODULATE THE FUNCTION OF PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IN VITRO AND IN VIVO

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Cardiomyoctyes (CMs) have highly organized physiological structure with ordered cellular alignments. We hypothesize that the microtopographical cues, coupled with the cell-cell interactions between CMs and endothelial cells (ECs), enhance cardiac function *in vitro* and *in vivo*. To engineer myocardial tissues that better mimic the highly ordered physiological arrangement and function of native CMs, we generated microfibrous scaffolds with either randomly oriented or parallel-aligned microfiber arrangement and co-seeded the scaffolds with human pluripotent stem cell-derived cardiomyocytes (iCMs) and endothelial cells (iECs). Assessment of iCM morphology, maturation markers, and contractility was performed in vitro, and the therapeutic potential of engineered myocardial tissue constructs was evaluated in the infarcted myocardium. Microfibrous polycaprolactone scaffolds with either randomly oriented or parallel-aligned fiber orientation were fabricated by electrospinning. Scaffolds were seeded with iCMs alone or in addition to iECs. For contractility measurements, scaffolds seeded with iCMs were field stimulated at a frequency of 1Hz. For assessment of iCM morphology, alignment, and sarcomeric length, the cells were immunofluorescently stained for cardiac troponin-T. To test the therapeutic efficacy of engineered myocardial tissue, cell-seeded scaffolds were transplanted as an epicardial patch over the infarcted myocardium of immunocompromised RNU nude rats. Aligned microfibrous scaffolds induced iCM alignment along the direction of the aligned microfibers, as well as promoted greater iCM maturation by increasing the sarcomeric length and gene expression of MYH7, in comparison to randomly oriented scaffolds at 5 days of culture. Furthermore, aligned scaffolds significantly increased the maximum contraction velocity of iCMs on the aligned scaffolds, compared to randomly oriented scaffolds. When transplanted to the epicardium of infarcted rats for 28 days, aligned scaffolds seeded with iCMs showed improved cardiac function based on ejection fraction. These findings demonstrate important roles of scaffold anisotropy and, to a lesser degree of endothelial interaction, in engineering cardiovascular tissues that maintain iCM organization and contractile function.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

W-2108

ANALYZING SHORT-TERM AND LONG-TERM OUTCOMES REGARDING STUDENT OPINION OF ACTIVE LEARNING INTERVENTIONS ON PERFORMANCE IN A LIFE SCIENCE GATEWAY COURSE

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As educators, we are constantly working on improving the courses we teach by introducing clear goals and objectives, problem-based learning, practice questions, review sessions, etc. We are thrilled when students grasp the concepts of the course, are able to apply their gained knowledge, and perform well in their assessments. Studies have shown that the utilization of active learning interventions and animations within a course increases long-term retention and deep learning. However, the vast majority of these studies focus on the use of one specific pedagogical element and assesses retention only up until a few months after the original course. Our departmental gateway course (SCRB 10: Human Developmental and Regenerative Biology) has been taught for over seven years. It focuses on the understanding of important embryological concepts, the ability to design experiments and think critically, and the ability to analyze and critique data. From 2009 to 2015, the course has undergone substantial re-organization and improvement. We recently included the use of online "clicker" software both inside and outside of the classroom, more animated slides to better visualize critical concepts, hands-on section exercises, and more interactive practice questions for exams. We wanted to investigate how the different active learning interventions were perceived by the students and how these exercises affected student opinion of their learning. In this first part of our two-part study, we asked what our students thought about the active learning interventions utilized in the course. By getting feedback from students from the first seven years, we gained a first insight on individual active learning techniques, how they were perceived by students, and how the students think the use of these exercises affected their learning. Our study assessed how students felt about these educational tools in retrospect, which for a large cohort meant after finishing college and starting a job. The data gathered in this study will help us set up the second part of our study in which we are planning to look at how long-term retention of course material and critical thinking skills are linked to the use of individual active learning interventions.

W-2110

REGULATING UNPROVEN STEM CELL THERAPIES IN AUSTRALIA

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There are for-profit private clinics proffering untested stem cell treatments all over the world including Australia. There have been reports of baseless claims of cures and adverse events including deaths. Previously, these stem cell treatments are available primarily in developing countries with less regulation or weak enforcement. However, Australia too has stem cell businesses since 2011, and it is growing to more than 60. This nation has among the world's highest concentration of stem cell clinics, with websites advertising medical procedures and anti-ageing therapies. In 2013, Mrs Sheila Drysdale died as a consequence of procedures from liposuction used to extract stem cells to treat her dementia condition. Australia's national drugs regulator is the Therapeutic Goods Administration (TGA). It is responsible for regulating the safety and efficacy of medicines, medical devices and the manufacturing and advertising of therapeutic goods in the country. In 2011, the TGA introduced an exemption for certain kinds of biologicals including 'autologous therapies'. To determine a suitable regulatory framework to govern the matter, TGA conducted public consultations with various stakeholders to seek their views. In late 2017, TGA announced that amendments to the law would be implemented in 2018. This presentation will explore these latest changes to the law.

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

W-2114

EVALUATION OF ADIPOSE DERIVED MESENCHYMAL STEM CELL THERAPY IN COMBINATION WITH ARTHROSCOPIC ABRASION ARTHROPLASTY IN ADVANCED KNEE OSTEOARTHRITIS : A PILOT STUDY

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Osteoarthritis is a significant cause of morbidity and the 4th leading cause of disability world wide. An increasing number of patients are undergoing total knee replacement under the age of 65. The purpose of this study was to prospectively assess the pain, functional and structural improvements after adipose derived mesenchymal stem cell therapy in combination with arthroscopic abrasion arthroplasty in advanced knee osteoarthritis. 20 patients with radiological diagnosis of Grade IV knee osteoarthritis underwent arthroscopic abrasion arthroplasty followed by post-operative intra-articular injections of autologous AdMSCs. Participants received a total of 2 intra-articular injections of 50×10^6 AdMSCs with the first within 1 week of the arthroscopy and the second at 6 months. Participants were placed on strict touch weight bearing for one month post arthroscopy and immediately commenced active low load range of motion exercises (cycling). Outcome was assessed over 12 months and included validated pain and functional outcome scores - Numerical Pain Rating Scale (NPRS) and Knee Injury and Osteoarthritis Outcome Score (KOOS) - and quatitative Magenetic Resonance Imaging (MRI) techniques including T2mapping, and cartilage volume. At 12months statistical and clinical significant improvement in pain and function was observed. MRI analysis showed significant cartilage regeneration with T2mapping indicating values of hyaline like cartilage morphology. Arthroscopic abrasion arthropalsty in combination with intra-articular AdMSC therapy results in reproducible pain, functional and structural improvements with regeneration of hyaline like cartilage. This may be a suitable option for patients with advanced arthritis who are unsuitable due to age or other factors for consideration of total knee replacement.

W-2116

ANALYSIS ON TUMORIGENICITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOR DEVELOPING CELL THERAPEUTIC PRODUCTS

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Cell therapeutic products derived from human induced pluripotent stem cells (hiPSC-CTPs) have been highlighted for the personalized regenerative cell therapy. However, because hiPSCs can form tumors which are known as the teratoma, there is a risk of tumor formation when undifferentiated hiPSCs remain in hiPSC-CTPs. Thus, understanding the features of tumor-formation of hiPSCs is essential to evaluate tumorigenicity of hiPSC-CTPs and to suggest an appropriate guideline for tumorigenicity evaluation. In this study, we transplanted hiPSCs to NSG mice which is the most immunodeficient to date and traced the tumor-forming characteristics up to one year. Before this long-term following, we optimized the transplantation conditions by comparing various parameters such as gender of mice, transplantation sites, and applicability of completely dissociated hiPSCs for accurate cell counting of transplanted cells. We prepared two kinds of cell group: one was composed of hiPSCs only (Basic), and the other was fibroblasts spiked with hiPSCs (Spiked) to mimic hiPSC-CTPs. For the Basic group, we transplanted 10 to 106 cells by increasing the cell number by 10-fold. For the Spiked group, we increased the hiPSC number by 10-fold in each sample and made total 106 cells by mixing fibroblasts. We examined the tumor formation by palpation in every week. After one year, we found that the number of tumors was increased by the amount of hiPSCs in a dose-dependent manner in both Basic and Spiked groups. Interestingly, we found the time point of no additional tumor formation is the 25th week with 104 hiPSCs in both groups. To compare the tumorigenicity of each sample, we adopted TPD50 value which means the statistically calculated cell dose producing tumors in 50% of mice at the endpoint. The last plateau TPD50 of the Basic group is 101.82, and the Spiked group is 102.34 explaining the tumorigenicity of hiPSC is decreased when mixed with fully differentiated cells. Based on our massive analysis, we propose that at least 25 weeks of observation is required to confirm the safety of hiPSC-CTPs in term of tumorigenicity.

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W-2118

SAFETY AND EFFICACY EVALUATIONS OF NOVEL AAV VARIANT BASED EX VIVO CELL-GENE THERAPEUTIC FOR HYPOXIC ISCHEMIC BRAIN INJURY

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Adeno-associated viral (AAV) vectors have been attracting attention in many gene therapy trials for treating various intractable disease due to their own safe features, such as their non-pathogenicity, and replication deficiency. In the previous study, novel AAV vector "AAV r3.45" was specially developed to overcome the limited tropism of traditional AAV serotypes and improve the transduction in human neural stem cells (hNSCs), and hNSCs are well known to exhibit therapeutic effects on the neonatal hypoxic-ischemia which is one of the severe disease with high mortality and serious aftereffects. In this study, the safety and efficacy of the AAV r3.45 and AAV r3.45 infected hNSCs for preparing interleukin-10 (IL-10) secreting hNSCs to improve the therapeutic effects on neonatal hypoxic-ischemia. Replication competent

AAV assay and contamination test were conducted by comparing with other traditional AAV serotypes and the tropism of AAV r3.45 were examined by analyzing the in vivo distribution of their expression. Moreover, the characteristics of AAV r3.45 infected hNSCs, such as their apoptosis, transduction, and genomic insertion were analyzed. Finally, the therapeutic effects of the IL-10 secreting AAV-hNSCs ex vivo therapeutic were evaluated by in vivo animal model studies. This research could contribute to establish the standard for the preparation of clinical trials of novel AAVs and AAV-mediated gene-cell therapies.

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W-2120

ADIPOSE DERIVED-STEM CELLS SHOW ABILITY TO DIFFERENTIATE INTO ALVEOLAR EPITHELIAL CELLS AND AMELIORATE ELASTASE-INDUCED EMPHYSEMA IN MODEL MICE

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Chronic obstructive pulmonary disease (COPD) is a leading cause of mortality throughout the world, with no effective therapy yet established. Recently, adipose tissue-derived stem cells (ADSCs) have been reported useful for ameliorating lung injury in animal models and other studies have shown that these cells have a capability to repair injured tissue by a variety of mechanisms. However, whether ADSCs differentiate into functional cells remains uncertain. In the present study, we examined whether ADSCs differentiate into lung alveolar cells and are able to ameliorate lung injury caused by elastase (PPE)-induced emphysema in model mice. After isolating ADSCs from subcutaneous adipose tissue of a GFP mouse, we confirmed their potential to differentiate into alveolar epithelial cells in culture by performing immunostaining and gene expression analyses, as well as observations with an electron microscope. Next, emphysema was induced in C57BL/6 mice by intratracheal administration of elastase (PPE, 1.5 IU/mouse). After 3 days, ADSCs were intravenously administered (1×106 cells/mouse) and their engraftment was observed histologically after 2 weeks. We divided the mice into the following 4 groups: (a) control, (b) PPE+/ADSCs-, (c) PPE-/ADSCs+, (d) PPE+/ADSCs+. Two weeks after administration of ADSCs, we evaluated their histological and functional effects. A greater number of ADSCs were found accumulated in the emphysematous lungs of the PPE+/ADSCs+ group as compared to the PPE-/ADSCs+ group. Histological evaluations using a mean linear intercept method showed improvement of emphysematous change in the PPE+/ADSC- (57.8 μm) and PPE+/ADSC+ (29.8 μm) groups P<0.0001. We also noted that some of the engrafted ADSCs were positive for an alveolar epithelial marker. Furthermore, ADSCs improved airway compliance shown in respiratory function test results (PPE+/ADSC-, 0.06 ml/cmH2O; PPE+/ADSC+, 0.05 ml/cmH2O; P<0.01), and gas exchange efficiency as shown by Hyperpolarized129 Xe MRI findings (PPE+/ADSC-, 4.8%; PPE+/ADSC+, 7.8%; P<0.05). These results show that ADSCs have an ability to differentiate into alveolar epithelial cells and accumulate in lungs affected by emphysema, thus indicating their therapeutic potential as a source of alveolar epithelial cells for treating pulmonary emphysema.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

W-2122

SPATIOTEMPORAL TRANSCRIPTOME FOR LINEAGE SPECIFICATION AND CELL FATE DECISION OF POST-IMPLANTATION MOUSE EMBRYO

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It is a fascinating process for animals to build up the body plan during gastrulation. The primary function of gastrulation is to generate a body plan that serves as a blueprint for the subsequent morphogenesis of the embryo. However, a comprehensive genome-wide molecular annotation of the mechanism that determine the lineage specification and cell fate decision has not been described. Here, we generated a series of spatial transcriptome dataset that encompass mouse embryos from early streak to late gastrulation. We obtained a completed gene expression atlas for three germ layer cells in real time and real space. Based on the high resolution four dimensional transcriptome data, we unraveled the regulatory mechanisms that control the lineage specification of the ectoderm, mesoderm and endoderm. This molecular map servers as an important foundation for understanding stem cell pluripotency and translational medicine.

W-2124

GLYCOLYSIS METABOLIC PATHWAY WAS REPRESSED IN TOTIPOTENT FRACTION IN ES CELL CULTURES

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In mammals, cells from very early-stage embryos have the ability to generate both embryonic and extra-embryonic cell types and thereby be defined as totipotent cells. In the mouse, the first specialization event occurs at around the 16-cell stage. At this stage, the polarized outer cells of the embryo begin to differentiate towards trophoblast while the inner cells start to differentiate to inner cell mass (ICM). Embryonic stem cells (ESCs) can be derived from ICM of blastocyst and maintain the capacity to make all the somatic lineages and the germ cells, but not the extra-embryonic lineages. Therefore, ESCs are thought to pluripotent cells, which lack the ability to make all extra-embryonic tissues. However, recent study revealed that a rare transient fraction within ESCs culture, that expresses high levels of murine endogenous retrovirus with leucine tRNA primer (MuERV-L). Importantly, MuERV-L expressing ESCs lack the pluripotency associated proteins Oct4, Sox2 and Nanog, and have acquired the ability to contribute to both embryonic and extra-embryonic tissues. In this study, we defined culture condition for ESCs that leads to increases the population of MuERV-L positive cells. We found that glycolysis metabolic pathway was repressed in MuERV-L positive cells. Since early preimplantation embryos require pyruvic acid as energy source rather than glucose, totipotent cells within ES cells share a similar feature with early preimplantation embryos.

W-2126

A NOVEL UNDIFFERENTIATED SPERMATOGONIA-SPECIFIC SURFACE PROTEIN 1 (USSP1) IN NEONATAL MICE

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Mammalian spermatogenesis is maintained by a rare population named spermatogonial stem cells (SSCs), which are very important for male fertility and sterility. SSCs remain a subset of undifferentiated spermatogonia, which could been isolated by a combination of several surface markers. There are still short of specific markers to identify and isolate undifferentiated spermatogonia effectively. Ussp1, a transcript that was previously annotated as long noncoding RNA, virtually encoded a membrane protein USSP1 in a highly testis-specific manner in mouse. We clearly demonstrated its expression on the membrane of undifferentiated spermatogonia by a homemade polyclonal rabbit antibody against the protein. In vivo, USSP1+ clusters consisted mainly of As, Apr (GFRα1+) and Aal (PLZF+) cells. USSP1+ cells exhibited enrichment of undifferentiated spermatogonia, as shown by the increased expression of SSC self-renewal molecular markers and the potential to form SSC clones in vitro. However, Ussp1 knockout did not affect the number of SSCs or spermatogenesis in mice. Thy1+ cells from Ussp1 null mice did not show any defect in the SSC colony formation capacity, indicating that USSP1 is not an essential factor for SSC self-renewal. Our data support that Ussp1 is specifically expressed in undifferentiated murine spermatogonia, indicating the potential to sort undifferentiated spermatogonia with USSP1 antibodies. Ussp1 might be a good maker for SSC enrichment in neonatal mice.

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TECHNOLOGIES FOR STEM CELL RESEARCH

W-2130

MULTIWELL MICROELECTRODE ARRAY TECHNOLOGY FOR THE EVALUATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTE AND NEURON DEVELOPMENT AND MATURATION

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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 lead 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 procedures, and the consumables (e.g., media) required for development. Here, we present data supporting the use of multiwell microelectrode array (MEA) technology as an efficient approach to quantification and optimization of human iPSC-derived neuron and cardiomyocyte production. 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. Quantification of the cellular electrophysiological activity, including spike amplitude and field potential duration, provides information on the depolarization and repolarization of the cardiomyocyte action potential. By comparison, the organization of the cellular activity across neurons within a network and across time define metrics of network bursting and synchrony that describe phenotypes of network electrophysiology. 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.

W-2132

COMPREHENSIVE PROTEOMIC CHARACTERISATION OF HUMAN ADIPOSE STEM CELLS

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Human adipose stem cells are widely used as treatments for a plethora of disorders, despite minimal evidence of their efficacy. Our understanding of human adipose stem cells is limited, in particular very little is known about their phenotype, as their proteome has not previously been characterised. Characterisation of proteins is crucial for developing a sound understanding of the complexity of biological samples as it allows for the analysis of functional changes due to post-translational modifications that can't be detected by genomics. It also provides more functional complexity than the analysis of the genome alone, because proteomes differs spatially and temporally within cells and tissues. In order to identify this valuable proteome, this project isolated and characterised human adipose stem cells from 10 healthy patient's abdominal lipoaspirates. These cells were passaged to achieve a homogenous cell culture and their proteome was characterised through a comprehensive analysis of cellular proteins, extracellular vesicles and secreted cytokines. A spectral library was developed for data independent acquisition mass spectrometry with a Q Exactive™ Plus Orbitrap Mass Spectrometer, resulting in a quantitative proteomic profile of human adipose stem cells. Identified cellular proteins provide 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. The stem cell derived extracellular vesicle proteome was also examined because stem cells secrete extracellular vesicles in substantial quantities and they are known to play a significant role in cancer, injury healing and immune suppression. Secreted cellular proteins such as cytokines also facilitate cellular communication of immune signals and warrant investigation. 27 cytokines were investigated through the utilisation of a Multiplex Immunoassay. This study produced a comprehensive data set of human adipose stem cell proteins, which is a unique resource that ultimately investigates the biological phenotype of human adipose stem cells. This is an invaluable tool for researchers and clinicians as it will assist in the development of efficacious cellular therapies.

W-2134

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

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There is an increasing emphasis on using complex, biologically relevant, and predictive cell-based assays to screen and evaluate compounds with potential for neurotoxicity. We used a high throughput 3D culture comprising human iPSC-derived neural spheroids composed of a co-culture of cortical glutamatergic and GABAergic neurons and astrocytes from a single source. This balanced cellular mix allows the development of a neural network enriched in synapses, creating highly functional neuronal circuitry. The neural spheroids are active, with spontaneous synchronized, readily detectable calcium oscillations. We used fast kinetic fluorescence imaging to measure the patterns and frequencies of the spheroid's Ca^{2+} oscillations as monitored by changes in intracellular Ca^{2+} levels with calcium-sensitive dyes. The assay was optimized for HTS in 384-well plates, with each well containing a single uniformly-sized spheroid, and allows for the characterization of oscillation profiles by using multi-parametric analysis including oscillation rate, peak frequency, peak width, amplitude, and waveform irregularities. The spheroids generated highly consistent performance across wells and plates. Spheroids were exposed to compounds in concentration-response for 60 min or 24 h. Cellular and mitochondrial toxicity was assessed by high-content imaging using cell viability markers and mitochondrial depolarization probes. The assay was validated using known neuromodulators, including agonists and antagonists of NMDA, KA, GABA and AMPA receptors, and produced effects as expected based on the biology of the correspondent compound. We applied a combinatorial in vitro screening approach for functional and mechanistic neurotoxicity profiling of 89 environmental chemicals and drugs including neurotoxicants noted in the literature. Over half of the tested compounds modulated Ca^{2+} oscillations. Concentration-response data for in vitro bioactivity phenotypes visualized using the ToxicologicalPrioritization Index (ToxPi) showed compound class-specific clustering of environmental chemicals. In conclusion, functional and morphological end-points using 3D iPSC-derived neural spheroids serve as a promising biologically-relevant tool to screen drugs and environmental toxicants for neurotoxicity.

W-2136

PROTEIN EXPRESSION SIGNATURES TO UNRAVEL HETEROGENEITY OF MESENCHYMAL STEM/PROGENITOR CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

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The dental pulp is considered as an important site for mesenchymal stem cells as stem cells derived from the pulp are pluripotent and might be ideal in tissue engineering. However, the inherent heterogeneity of these cells may preclude their utility in several clinical conditions requiring bone growth and repair. Whether or not human dental pulp stem cells obtained from subjects with a disease have the same multi-lineage capability for differentiation as human dental pulp stem cells from healthy subjects has not been extensively studied. This study aims to characterize different mesenchymal stem/progenitor cells from human exfoliated deciduous teeth (SHED). We have isolated mesenchymal stem cells from dental pulp of recently extracted healthy deciduous teeth, recently extracted deciduous teeth from osteopetrosis patients and commercially available SHED cell line Ax 3901. The cells were characterized using different analysis platforms including flow cytometry and expression proteomics by labeled free-quantitative liquid chromatography tandem mass spectrometry (LC/MS/MS). An average of 822 proteinshave been identified from each of the different analyzed cells representing the SHED subtypes. About a third of these proteins (290) were significantly differentially expressed ($\geq$ 2 to ∞ - fold change & $p < 0.05$) between the different sub populations of the analyzed cells and 32 of them are membrane/stem cell-related proteins. A review of some of these SHED specific protein have shown an overlap between a sizable protein panel with previously reported proteins characterized from SHED thus indicating their potential for the identification of dental pulp mesenchymal stem/progenitor cells markers. We have identified unique proteins that might be specific as novel dental pulp mesenchymal stem cell markers and some of them have been implicated as putative stem cell markers.

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W-2138

IDENTIFICATION OF PROGENITOR CELLS IN UMBILICAL CORD BLOOD MONONUCLEAR CELLS USING HIGH THROUGHPUT SINGLE-CELL PROFILING

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Despite recent advances in stem cell transplantation therapies, donor matching remains a challenge. A promising solution is through the use of cells isolated from umbilical cord blood, as a close match is not required and these cells are readily available. However, the realization of using cord blood for therapies requires deeper understanding of the heterogeneous populations and how differentiation is regulated. Recent advancements in single-cell sequencing technology, with the ability to analyze the transcriptomes of thousands of cells simultaneously, provides a powerful high parameter tool to study these cell populations. However, the cost of sequencing the entire transcriptome limits the number of biological samples that can be studied, especially if functionally important genes are expressed at relatively low levels. In this study, we took a targeted sequencing approach and used the BDTM Rhapsody Single-Cell Analysis System to analyze ~12,000 cord blood mononuclear cells (CBMCs). First, we subsampled 6,000 of the captured cells for immune profiling using a gene panel of ~400 genes. We were able to identify all the major immune cell types and examine their gene expression. A few stem-cell like clusters drew our attention and we analyzed the remaining archived cells using a custom stem cell panel of ~400 genes in order to further characterize the stem-cell like clusters in our sample. Our study demonstrates the flexibility of the BD Rhapsody system and cost efficiency and sensitivity of a targeted approach to single cell RNA-seq. It also demonstrates how the approach can be used to advance our understanding of heterogeneous populations in rare samples. For Research Use Only. Not for use in diagnostic or therapeutic procedures.

W-2140

ELECTROPHYSIOLOGICAL PROPERTIES OF IPSC-DERIVED MOTOR NEURONS WITH AAV TRANSFECTION OF OPTOGENE

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Optogenetics is a technology that allows targeted and fast control of biological systems with noninvasive optical stimulation. Channelrhodopsin-2 (ChR2) is a membrane protein that enables cellular regulation of transmembrane ion conductance through light-gated pores. Previous methods of transgenic approach may limit the clinical application of optogenetics in human induced pluripotent stem cells (iPSCs). This study tested the hypothesis that ChR2 can be expressed in iPSC-derived neurons through AAV transfection to the neuronal progenitor cells (NPCs). The cultured NPCs were transfected with ChR2 optogene using AAV with a vector concentrations of 3.25×1013 vg/mL. The experimental procedure included cloning of vectors, virus purification, transfection of cultured neurons cells and patch-clamp electrophysiological measurement of the derived neurons. The results showed that ChR2 was stably expressed for at least 20 days in human iPSC-derived motor neurons through AAV transfection to the NPCs. The neurons exposed to 470 nm blue light stimulation enabled regulation of membrane voltage and depolarized the cells from −55 mV to −15 mV observed with the whole-cell patch-clamp technique. The optically activated neurons also showed a spontaneous firing pattern consistent with that of the motor neurons. Our results demonstrated that iPSC-derived NPCs carrying ChR2 optogene differentiated into motor neurons can maintain ChR2 gene expression in an extended period of time, thus allowing optical manipulation of activity in this specific cell population. The AAV optogene transfection method may benefit iPSC research and future clinical applications.

W-2142

USE OF THE NANOBRIDGE SYSTEM AS A SCALE-UP PLATFORM FOR PLURIPOTENT HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) are viewed as attractive sources for disease modelling, clinical and regenerative medicine therapies due to their proliferative and pluripotent capacity. However, current, conventional, methods where hESCs are cultured as monolayers have inherent capacity limitations mainly due to restricted surface areas and the requirement for

manual manipulation, making the methods cumbersome to scale and thus less efficient. In addition, the use of enzymes and small molecule inhibitors, such as the Rho Kinase inhibitor, have been reported to adversely affect the genetic stability of the cells, thereby raising concerns regarding their use. Therefore, the need for a robust, scalable, system that does not utilize small molecules or enzymes has led to recent developments in scale-up platforms of hESCs as 3-dimensional suspension cultures. We have previously published the Nanobridge system which is comprised of a 2-component thermoresponsive polymer. We have demonstrated that this system is capable of supporting hESC proliferation while maintaining their pluripotency using a 96-well platform. To demonstrate the feasibility of this technology for use on a scale-up platform, we report the expansion to larger volumes in multi-well dishes. High cell viabilities of > 80% were obtained at each passage, with aggregate size distribution studies demonstrating the dissociation of aggregates during passaging and continued proliferation between passages. Additionally, cell characterisation studies demonstrated that hESCs maintained a normal karyotype and expression of key pluripotency markers. While this proof-of-principle study has demonstrated the feasibility of the Nanobridge system in maintaining hESC as suspension cultures, it remains crucial to demonstrate that the system is amenable to further scalability and automation to generate clinical-grade hESC. Current work using a 24-well minispinner platform is ongoing to establish an optimal method to utilise the shear for passaging of hESC aggregates in the Nanobridge system while maintaining their pluripotency.

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W-2144

ISCANDAR: AN INTERACTIVE REPORT FOR SINGLE CELL RNA-SEQ DATA

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Iscandar is an interactive report that can be generated by a bioinformatician to quickly involve the bench biologist in exploring single cell RNA-sequencing data. It provides interactive cell clustering, with t-SNE and PCA plots as standard, providing users with the ability to overlay gene or gene set expression as colour gradients on the cells. Using gene vs gene expression plots to generate a 'virtual FACS' (which can also work at the gene set level), users can explore correlation between key genes in the dataset. An example usage of this is to identify subpopulations of embryonic day 5 cells from Petropoulos et al (Cell 2016) dataset, based on anti-correlation of trophectoderm genes vs inner cell mass genes as described in the publication. Iscandar is the standard reporting tool for single cell RNA-seq data being uploaded into Stemformatics (stemformatics.org), which is a large curated repository of gene expression datasets focusing on stem cells. Iscandar is open source software and freely available from github.com/jarny/iscandar.

W-2146

HIGH THROUGHPUT OPTIMISATION OF RENAL LINEAGE PATTERNING FROM HUMAN PLURIPOTENT CELLS USING MICROBIOREACTOR ARRAYS

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Creating complex multicellular kidney organoids from pluripotent stem cells show great promise. Further improvements in differentiation outcomes, patterning and maturation of specific cell types are however intrinsically limited by standard tissue culture approaches. To address this limit, and expose new factor combinations and factor interplay, we recapitulated early kidney tissue patterning events within a full factorial microbioreactor array (MBA), exploring over 1000 unique conditions in an unbiased and quantitative manner. Single cell resolution identification of distinct renal cell types, coupled with multivariate analysis, defined the roles and interplay of Wnt, FGF and BMP signalling in their specification, while exposing retinoic acid as a minimal effector of nephron patterning. We reveal critical contributions of induced paracrine factors on cell specification and patterning into multilayered kidney organoids, along with media combinations that achieve near pure renal cell types. This study, in probing the relevance and interplay between the pathways stimulated by staged soluble factor-based protocols, provides the necessary insight for the development of improved protocols for directed differentiation of kidney lineage cells for use in drug screening and regenerative medicine. Importantly, this sophisticated but facile MBA-based methodology cannot only be used for tuning nephron segmentation but should prove useful for interrogating other complex multicellular differentiation processes.

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W-2148

ENGINEERING NONHUMAN PRIMATE PLURIPOTENT STEM CELLS WITH CLINICALLY RELEVANT FUNCTIONAL ELEMENTS TO DEVELOP A CELL THERAPY FOR STROKE

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One challenge in developing regenerative cell therapies is a lack of highly adequate clinically-relevant animal models for many human diseases or conditions. There is a significant unmet need in particular for brain disorders, which are among the largest causes of disease burden worldwide. Pre-clinical studies performed in rodent models of central nervous system (CNS) diseases often fail to translate to humans, likely due to primate-specific neural diversity and evolutionary distance in brain function and development. Nonhuman primate (NHP) models offer a more faithful representation of the pathophysiological features that occur in the human brain in terms of temporal and cellular response to injury and disease. With this in mind, we have generated induced pluripotent stem cells (iPSCs) from marmoset skin fibroblasts and propose to differentiate these marmoset iPSCs into cortically-specified neural stem/progenitor cells and characterize these cells for future applications as a transplantable cell therapy in a marmoset stroke model. We are also incorporating pre-engineered functional elements into our NHP stem cells to confer novel therapeutic features, such as allograft tolerance (see J Harding's abstract), live cell tracking, and cells expressing local-acting secreted biologics. These functional elements act as building blocks, which can be combined and customized for cell therapy applications across various disease models. Another major challenge facing the translation of cell therapies into patients is the tumorigenic risk of grafted cells. We have also developed a strategy to tackle this issue using our recently patented technology called "FailSafe," (see C Monetti's abstract) where we genetically link a suicide gene to a cell division essential locus (CDEL). We are genome editing marmoset stem cells to incorporate fail-safe modifications, which have already been validated in mouse and human PSCs. We have also expressed luciferase in marmoset PSCs to allow for live cell tracking of these cells in tissue grafts in vivo.

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W-2150

A UNIQUE DEFIND PRE-COATING-FREE CULTURE PLATFORM FOR ISOLATION AND EXPANSION OF HMSC TOWARDS CLINICAL APPLICATIONS

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Human mesenchymal stem cells (hMSC) hold great promise as tools in cell therapy. Since hMSCs are rare in adult tissues, the isolated cells must be expanded in-vitro to generate sufficient cells numbers for clinical use. The use of serum-free (SF), xeno-free (XF) culture system is an advantage in order to minimize the health risk of using xenogeneic compounds, and to limit the immunological reactions in-vivo. Under SF, XF culture condition, usually a coating step is required to enable cells attachment, spreading and proliferation in-vitro. The coating procedure is an obstacle step for scale up towards cells therapy. Thus, having an optimal coating-free culture platform may provide efficient, user-friendly and economical hMSC manufacturing process. In the present study, different combinations of treated plastic ware (uncoated) and SF, XF media were evaluated. Results show that MSC NutriStem® XF (BI) together with Corning CellBIND® (uncoated culture ware) is a superior platform for the expansion and proliferation of hMSC from a variety of sources.

W-2152

SCCHEMRISC: A JAPANESE CONSORTIUM FOR SHARING STEM CELL-BASED CHEMICAL RISK INFORMATION ASSESSED BY GENE NETWORKS

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Aiming the achievement of efficient drug development, which costs several hundreds of million dollars per compound, we propose a novel alternative toxicity test with high extrapolation efficiency to humans, towards the creation of highly safe medicines, by reducing the development cost and time. In recent years, alternative methods using human differentiated cells have been developed, but the robustness of experimental method and the control of cell quality are challenging, indicating that it may take nearly 10 years until practical use. In the past, mouse embryonic toxicity test (EST) using ES cells and two kinds of differentiated cells has been promoted in Europe, but it has not been sufficient in terms of extrapolation efficiency to humans. Here we propose a method that enables alternative toxicity tests to be carried out quickly, accurately, and at lower cost than conventional methods using human ES cells that are easy to control quality. We have recently developed a novel method "hEST" to predict the categories of neurotoxicity, genetic / non-genotoxic chemicals with a prediction accuracy of 95% or more by machine learning of gene network using only human ES cells (Nucleic Acids Res. 2016). Inspired by this innovative technique, about 100 of Japanese toxicologists have organized a consortium, scChemRISC, where we will develop a gene expression database and an analysis system against various organ toxicity such as heart, nerve, liver, blood, etc. We have also introduced germ line stem cells to the system to further expand the testable chemicals. Ultimately, tests using human iPS cells that exhibit donor diversity and less ethical problems will be conducted and verified the validity of iPS cell tests on the ES cell database. Once this project is accomplished, a low-cost, accurate and robust alternative toxicity testing method that reduces more than half the current animal study cost and time will be achieved to contribute to our goal of highly safe drug development.

W-2154

DEVELOPMENT OF REMOVAL TECHNIQUE OF TUMORIGENIC RESIDUAL PLURIPOTENT STEM CELLS

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In order to promote practical use and industrialization of regenerative medicine, establishment of quality control technology is generally required. In order to ensure the safety of products for regenerative medicine made from human pluripotent stem cells (ES / iPS cells), residual undifferentiated cells must be eliminated in the manufacturing process. To reduce the risk of teratoma formation, recent studies have focused on developing efficient techniques to isolate and purify the objective cells and to eliminate and kill residual undifferentiated cells. We reported the lectin probe rBC2LCN, which binds harmlessly and specifically to the cell surface of human pluripotent stem cells, and is working on the development of related technology using this lectin probe. In this presentation, we will report on the method for separating residual tumorigenic cells by rBC2LCN bound magnetic beads. Using biotin-labeled rBC2LCN and streptavidin-bond magnetic beads, the cells that have the potential to form teratoma in heterogeneous cell population could be eliminated. We propose that removal of residual tumorigenic cells based on rBC2LCN can be a useful option for preclinical and clinical therapy trials using human pluripotent stem cells.

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W-2156

AUTOMATED ELIMINATION OF SPONTANEOUS DIFFERENTIATED HUMAN INDUCED PLURIPOTENT STEM CELLS BY A HIGH-SPEED LASER MEDIATED BY A LIGHT-RESPONSIVE POLYMER

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The purification of different types of cultured cells is critical in various biomedical fields, including basic research, drug development, and cell therapy. Conventionally, fluorescence activated cell sorting (FACS), affinity beads, gradient centrifugation, and elutriation have been used for cell purification. However, the process of detaching, dissociating, sorting, and reseeding can result in low yield and in altered cell characteristics for adherent cells. We have developed a Laser-Induced, Light-responsive-polymer-Activated, Cell Killing (LILACK) system enabling high-speed and on-demand adherent cell sectioning and purification. A visible laser beam, which does not kill cells directly, induces local heat production in only the irradiated area of a light-responsive thin layer. This scheme enables effective cell killing even at very fast beam scanning without damaging neighboring unirradiated cells. We examined the effect of the long-term culturing of human induced pluripotent stem cells (hiPSCs) using this LILACK system. After 10 passages through the system, the hiPSCs were characterized. The karyotype of these hiPSCs was maintained in all the cells. These cells expressed self-renewal markers of pluripotent stem cells. When differentiated using embryoid body (EB) formation, these cells differentiated into three germ layers. We also developed a label-free cell elimination system based on deep machine-learning imaging analysis. We applied the trained classification algorithm to our laser-mediated cell elimination with only phase-contrast images. After the automated cell elimination, we found that the TRA1-60-positive cell ratio increased after laser irradiation to eliminate the “differentiated” cells classified by this algorithm. These results indicate that in situ label-free cell purification was achieved using our LILACK system combined with imaging analysis based on deep learning. We believe that our methods can be widely used in various biomedical fields, including basic research, drug development, and cell therapy. Since the classification of cell types based on deep-learning methods is advancing rapidly, the importance and functionality of our technology will be further enhanced in the near future.

W-2158

PHYSIOXIC CONTROL OF CELL HANDLING CONDITIONS REDUCES VARIABILITY FOR HUMAN MSC

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The importance of incubating clinically valuable Mesenchymal Stem/Stromal Cells (MSC) at low oxygen levels has been well established, but many cell culturists still handle MSC in conventional room air biological safety cabinets (BSC). This practice causes stress in MSC as they experience large swings in conditions and increases experimental variability. MSC utilize aerobic glycolysis, which consumes less O2 than oxphos even when O2 is abundant. We have previously published that room air cell handling reduces MSC yields and speeds culture senescence. For this project, we sought to establish how long it takes pericellular O2 to return to optimum low levels after MSC undergo routine passaging in a room air atmosphere. Using the Xvivo System, in which we control gas levels and temperature for the cells during all cell handling steps as well as incubation, we sealed an immersion oxygen probe into a vented-cap T-75 flask of human bone marrow MSC cells immediately after passage. We kept the flask in the cell handling chamber, which was at 37 degrees C and 20%O2, and changed the atmosphere to 3% O2. We monitored pericellular oxygen levels as the chamber atmosphere, the vessel headspace, and the cell culture medium equilibrated to 3% O2. With 15ml of medium, sitting undisturbed, we found that it took between 1 and 2 hours for pericellular oxygen levels to equilibrate to chamber oxygen levels. This is a long period of time for MSC to be at stress-inducing supraphysiologic oxygen levels. When cell culture medium was pre-equilibrated to 3%O2, this time was shortened, but cells were still out of optimum after passage in room air oxygen atmosphere. Only when handled at 3% with pre-equilibrated media did the cells stay at optimal levels. We concluded that full-time control of cell handling atmosphere is necessary to reduce variability for MSC and keep them in optimal conditions.

W-2160

P53 INHIBITS CRISPR/CAS9 ENGINEERING IN HUMAN PLURIPOTENT STEM CELLS

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CRISPR/Cas9 has revolutionized our ability to engineer genomes and to conduct genome-wide screens in human cells. While some cell types are easily modified with Cas9, human pluripotent stem cells (hPSCs) poorly tolerate Cas9 and are difficult to engineer. Using a stable Cas9 cell line or transient delivery of ribonucleoproteins (RNPs) we achieved an average insertion or deletion (indel) efficiency greater than 80%. This high efficiency made it apparent that double strand breaks (DSBs) induced by Cas9 are toxic and kill most treated hPSCs. Cas9 toxicity creates an obstacle to the high-throughput use CRISPR/Cas9 for genome-engineering and screening in hPSCs. We demonstrated the toxic response is tp53-dependent and a functional tp53 severely reduces the efficiency of precise genome-engineering in hPSCs. Our results highlight that CRISPR-based therapies derived from hPSCs should proceed with caution. Following engineering, it is critical to monitor for tp53 function, especially in hPSCs which spontaneously acquire tp53 mutations.

PLURIPOTENCY

W-2164

THE ROLE OF DPPA4 IN HUMAN PLURIPOTENT STEM CELLS

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DPPA4, a SAP domain protein, is expressed in murine and human naïve and primed pluripotent stem cells, which is down regulated upon differentiation. SAP domain proteins are mainly implicated in chromosomal organization, DNA repair and RNA metabolism. Hence, potential roles in pluripotency might be mediated by entirely novel mechanisms. To investigate the role of DPPA4 in human pluripotent stem cells, we performed an in silico analysis which predicted many potential post-translational modifications like phosphorylation and a SUMOylation site. However, we failed to demonstrate experimentally the SUMOylation of DPPA4. Moreover, the in silico analysis revealed a significant stretch of homology to a rat protein that exhibits high similarity to human HDAC9b, which in turn binds HISTONE3. Indeed, we show for the first time that human DPPA4 interacts with HISTONE3 using immunoprecipitations. In addition to DPPA4's association with chromatin throughout the entire cell cycle, we show that it is also localized in cytosolic peri-nuclear foci during interphase using a DPPA4-EGFP fusion protein for live cell imaging and immunocytochemistry using a customized antibody. DPPA4 knock-down experiments resulted in differentiation as judged by alteration of morphology, reduced growth curve and reduced plating efficiency. Of note, however, key markers like OCT3/4, SOX2 and NANOG as well as the cell surface antigens SSEA3 and TRA1-60 were unaffected. Also hCG, a marker for trophoectoderm was unaffected, while CDX2, a marker for both, trophoectoderm and intestinal gut was significantly upregulated. These results suggest that DPPA4 acts downstream of OCT3/4, SOX2 and NANOG and that the differentiation caused by *DPPA4*'s depletion is uncoupled of any negative feedforward mechanisms to these key pluripotency markers. DPPA4

appears to play multiple roles in the nucleus and the cytosol. We hypothesize that among its nuclear roles is the prevention of heterochromatin formation by HISTONE3 binding to block sterically the recruitment of heterochromatin inducing factors like presumably HISTONE3 methyltransferases of (H3K9 and/or H3K27), HDACs of H3K27 and/or histone de-methylases of H3K4. The results obtained in this study will contribute to elucidate the poorly understood role of DPPA4 in human PSCs.

Funding Source: King Faisal Specialist Hospital & Research Centre, King Abdulaziz City of Science and Technology, University of Sheffield (Centre of Stem Cell Biology)

W-2166

CELL CYCLE ANALYSES DISSOCIATE LINEAGE COMPETENCY (POTENCY) AND PROLIFERATIVE COMPETENCY (FOUNDING CAPACITY) AS INDEPENDENT FATE DECISIONS IN MURINE ESCS

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Stem cells are defined classically by the maintenance of two hallmark features that are, arguably, independently regulated: lineage competency (potency) and proliferative competency (founding capacity). The classic definition, though, seems at odds with the onset of development where there are no discernable cell fates and/or subpopulations. As a result, the term stem cell was reworked tethering lineage and proliferative competencies as a solitary-and-defining fate decision. Despite the revision, a paradox exists: not all pluripotent cells found the embryo, whether that cell is an in vivo epiblast cell or an embryonic stem cell (ESC). This begs the question, are all pluripotent cells bona fide stem cells? Utilizing the cell cycle (CC) framework, we provide evidence that no, not all ESCs are stem cells and those that are, adhere to the classic definition. Only a subset (36%) of individually plated ESCs can found a new ESC colony. This modest founding capacity is independent of the cell's CC position at plating. CC position does, however, influence colony size: S>G2/M>G1. In addition, the expression of OCT4 is dynamic during mitosis with many cells in G2/M expressing low to negligible levels. Together, these data illuminate a weak point for the pluripotent state during G2/M. ESCs were thus exposed to drugs conventionally used to arrest cells in G2/M and assessed for both, ES cell colony and primitive neurosphere (pNS) formation. The ESC to neural transition is key. First, it occurs via a default mechanism precluding the requirement for a differentiation signal. Second, it allows decisions regarding proliferative and lineage competencies to be dissected: both lineages possess founding capacity. Chemically increasing the CC time of ESCs during G2/M dramatically depletes both, ESC colony and pNS formation. Notably, ESC lines mutant for cell death genes AIF or Apaf1 cannot rescue ESC colony formation. Apaf1-/- ESCs, however, can rescue partially (63%) pNS formation consistent with a change in lineage competency. These data therefore dissociate cell fate decisions pertaining to lineage and proliferative competencies in ESCs. Furthermore, they introduce subpopulations of pluripotent cells—stem and progenitor—into ESCs thereby providing a unique functional explanation for the lack of founding capacity.

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W-2170

STUDYING EFFECTORS OF Wnt/β-CATENIN SIGNALING PATHWAY FOR THEIR ACTIVITY TO CONTROL EMBRYONIC STEM CELL SELF-RENEWAL AND PLURIPOTENCY

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Mouse embryonic stem cells (mESC) require extracellular signals to induce the expression of intrinsic core pluripotency transcription factors (Oct4, Sox2 and Nanog). Transient activation of the Wnt/β-catenin pathway through the inhibition of the transcription factor, Tcf3, leads to activation of the core pluripotency network. On the contrary, inhibition of the Wnt pathway leads to ESC differentiation into epiblast stem cells (EpiSCs). Additionally, previous studies have shown that Wnt pathway activation enhances somatic cell reprogramming via cell-fusion in vivo and in vitro. However, the mechanism of Wnt pathway activity in these processes and its downstream effectors still remain to be fully elucidated. For this, we generated Wnt-induced ESC gene expression profiles by sequencing several ESC clones where the Wnt pathway was perturbed constitutively. We performed a regulatory network based analysis to identify potentially novel 'pluripotency factors' or re-programmers. This method measures the activity of the transcription factors by computing the differential expression of their targets and thus allows for an unbiased prediction of causal driver genes of phenotypic changes. Interestingly, our approach allowed us to identify novel genes in addition to the previously described transcription factors (TFs) such as Esrrb, Nanog and Prdm14 that were among the top active predicted genes. We also interrogated a pre-implantation embryo RNA-seq dataset and observed that the activity profile of the newly identified TFs was highly similar to Nanog, Esrrb and Klf4. We are currently

studying the activity of these novel TFs and their role in the control of stem cell pluripotency and self-renewal. Understanding the mechanism by which these TFs exert their function will be key to fully decipher the role of the Wnt pathway in pluripotency and differentiation.

Funding Source: Severo Ochoa

W-2172

TRANSGENERATIONAL EPIGENTIC CONTROL OF PLURIPOTENCY BY ESC PLURIPOTENCY FACTORS

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The pluripotent state of embryonic stem cells critically depends on the maintenance of a proper epigenetic landscape. In this context, both activating (H3K4me3) and repressive (H3K27me3) histone modifications play crucial roles. Undifferentiated embryonic cell transcription factor 1 (Utf1) belongs to the core pluripotency factor network in embryonic stem cells (ESCs) and is also expressed in primordial germ cells (PGCs) during embryonic development. Utf1 expression is directly controlled by pluripotency factors Oct4 and Sox2, which form a ternary complex with the Utf1 enhancer. The Utf1 protein plays a role in chromatin organization and epigenetic control of bivalent gene expression in ESCs in vitro, where it promotes effective cell differentiation during exit from pluripotency. Specifically, the epigenetic function of Utf1 in ESCs involves the control of H3K27me3 deposition at bivalent genes via binding to thousands of loci around transcriptional start sites. We recently demonstrated that Utf1's proposed epigenetic role in ESC pluripotency in vitro may be linked to intergenerational epigenetic inheritance in vivo. We proposed that the protein contributes to H3K27me3 deposition in PGCs (and in spermatogonial stem cells and oocytes) in a manner similar to its role in ESCs. Here, we provide evidence which supports such a crucial role of UTF1 in deposition of both activating and repressive epigenetic marks in PGCs of E14-E16 ovaries. Hence, Utf1 now emerges as a key factor in the regulation of the epigenetic landscape in PGCs which controls transgenerational inheritance of pluripotency.

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W-2174

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

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Chromosomal aberrations in cultured human pluripotent stem cells (hPSCs) such as numerical aneuploidy, chromosomal rearrangements and sub-microscopic changes have been widely reported. Genetic variants can affect hPSC growth rates, cell survival and differentiation potential. Recurrent genetic abnormalities observed in hPSCs are also observed in human cancers, an observation that raises concerns for downstream clinical applications. 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 detect the 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-banding karyotyping. As a case study, we analyzed the WLS-4D1 human induced pluripotent stem cell (hiPSC) line using G-banding, fluorescent in situ hybridization (FISH), and the hPSC Genetic Analysis Kit. Although this hiPSC line was found to be karyotypically normal by G-banding, duplication of 20q11.21 was detected using the hPSC Genetic Analysis Kit and confirmed by FISH. To determine assay sensitivity, fluorescently-labelled hPSC lines known to be abnormal for 10p, 12p and 20q were mixed with unlabelled normal 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 pre-screen the hPSC lines in their laboratory for recurrent genetic abnormalities.

W-2176

THE DEVELOPMENT OF A COMBINATORIAL MATRIX MICROARRAY TO STUDY THE EFFECT OF EXTRACELLULAR MATRIX AND SUBSTRATE STIFFNESS ON CELL FATE

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In vivo, stem cells are subjected to a complex interplay of biochemical cues and biophysical forces that act in concert to orchestrate cell fate. Interrogating the role of each factor in the cell microenvironment, however, remains difficult due to the inability to study microenvironmental cues and tease apart their interactions in high throughput. To address this need, we have developed an extracellular matrix (ECM) microarray screening platform capable of tightly controlling substrate stiffness and ECM protein composition to screen the effect of these cues and their interactions on cell fate. As a proof of principle study, we used this platform to screen for optimal conditions that can maintain human pluripotent stem cell (hPSC) pluripotency in defined, xeno-free conditions. While xeno-free culture media are generally effective, xeno-free culture substrates are poorly-defined and/or not effective for all hPSC sources. Combinations of ECM proteins (fibronectin, vitronectin, laminin-521, and collagen IV) were deposited on PDMS substrates with elastic moduli ranging from 3-40 kPa using a high throughput protein plotter. hPSCs (CA2, PB110, H9) were seeded on the arrays and grown in E8 media for four days prior to evaluating cells for Oct4 expression. The number of Oct4+ cells per condition was modeled as a function of matrix composition and substrate stiffness to investigate single factor effects and multifactorial interactions. For all cell lines tested, fibronectin, laminin-521, and vitronectin had the most prominent positive effect on Oct4+ cell counts. Interestingly, we observed a synergistic interaction with fibronectin and laminin-521, whereas vitronectin and laminin-521 had a pronounced antagonistic interaction. Stiffness also played a significant role, with stiffer substrates predicted to be better for cell attachment and pluripotency. Cells grown on optimized PDMS substrates remained pluripotent for over 25 passages and expressed high levels (>90% cells positive) of Nanog, Oct4, and Sox2. Cells grown on these substrates also retained the capacity to differentiate into the three germ layers. This proof of principle study shows the promise of our matrix microarray platform and its unique ability to tease out single factor and combinatorial effects of ECM protein and substrate stiffness on cell fate.

PLURIPOTENT STEM CELL DIFFERENTIATION

W-3002

PRODUCTION OF "GMP-COMPLIANT" iPSCs FROM CRYOPRESERVED HUMAN UMBILICAL CORD BLOOD

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An iPSC bank derived from cord blood donors selected on the basis of human leukocyte antigens (HLA) homozygosity could form the basis of future cell based therapies. However, the establishment of such a "haplobank" will require the development and validation of protocols to generate iPSC lines under good manufacturing practice (GMP) conditions. We investigated the use of banked cord blood samples for the creation of GMP compliant HLA haploidentical iPSC lines for cellular therapies. We established an Animal Component Free (ACF) protocol to generate iPSC lines from 50 ul of cryopreserved CB buffy coat using defined reagents manufactured under GMP. Thawed CB cells were washed and an erythroblast cell population expanded in ACF erythroblast growth medium prior to reprograming. Up to 0.11% reprogramming efficiency was obtained with ThermoFisher CytoTune-iPS 2.0 Sendai Reprogramming kit. iPSC colonies formed on vitronectin coated plates were manually picked and passaged on the same matrix in defined Xeno-free E8 flex medium. Karyotype integrity of the generated iPSC lines was confirmed with SNP Illumina Infinium CoreExome-24 v1.1 array at 0.50Mb resolution. Flow cytometry was used to show that iPSCs expressed the known pluripotency markers SSEA4, SSEA4, TRA-1-81 and TRA-1-60R but lacked expression of SSEA1. In vitro differentiation experiments indicated that cord blood derived iPSCs could generate beating cardiac cells, with the cells expressing relevant cardiomyocytes markers. We are currently assessing different types of recombinant matrices for their ability to increase iPSC colony formation and improve iPSC expansion. Overall, our study demonstrates that GMP-compliant iPSC lines can be reproducibly generated from small volumes of

cryopreserved umbilical cord blood. Technical protocols established through this work lay the foundations for the creation of an Australian CB-derived iPSC "haplobank" for cellular therapies (see abstract by Abberton et al).

Funding Source: This work is supported by the Lions Cord Blood Foundation and the Fight Cancer Foundation.

W-3004

TELOMERASE ACTIVATES A LATENT STEM CELL POPULATION THAT DRIVES CLONAL REGENERATION OF PODOCYTES IN THE ADULT MOUSE KIDNEY

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Kidney podocytes serve a crucial role in supporting and maintaining the integrity of the glomerular filtration barrier by extending numerous foot processes that surround the glomerular capillary basement membrane. These highly specialized, quiescent epithelial cells are the targets of injury in a variety of kidney diseases, which results in podocyte depletion. The inability of podocytes to proliferate and replace those lost in response to injury ultimately leads to the development of glomerulosclerosis and end-stage renal failure. Recent reports have shown that podocytes possess a regenerative capacity, although this potential does not match podocyte loss observed in the models studied. To date, the only model in which extensive and efficient podocyte regeneration is observed consists of a transgenic mouse model in which the protein component of telomerase TERT is overexpressed in a transient manner. In this study, we used this original model in order to determine how efficient podocyte regeneration is achieved in the adult organism. Using a lineage tracing approach, we found that podocyte duplication synergize with recruitment of stem cells in order to yield efficient podocyte renewal. We further found that efficient podocyte regeneration occurs in physiological conditions following injury, and that this process is associated to upregulation of endogenous TERT in specific cellular compartments within the adult kidney. To further identify podocyte stem cells, we used an unbiased stochastic multicolor tracing approach that allows random lineage tracing within the adult kidney during TERT-enforced podocyte regeneration. Such study revealed striking appearance of clonal podocytes that yield mono-colored glomeruli upon TERT-enforced regeneration. Moreover, examination of kidneys along the regeneration process unveiled initial expansion of clones in specific nephron segments that subsequently colonize kidney glomeruli to give rise to clonal podocytes. Those data show that efficient podocyte regeneration within the adult mammalian kidney relies on mobilization of stem cells located within specific nephron compartments. Moreover, our data demonstrate that transient telomerase reactivation is an efficient way to stimulate regenerative potential of those podocyte stem cells identified hereby.

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W-3006

CONDITIONED MEDIUM FROM HUMAN TESTIS DERIVED CELL CULTURES AS AN IN VITRO NICHE FOR MALE GERM CELL DEVELOPMENT FROM HUMAN EMBRYONIC STEM CELLS

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Existing literature reported inductive effect of the numerous growth factors on in vivo spermatogenesis in the testis. This component secreted by various types of cells including, Sertoli cells, Leydig cells, spermatogonial stem cells (SSCs), spermatocytes, and other testicular somatic cells. Reports showed that CM from testicular cells could improve the differentiate capacity of embryonic stem cells (ESCs) to form germ cells in animals such as Buffalo. Hence, the mixture of various cell types in the testicular derived cell cultures to produce the growth factors in the testicular derived cells conditioned medium (TCCM). Five Non-obstructive azoospermia TESE biopsies that contained sperm were used after fully-informed patient consent. After enzymatically treatment, tissue fragments minced mechanically into small pieces and were pelleted by centrifugation for 5 min at 1000 rpm. Pellet was seeded in the tissue culture flasks containing DMEM /20%FBS medium. CM were collected 4 days after culture of TESE samples at passage 1 and subsequent subculture and filtered through 0.22-mm syringe filter following stored at -20 °C after collection. After embryoid body (EB) production from Yazd2 and Yazd3 (hESC lines) cell were induced to differentiate for 14 days.

Experimental groups were divided into spontaneously and TCCM (40% TCCM in EB medium) groups. Gene expression profile of EBs was evaluated by IF. However, interestingly, a very small number of cells were shown to be positive for SCP3 similar to previously reported studies. Our preliminary data so far, using IF for cKIT, DAZL and SCP3, however, indicates the signs of male germ cell differentiation in both groups, but, more work is in progress with more markers using IF and Q-PCR. In vitro spermatogenesis via using pluripotent potential of hESCs with TCM as testicular environment modeling will advance for cell based therapies which expected in the future. Our finding may help to clear the obstacles about spermatogenesis disorders and help to solve this problem for infertile patient.

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W-3008

INVESTIGATING THE ROLE OF THE WNT/β-CATENIN PATHWAY IN LINEAGE DETERMINATION VS SELF-RENEWAL IN NAÏVE VS PRIMED STEM CELLS

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The role of Wnt/β-catenin signaling in stem cell differentiation remains controversial. Several studies have produced conflicting results: some indicate that activation of the Wnt/β-catenin pathway promotes self-renewal while others indicate that it promotes differentiation. To further confound the issue, some studies were performed using mouse ES cells (naïve) and others used human ES or iPS cells (primed). We investigated the apparently dual role of Wnt/β-catenin signaling by studying its effect on human naïve stem cells versus primed state stem cells. The stem cells were cultured in mTeSR, E8, or a naïve $NME7_{AB}$ media. These media have different levels of active nuclear β-catenin, with mTeSR having the lowest level and $NME7_{AB}$ media having the highest. In addition, we grew stem cells in these different media in the presence or absence of inhibitors or agonists of the β-catenin pathway. We then compared the ability of each to differentiate down all three germlines: retinal progenitors, hepatocytes, vascular progenitors, or mesenchymal stem cells, which were then further differentiated into chondrocytes, osteogenic cells and adipocytes. Whether activation of the Wnt/β-catenin pathway induces differentiation or promotes pluripotency was explored. First, stem cells were allowed to spontaneously differentiate by withholding growth factor, but adding Wnt3A or a β-catenin inhibitor. Markers of pluripotency vs. differentiation were measured over time. To test the ability of the Wnt/β-catenin pathway to promote self-renewal, we cultured naïve or primed state stem cells in Wnt3A or in a β-catenin inhibitor, but in the absence of their respective growth factors. Naïve, primed, pluripotency and differentiation associated genes were measured by PCR. In the naïve stem cells, we analyzed the effect of Wnt3A, in the absence of $NME7_{AB}$, on the transition of XaXa cells to XaXi cells. Results of these experiments showed that for some classes of stem cells, levels of active β-catenin played a critical role in lineage determination, while in other classes it had no effect. Similarly, for one class of stem cells activation of the Wnt/β-catenin pathway promoted self-renewal.

W-3010

SINGLE-CELL RNA-SEQ AND ARCLIGHT ANALYSIS IMPLICATE FHL1 IN REGULATING ATRIAL-LIKE ELECTROPHYSIOLOGICAL PROPERTIES OF HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a valuable platform for disease modeling, regenerative studies, and drug testing. Nonetheless, numerous challenges face this field, including the production and accurate phenotyping of mature populations of ventricular, atrial, or nodal cardiomyocytes. We sought to examine the relationship between transcriptional and electrophysiological markers of hiPSC-CM subtypes, and examine how these properties change as hiPSC-CMs mature with time in culture. Using the genetically encoded voltage indicator ArcLight, we profiled increases in action potential amplitude and maximum upstroke velocity with time in culture, as well as an increased prevalence of cells with atrial-like action potential morphology after ~30 days of differentiation. In order to profile the cardiomyocyte subtypes within the culture system at both an electrophysiological and transcriptional level, we performed ArcLight analysis and single cell RNA-seq at Day 12 and Day 40 of differentiation. We were able to detect gene expression changes consistent with a more mature phenotype in the Day 40 cells; however, we could not delineate the cardiomyocytes into distinct ventricular, atrial, or nodal populations via standard transcriptional markers. We did observe increased Day 40 expression of SLMAP and FGF12, both of which impact sodium channel function,

along with FHL1, which has been reported to modulate the atrial-enriched ion current IKur in a heterologous expression system. Subsequently, we demonstrated that knockdown of FHL1 shifts the action potentials of hiPSC-CMs towards more ventricular-like properties. Our findings support the evaluation of both molecular and functional parameters for accurate phenotyping of hiPSC-CM subtypes, particularly considering that FHL1 expression can impact the electrophysiological behavior of hiPSC-CMs. Ion channel modifiers such as FHL1, SLMAP, and FGF12 may allow us to better understand and even modulate hiPSC-CM electrophysiology.

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W-3012

HIGHLY EFFICIENT IN VITRO PROLIFERATION AND ANGIOGENIC POTENTIAL OF DENTAL PULP STEM CELLS IN HUMAN PLATELET LYSATE SUPPLEMENTATION

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The principal role of mesenchymal stem cells (MSCs) in clinical trials and prospective regenerative therapies demands an in-vitro cell expansion at large scale. The culture of MSCs in-vitro depends on supplementing culture medium with foetal bovine serum (FBS) that contains numerous growth factors inducing cell attachment, proliferation, and differentiation. FBS is known to pose a risk of occurrence of unknown infections from xenogenic agents rendering it unsuitable use of FBS in clinical trials. Human-derived medium additives like platelet-rich plasma (PRP) and human platelet lysate (HPL) are known to contain a cocktail of autologous growth factors optimum for cell culture and expansion. This study was aimed to first, compare the effects of HPL and FBS on proliferation and angiogenic differentiation of DPSCs. Second, to determine the optimum concentration of HPL inducing DPSCs proliferation and proangiogenic differentiation. The methodology involved isolation of DPSCs from dental pulp of extracted human premolars. Mesenchymal characterization of isolated cells was confirmed by trilineage differentiation and flow cytometry analysis. HPL was prepared by repeatedly freezing and thawing pooled PRP derived from whole blood of eight healthy donors. Isolated DPSCs were divided into two groups. DP first group was expanded in FBS and those in the second group were expanded in different concentrations of HPL for 2, 4, 6 and 8 days. Proliferative effect of FBS and HPL on DPSCs was assessed by Alamar blue assay. qPCR and ELISA were used to evaluate the expression of pro-angiogenic markers. Results showed that proliferation of DPSCs was significantly high at 10% HPL concentration compared to FBS group. qPCR and ELISA assessment showed that 20% HPL induces angiogenic differentiation in DPSCs indicated by significantly high ($p < 0.05$) expression of proangiogenic markers. It could be concluded that 10% HPL is optimum for enhancing DPSCs proliferation and 20% HPL is optimum for inducing pro-angiogenic differentiation of DPSCs in vitro. This exploratory evidence may encourage the relative clinical and laboratory application of HPL as a non-xenogenic adjuvant to FBS for expansion of MSCs for regenerative therapies. However, the interpretation of the molecular pathways triggered by HPL requires further investigation.

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W-3014

HIGHLY SENSITIVE AND QUANTITATIVE DETECTION OF REMNANT OCT3/4 PLURIPOTENT CELLS IN DIFFERENTIATED CELL PREPARATIONS FOR STEM CELL BASED THERAPIES

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A challenge of the manufacturing process for cellular therapies is the exact analysis of the cell product's identity after the differentiation process. Detection of non-differentiated cells that might contaminate the cell product is especially important due to the teratogenic potential of pluripotent cells. We developed a flow cytometry based assay for highly sensitive and quantitative detection of remnant Oct3/4 pluripotent cells in differentiated cells. Flow cytometry enables a standardized and fast readout of protein levels on single cells and the absolute count of cells positive for a given marker. In our assay, cells were labeled with the Oct3/4 specific clone REA622 conjugated to APC, measured using the MACSQuant Analyzer 10 and analyzed with the MACSQuantify Software. To define the assay's sensitivity, the Limit of Blank (LoB), Limit of Detection (LoD) and the Limit of Quantification (LoQ) were determined. Whereas the LoB defines the highest apparent analyte concentration that can be found in a

sample that contains no analyte (LoB = Mean blank + 3.09 (SD blank)), the LoD describes the lowest analyte concentration that can be reliably distinguished from the LoB (LoD = LoB + 3.09 (SD low concentration)) with an error probability of 0.1% assuming a Gaussian distribution of the samples (Armbruster & Pry, 2008). Furthermore, the LoQ is the lowest concentrations that can be reliably quantitatively determined, but at which some predefined goals for imprecision are met (coefficient of variation (CV) ≤ 20%). The LoB was determined by measuring 20 replicates of unstained cells. To define the LoD and LoQ, 0.01%, 0.025%, 0.05%, and 0.1% of Oct3/4 positive pluripotent, stained cells were spiked into unlabeled cells (n=20, 10E6 cells/sample) and the percentage of Oct3/4 positive was determined. The blank sample showed a very low background in the APC channel and the Limit of Blank was determined as 0.002%. Percentages of positive cells detected in the spike-in samples nicely matched the theoretical values resulting in a Limit of Detection of 0.008% and a Limit of Quantification of 0.01%. In summary, we developed a highly sensitive and quantitative assay for detection of potentially contaminating pluripotent cells in differentiated cells with defined settings for flow cytometric acquisition and data analysis.

Funding Source: Research activities were supported by Neurostemcellrepair (European Community's Seventh Framework Programme grant agreement nr. 602278)

W-3016

A SYSTEMS LEVEL VIEW OF MIRNA-124 FUNCTION DURING NEURONAL DIFFERENTIATION OF HUMAN STEM CELLS

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The underlying gene regulatory programs of induced neurogenesis in human induced pluripotent stem cells (iPSCs) are mostly enigmatic. Non-coding miRNAs have been implicated as potent players, especially as the neuron specific miR-124 is considered essential for neurogenesis. However, very few of the 4000 miR-124 targets and regulatory features have been identified yet. To systematically chart miR-124-dependent functions during neuronal differentiation of iPSCs, we used CRISPR/Cas9 gene editing to disrupt all six miR-124 alleles and created a monoclonal miR-124 knockout cell line. Upon neuronal induction, miR-124 depleted cells still underwent neurogenesis and matured into functional neurons, albeit with altered morphological features and neurotransmitter specification compared to their isogenic wildtype (WT) control. By AGO2 precipitation followed by RNA-Sequencing (AGO2-RIP-Seq), we could identify 100 biologically-active miR-124 targets in parallel. Many of these targets took over direct physiological functions such as long-term viability, which was decreased in miR-124-depleted neurons. Another fraction of active miR-124 targets were transcription factors (TF) that could lead to indirect miR-124 regulatory impacts. We studied these TFs by gene regulatory network analysis of transcriptomic data (RNA-Seq) over the entire time course of differentiation. By comparing WT and miR-124 knockout data sets, we revealed that certain miR-124-regulated TF networks drove neurogenesis towards different neuronal subtypes. We generated the first complete miR-124 cellular knockout model and revealed that neuronal differentiation from human iPSCs is miR-124-independent. Though, miR-124 has neuroprotective features in stem-cell-derived neurons and influences neuronal cell fates via targeted TF networks. In general, our data emphasizes the feasibility but also the need of systems level analyses to comprehensively understand neuronal differentiation from human iPSCs.

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W-3018

OVER-EXPRESSION OF SFRP2 ENHANCED THE OSTEOGENIC DIFFERENTIATION OF STEM CELLS FROM APICAL PAPILLA

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Mesenchymal stem cells (MSCs) isolated from tooth tissues are a reliable resource for dental tissue regeneration. Explored the regulation mechanism for their differentiation is helpful for potential clinical applications. Previous study discovered that Wnt inhibitory SFRP2 is highly expressed in apical papilla tissues compared with stem cells from apical papilla (SCAPs) by microarray analysis. However, function of SFRP2 in SCAPs remains unclear. In present study, we used SCAPs to investigate the function for osteogenic differentiation. Then alkaline phosphatase (ALP) activity, Alizarin Red staining, quantitative calcium analysis and osteogenic -associated gene expression were performed to investigation of the osteogenic differentiation potentials of SCAPs in vitro. The transplantation experiments were used to study osteogenic potential in vivo. Results: Real Time RT-PCR results showed that SFRP2 was highly expressed in apical papilla tissues compared with SCAPs, and its expression was increased along with osteogenic differentiation process. Over-expression of SFRP2 promoted ALP activity and mineralization in vitro, and enhanced the expression of DSPP, DMP1 and OSX in SCAPs. Moreover, in vivo transplantation experiments discovered that SCAPs osteogenic was enhanced by SFRP2 over-expression. Taken together, these results suggested that SFRP2 could enhance the osteogenic differentiation potential in dental MSCs.

Funding Source: National Natural Science Foundation of China, Dr Yu Cao(81670948)

W-3022

ENHANCED MYELINATION OF HUMAN STEM CELL-DERIVED OLIGODENDROCYTES AND NEURONS ON EXTRACELLULAR MATRIX-FUNCTIONALIZED NANOFIBROUS SCAFFOLDS

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Central nervous system-related neurological disorders and neurodegenerative diseases are known to be caused by demyelination due to the defects of oligodendrocytes. However, there have been few attempts to enhance myelination process in neurons by combining human stem cells and biomaterials. Here, we report the extracellular matrix (ECM)-modified polymer nanofibrous scaffolds as a functional culture platform to facilitate myelination process between induced neuronal (iN) cells generated by direct conversion and human induced pluripotent stem cell (hiPSC)-derived oligodendrocytes. Our results indicated that ECM-functionalized nanofibrous scaffolds efficiently guided myelination of by promoting cellular alignment and interactions along the nanostructures. We also observed that our platform upregulated expression of O4 and myelination binding protein (MBP), the most important indicators in oligodendrocyte maturation or functionality, and increased the number of MBP-positive oligodendrocytes which form myelin along the axons. Lastly, we may conclude that biomimetic scaffolds can successfully provide a functional myelination platform, which may be applied for demyelination disease modeling and regenerative medicine for the treatment of demyelination diseases.

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W-3024

THE ROLE OF INTRACELLULAR CALCIUM ION IN HUMAN EMBRYONIC STEM CELLS DURING EARLY NEURAL DIFFERENTIATION

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Calcium ion (Ca2+) is a ubiquitous second messenger in all kinds of cells. Ca2+ transients are spontaneously arising in the neuroectodermal region during embryonic development and involved in overall processes of neuronal development. However, the role of Ca2+ affecting to early neuroectodermal differentiation remains still unclear. Although spatio-temporal information of intracellular Ca2+ transient is one of the crucial determinants for which calcium signaling is activated or inactivated, most studies has been limited to treatment of chemicals which irreversibly regulate concentration of intracellular Ca2+. On the other hand, since early neural differentiation is a dynamic process orchestrated by multiple signaling pathways of specific time point, a more robust approach is required to study differentiation of human embryonic stem cells (hESCs). In this study, we adopted optogenetic system called OptoSTIM1 to explore the spatio-temporal role of Ca2+ in hESCs during neural development. OptoSTIM1 is a recombinant STIM1 protein that works as Ca2+ influx inducer via endogenous ORAI1 channel upon 488nm optic stimulation. In order to establish stable human pluripotent stem cell line with light-inducible Ca2+ entry system, we knocked-in OptoSTIM1 into AAVS1 genomic locus of hESC H9 by utilizing CRISPR-Cas9 system. OptoSTIM1 was stably expressed in transgenic hESCs during differentiation to neural progenitor cells. Ca2+ influx upon blue light illumination was checked in the transgenic hESCs by X-rhodamine staining. Furthermore, the transgenic hESCs showed translocation of NFATc1 reporter from cytoplasm to nucleus after optic stimulation. This result demonstrate that the optogenetic system activate downstream Ca2+ signaling pathway in hESCs. This optogenetic system can be utilized as a novel model to uncover the specified role of intracellular Ca2+ transient during early neural differentiation.

W-3026

EMERGING ALLIANCES: SIGNALING PATHWAYS REGULATE HISTONE MODIFIERS DURING PANCREATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Pluripotent stem cells (PSCs) have the capacity to differentiate into pancreatic cells upon stimulation by signaling ligands such as FGFs, TGF-β, WNT, Retinoic acid, Hedgehog and Notch. Retinoic acid and sonic hedgehog pathways provide instructive signals for patterning of the posterior foregut endoderm and establishing pancreatic organ limits respectively. Studies on mouse embryos have shown that ectopic activation of hedgehog signaling results in severely disrupted endocrine as well as exocrine pancreas while, deficiency in retinoic acid signaling results in failure to form pancreas. Polycomb group (PcG) proteins are important epigenetic silencers that mediate chromatin compaction and bring about gene silencing. PcGs function as multimeric units consisting of subunits BMI1, RING1B, EZH2 and SUZ12, through which they mark key developmental genes with the histone marks H3K27me3

and H2AK119ub1 and influence pancreatic differentiation. PcG subunits oocupy pancreas-specific regulatory elements and restrain the extent of ventral pancreatic specification in the endoderm. Several cancers have shown an association between GLI1, a sonic hedgehog downstream effector molecule and BMI1. In the present study, we cultured human pluripotent stem cells (hPSCs) and differentiated them into pancreatic progenitor cells. Our aim was to investigate whether sonic hedgehog and retinoic acid pathways regulate PcG proteins during the course of pancreatic differentiation. hPSCs were differentiated in the absence and presence of sonic hedgehog inhibitor, SANT-1 and were characterized using qRT-PCR. Expression of BMI1 and RING1B were studied at the transcript and protein levels and we found that in the presence of the inhibitor, PcG subunit levels were higher as compared to cells differentiated in the absence of the inhibitor. Our study indicates that sonic hedgehog pathway can directly regulate PcG proteins ultimately influencing hPSC differentiation and pancreatic cell fate.

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W-3028

DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO MESENCHYMAL STROMAL CELLS IS SUPPORTED BY SOFT HYDROGELS

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Induced pluripotent stem cells (iPSCs) can be differentiated towards mesenchymal stromal cells (MSCs) - but on molecular level, this transition remains incomplete. It has been demonstrated that matrix elasticity directs fate decisions in stem cells. Therefore, we followed the hypothesis that generation of iPSC-derived MSCs (iMSCs) is supported by fibrin-based hydrogels. We demonstrate that hydrogels generated with human platelet lysate (hPL-gel) and fibrin-gel support differentiation of iPSCs towards MSCs. During culture on these soft substrates, iMSCs showed enhanced growth with pronounced deposition of extracellular matrix as compared to tissue culture plastic (TCP). Unexpectedly, lineage-specific differentiation was not affected by soft hydrogels: iMSCs generated either on TCP or hPL-gel had the same morphology, immunophenotype, differentiation potential, and gene expression profiles. Furthermore, global DNA methylation patterns of iMSCs generated on TCP or hPL-gel were essentially identical, indicating that they were epigenetically alike. In continuation of this work, we have recently trapped iPSCs inside fibrin-gel where they formed cell aggregates with cavities, comparable to embryoid bodies (EBs). The cells surrounding these cavities revealed heterogeneous expression of Oct4 and E-cadherin. It is suggested that these cells are undergoing early mesodermal differentiation in EBs. Accordingly, we observed MSC-like cells that migrated from these cell aggregates and we are currently analysing the molecular impact of this 3D culture condition on iPSCs embedded into fibrin-gel and on the differentiation process into iMSCs. Taken together, hPL-gel and fibrin-gel provide powerful matrices to support growth and differentiation of iMSCs. Surprisingly, substrate elasticity did not have any molecular impact on differentiation if iMSCs were generated on soft hydrogels. On the other hand, culture inside fibrin-gel seemed to direct iPSCs towards early mesoderm and may therefore be more suitable for differentiation towards MSCs.

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W-3030

PARASPECKLES REGULATE NEURONAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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There is increasing evidence that post-transcriptional gene regulation is crucial to orchestrate the switch from pluripotent embryonic stem cells (ESCs) to early lineage progenitors. In many instances, RNA binding proteins (RBPs) organize themselves in membrane-less higher-ordered structures that can regulate gene expression. We focus on understanding the molecular function of paraspeckles, nuclear aggregates of a plethora of RBPs clustering onto NEAT1, a long non-coding RNA that is

crucial for paraspeckle integrity. While the majority of examined cell lines contain paraspeckles, they are absent in ESCs. Previously, we identified NEAT1 as one of only 18 transcripts that is lineage-independently upregulated during exit from pluripotency and we showed that paraspeckles form upon applying various differentiation stimuli to human ESCs (hESCs). This raises the question, whether paraspeckles might have a function during the exit of the pluripotent state and lineage acquisition. By performing differentiation time courses towards cardiomyocytes, hepatocytes and astrocytes, we demonstrated that paraspeckles form during the transition from a pluripotent to a multipotent state and disintegrate upon terminal lineage commitment. To analyze function of paraspeckles during differentiation, we engineered hESCs that are either devoid, or overexpress paraspeckles, by deleting parts of NEAT1 promoter including transcription start site or deleting the polyA site, respectively. We showed that in both, paraspeckle KO and overexpression cell line, the differentiation towards neuroectoderm, including neurospheres, neuronal rosettes and neuronal progenitor cells is compromised. This is the first evidence that paraspeckles have a function in embryonic development.

W-3032

CHARACTERIZATION OF DERMAL PAPILLA CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Throughout history hair has shown considerable symbolism. Hair loss is associated with many psychological implications such a lowered self-esteem, increased distress, and depressive episodes. The discovery of an effaceable strategy to manage hair loss has both cosmetic and psychiatric implications. We propose to use subject-specific induced pluripotent stem cells (iPSCs) to generate dermal papilla (DP) cells, which can then be co-transplanted with Keratinocytes to form functional hair follicles. We focused on dermal papilla because of the dominant role it plays in determining hair thickness, length, and lifecycle. Based on prior genetic evidence of NC contribution to DP cells in-vivo, we adopted a novel strategy of obtaining DP cells from iPSCs through a Neural Crest (NC) intermediate. NC cells are developed through a three-dimensional sphere protocol. Based on the developmental pathways uncovered in mice, we sought to determine the signaling pathways that enable efficient NC differentiation into DP cells, by systematically investigating the effects of growth factors and extracellular matrix on the differentiation process. Specifically, we investigated the role of Wnt, BMP and FGF pathways, known to be key players in hair follicle morphogenesis. We observed enhanced DP differentiation when cells were treated with a combination of Wnt10b and R-spondin for 2 weeks, as shown by the expression of important DP markers, such as Versican and Alkaline Phosphatase. With the use of RT-PCR, we confirmed the up-regulation of relevant DP genes (Nexin, Corin, SDC, ALK4, HEY1, EGR3). Moreover, we implemented robust cell purification and enrichment strategies such as FACS Analysis and Immunobeads to generate a homogeneous DP population, based on the expression of surface markers such as syndecan-1 and integrin-α9. In conclusion, our data revealed the critical molecules and pathways responsible for DP cell induction from iPSC-derived NC cells. The major novelty and strength of this approach is its exploitation of the ability of patient-specific iPSCs to act as a virtually unlimited source of DP cells for transplantation to induce de novo formation of hair follicles.

W-3034

EXPRESSION PATTERNS OF MITOCHONDRION-RELATED GENES CAN BE AN INDICATOR FOR THE DEGREE OF DIFFERENTIATION

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During the embryonic development process, cells undergo various changes involved in gene expression and signaling pathways. Metabolism and intracellular organelles are also changed during the development. One of the dramatic changes in cells was shown in mitochondria. Mitochondria is an essential organelle in animal cells and involved in various cellular processes, including energy metabolism, apoptosis, aging, and differentiation. The shape, function, and maturity of mitochondria vary from cell type. Pluripotent stem cells have globular-shaped immature types of mitochondria. On the contrary, somatic cells contained elongated types of mature mitochondria. Mitochondrial morphology is changed between elongated tubular and fragmented globular shapes by fusion and fission processes. Mitochondrial fusion is known to be mediated by Mfn1, Mfn2, and Opa1, whereas mitochondrial fission is mediated by Fis1 and Dnm1L. In this study, we investigated the expression patterns of mitochondrion-related genes during the differentiation of mouse embryonic stem cells (ESCs), which entail the change of mitochondrial morphology. We confirmed that the mitochondrial length became longer as differentiation progressed. Thus, we quantified the expression level of fusion- (Mfn1, Mfn2, and Opa1) and fission- (Fis1 and Dnm1L) related

genes during the ESC differentiation. We found that gradual increase of Mfn2/Dnm1L and gradual decrease of Mfn1/Fis1 ratio were closely matched to the extent of differentiation of ESCs. Consequently, we suggest that these two ratios could represent the differentiation of pluripotent stem cells as well as mitochondrial shape changes. Thus, these ratios can be a reliable index for the extent of age and differentiation of cells.

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PLURIPOTENT STEM CELL: DISEASE MODELING

W-3038

A COMPARISON OF HAEMOPOIETIC CELL TRANSPLANTATION AND IN VIVO LENTIVIRAL GENE TRANSFER FOR THE CORRECTION OF SEVERE BETA-THALASSEMIA IN A MURINE MODEL OF INTRAUTERINE THERAPY

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Major haemoglobinopathies place tremendous strain on global medico-economic resources particularly where standard curative transplantation is unavailable. Intrauterine haemopoietic cell (IUHCT) and gene (IUGT) therapies can potentially prevent end-organ damage in perinatally-lethal α-thalassaemia major and reduce chronic morbidity in β-haemoglobinopathies, leveraging on immune-naïveté and low cell and vector requirements. Challenges include genotoxicity and competitive physiological barriers to engraftment. We compared IUHCT and lentiviral (LV) IUGT in the HbbTh3/+ murine model of thalassaemia. Murine fetuses were injected intraperitoneally at E13-14 with 2E+5 human fetal liver-derived haemopoietic cells in a xenogenic model, and with fetal murine Lin-negative mononuclear cells (MNC, 20% Lin-) at low (2E+6 cells) and high (5E+6 cells) doses in a congenic model. IUHCT alone produced transient low chimerism, which improved in the congenic model with selective postnatal challenge (~1-4%) in microchimeric pups, especially after fludarabine immunosuppression (~2-15%), but did not produce phenotype reversal. Unchallenged chimeric pups showed a steady decline in engraftment to <1% by 16 weeks. Intravenous injection of 5E+6 transforming units of LV-MA821 ("GLOBE") encoding the mini-human-β-globin transgene at E15-16 significantly improved haematological indices at <0.4 vector copies per transduced cell, though without full phenotype rescue. These experiments demonstrate that, at the doses utilised, LV-IUGT appears more effective than IUHCT with postnatal transplantation, although neither was sufficient to correct thalassaemia. These strategies require further optimisation to overcome the altered hyperplastic microenvironment encountered in thalassaemic subjects.

W-3040

THE NATIONAL PHENOTYPIC SCREENING CENTRE: DEVELOPING COMPLEX, PREDICTIVE, DISEASE-RELEVANT BIOLOGY FOR DRUG DISCOVERY

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The National Phenotypic Screening Centre (NPSC) was created with ~£9M of Scottish Government investment and launched in 2015, with labs in three highly research-intensive UK Universities: Dundee, Edinburgh and Oxford. A key aim of the centre is to redress the balance in drug discovery, moving away from the types of target-centric approaches that have generally shown poor translational efficacy. NPSC is focusing its efforts on advanced phenotypic screening approaches at all levels: developing the most pathophysiologically-relevant assays possible; leveraging the latest developments in modelling human biology such as hiPSC/stem cell technology; CRISPR/Cas9; 3D cultures and organoids; implementing multiparametric profiling and data analysis methods as well as facilitating technology advancement. There is a deep well of biology in the academic and clinical community that remains somewhat untapped and often lacks translational direction. We have formed a public-private consortium which has Janssen as its founding partner (but open to all Pharma/Biotech) called the Phenomics Discovery Initiative (PDi) that allows the pre-competitive de-risking of phenotypic assay development. PDi leverages NPSC's world class facilities, industry standard operation and extensive global networks to crowdsource and develop the best biology from the academic, clinical and SME community. PDi's experts select the projects and the NPSC funds their in-house development and validation (using annotated and diversity-based compound libraries) working

very closely with the assay proposers throughout. PDi has a growing portfolio of disease-relevant assays. Current projects cover a spectrum of therapeutic areas including oncology and immuno-oncology, dementia and neuropsychiatric diseases, cell stress, infection and immunity. NPSC also is active in driving the

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W-3042

USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELL-CARDIOMYOCYTE MODELS TO INVESTIGATE THE GENETIC AND MOLECULAR MECHANISMS OF INHERITED HEART DISEASE

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Inherited heart diseases, including hypertrophic cardiomyopathy (HCM), arrhythmogenic cardiomyopathy (ACM) and catecholaminergic polymorphic ventricular tachycardia (CPVT) can have devastating outcomes including heart failure and sudden cardiac death. Induced pluripotent stem cells can be differentiated into iPSC-cardiomyocyte (iPSC-CM) models of disease providing a unique source of patient specific tissue. We aimed to create iPSC lines from inherited heart disease patients that demonstrate successful differentiation into iPSC-CMs, and can be used to model disease. Blood was collected from families with HCM, ACM and CPVT. Peripheral blood mononuclear cells were isolated, expanded and transfected with reprogramming factors. iPSCs were assessed for pluripotency marker expression, tri-lineage differentiation capacity, purity, chromosome integrity and loss of episomal vector expression. iPSCs were differentiated into iPSC-CMs, and cardiomyocyte function assessed. The pluripotent nature of the iPSC lines was demonstrated by expression of pluripotency markers SOX2, OCT4, SSEA-4 and TRA-1-60. Tri-lineage differentiation potential was confirmed by expression of CXCR4 and SOX17 (endoderm), PAX6 and Nestin (ectoderm), and NKX2.5 and TNNT2 (mesoderm). Cell line identity was confirmed through identification of genetic variants of interest, and comprehensive STR marker analysis. Absence of episomal reprogramming vectors in cell lines was verified. Genomic integrity was shown to be intact through molecular karyotyping. iPSC-CMs spontaneously contracted from day 12 post-differentiation, expressed cardiac markers and action potentials, and the ability to be continuously cultured. We have successfully developed iPSC-CM models from patients with a range of inherited heart diseases. This work provides a platform for assessment of genetic and molecular mechanisms that underlie disease and ultimately findings that can be translated into better patient outcomes.

W-3044

DEVELOPMENT AND OPTIMIZATION OF AN EFFICIENT METHOD FOR CRISPR/CAS CORRECTION OF PATIENT-SPECIFIC IPSCS

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Patient specific induced pluripotent stem cells (iPSCs) can be differentiated into any cell type of the body and harbour disease-specific genetic profiles, making them an ideal platform for modelling inherited diseases. Although combining iPSCs with CRISPR/Cas technology has been championed as attractive strategy in the development of gene-based therapies, given the low frequency of homology directed repair (HDR) pathway, the generation of isogenic gene-corrected iPSCs is generally laborious. Here we report the optimization of an efficient protocol for CRISPR/Cas correction of patient-specific iPSCs. We nucleofect a fluorescent-labelled sgRNA with high fidelity form of Cas9 as a CRISPR ribonucleoprotein (RNP) along with an asymmetric repair donor to target the mutation site of iPSCs. Post-electroporation, the cells are cultured with L755507 for 48h to enhance the HDR efficiency, then rather than manually picking single clones, Fluorescence Assisted Cell Sorting (FACS) is used to seed individual cells into vitronectin coated 96-well plates. Following adaptation to CloneR supplement and a plate centrifuge process, we observed a final single cell viability of at least 50% on feeder-free culture. Screening of single cell clones is initially performed with PCR amplification and single nucleotide polymorphism (SNP) genotyping. Clones are finally screened via bi-directional sanger sequencing. Using this approach, isogenic corrected patient-specific iPSCs can be generated within 5 weeks for a cost of approximately $AUD 3000. The combination of these optimizations has allowed the development of a rapid, easy, and efficient means for gene correction in iPSCs.

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W-3046

HUMAN INDUCED PLURIPOTENT STEM (IPS) CELLS IN GLAUCOMA MODELLING WITH AN AUTOMATED PLATFORM

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Glaucoma is an optic neuropathy characterized by gradual degeneration of retinal ganglion cells (RGCs) and their axon leading to irreversible vision loss. Studies involving human RGCs are hampered by the inability to obtain the RGCs from living donors in a non-invasive manner. This issue can now be circumvented by the use of human iPS cells as a source of RGCs. Nevertheless, disease modelling necessitates examination of large number of samples to achieve significant statistical power of the analysis. To fulfil this requirement 300 patients - 150 affected by glaucoma and 150 healthy controls - were recruited to participate in the study. Subsequently, they underwent skin biopsies, which were then used to generate patient-specific iPS cells. This challenging task was greatly facilitated by the automated platform (TECAN Freedom EVO200) and is now complete. Currently, all cell lines are undergoing quality control analysis to confirm their pluripotency and lack of karyotypic abnormalities. We are also optimizing the differentiation protocol to ensure that enough RGCs are obtained for downstream applications. In particular, we investigated feasibility of using a three-dimensional organoid approach, and sort RGCs from neural retina using THY1 selection. Further, our work already indicated that iPSC-derived RGCs can be used for single-cell RNA sequencing and have a transcriptional signature similar to that of human RGCs.

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W-3048

GENERATION OF ISOGENIC HUMAN INDUCED PLURIPOTENT STEM CELL LINES FOR HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the expansion of a CAG repeat in the huntingtin gene, HTT. The length of the CAG repeat is inversely correlated with age at motor onset but other factors influence onset including genetic variation elsewhere in the genome. Recent genome-wide association studies (GWAS) have identified genetic variants in or near DNA repair genes as modifiers of age at onset. We hypothesise that DNA repair processes trigger post-mitotic CAG repeat expansion in medium spiny neurons (MSNs), the cells most susceptible to the disease, leading to their degeneration. Evidence from post-mortem human HD brains and HD mouse models suggests that CAG expansion in neurons may drive HD pathogenesis, but thus far has been difficult to study in vitro. Induced pluripotent stem cells (iPSCs) derived from patients with HD provide a unique opportunity for modelling HD pathogenesis and HD-iPSCs recapitulate a HD disease-like phenotype. Here we show that HD-iPSC lines with a CAG repeat tract of >100 CAG are unstable in culture, with the repeat tracts undergoing further expansions in pluripotent cells and upon neuronal differentiation. Using these cell lines as models of CAG repeat expansion we can characterise how genetic variants in DNA repair genes affect cells harbouring expanded CAG repeats. To study the precise contribution of specific genetic factors to disease processes we report the generation of isogenic pairs of iPSCs, that differ only in the length of the CAG repeat. Employing CRISPR-Cas9 and a piggyBac transposon-based homologous recombination approach we show the seamless correction of the HD-iPSC lines with a CAG repeat length of 109 to a 'wild-type' repeat length of 22 CAG. Corrected HD-iPSCs maintain a normal karyotype and pluripotency, demonstrated by a SNP array and immunohistochemistry. Corrected clones were further able to differentiate towards neurons expressing DARPP-32 and CTIP2, indicative of MSNs. We anticipate that correction of the disease-causing mutation will rescue the disease-like phenotype previously reported and our isogenic stem cell model will thus provide a valuable platform to elucidate the role of DNA repair in HD pathogenesis.

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W-3050

HYPOPLASTIC LEFT HEART SYNDROME: COMPLEX GENETICS, SHARED PATHOGENIC PATHWAY

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Hypoplastic left heart syndrome (HLHS) is a genetically complex disease, characterized by hypoplasia of the left side of the heart. Although it is one of the most severe forms of congenital heart defects, our current knowledge of the molecular underpinnings of the disease is very limited. Here, we have generated an in vitro model of HLHS using human induced pluripotent stem cells (hiPSCs) to uncover disease-causing factors. hiPSCs were generated from 10 unrelated HLHS patients and their normal 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 using both embryoid body and small moleculte cardiac-directed differentiation methods, and their cellular populations and gene expression were studied. Gene expression analysis of spontaneously differentiated cells showed lower expression of both cardiac and vascular smooth muscle markers in patients compared to controls. Flow cytometry analysis performed on iPSC cultures after directed cardiac differentiation at 5-day intervals (day 0-30) showed that ventricular cardiomyocyte differentiation in HLHS-hiPCSs was perturbed. Time course RNAseq of 5 HLHS families revealed that the highest differences between patients and parents were at day 20 post-differentiation initiation, with down-regulation of cell cycle related pathways being the main driver. This finding was further confirmed using another 5 indipendent 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 and vasculogenesis in HLHS-hiPSCs is perturbed, which may include problems in the cell cycle. Furthermore, the functionality of cardiomyocytes derived from HLHS-hiPSCs with respect to calcium flux properties was altered, suggestive of cardiomyocyte immaturity. Our data suggest a common pathogenic pathway underlying the formation of HLHS despite the genetic heterogeneity of disease causation.

W-3052

INSULIN SIGNALING AND RESISTANCE IN HUMAN PLURIPOTENT STEM CELL-DERIVED MODELS OF METABOLIC DISEASE

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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 generated 5 genetic models in human pluripotent stem cells (hPSCs). These models replicate both impaired and constitutively activated insulin signaling through the knockout or modification of genes involved in the insulin signaling pathway. To mimic impaired signaling, we knocked out the insulin receptor INSR, as well as AKT2, the main intracellular insulin signaling node, and FOXO1, a major insulin-regulated metabolic transcription factor. To model activated insulin signaling, we have knocked out the pathway's brake, PTEN, and knocked-in a mutation in AKT2 (E17K). We have characterized these genetic models in 8 relevant metabolic cell types differentiated from hPSCs. These include adipocytes, hepatocytes, skeletal muscle, as well as endothelial and vascular smooth muscle cells, which are tasked with insulin transport from the bloodstream to underlying organs. The differentiated cells are comprehensively analyzed via RNA-Seq, metabolomics profiling and insulin stimulation assays to generate a global overview of the effects of altered insulin resistance on the behavior of

vital metabolic cells. Many processes are genetically and metabolically differentially regulated, such as cholesterol homeostasis, mitochondrial metabolism, differentiation capacity and cellular communication. 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.

W-3054

GENERATION OF IN VITRO MODELS OF THE AICARDI-GOUTIÈRES SYNDROME TO DISSECT MECHANISMS OF PHYSIOPATHOLOGY

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Aicardi-Goutières Syndrome (AGS) is a rare monogenic inflammatory encephalopathy that can be caused by mutations in any one of seven genes encoding for proteins involved in the metabolism of RNA/DNA. Aberrant sensing of nucleic acids (NA) deriving from endogenous retroelements or accumulating DNA damage and the consequent induction of type I IFN have been suggested to be a primary driver of AGS pathogenesis. However, the molecular mechanisms triggering disease remain elusive due to the lack of viable animal models recapitulating the human neuropathology and thus far few molecular studies have been conducted in physiologically relevant cell types. Here, we took advantage of CRISPR/Cas9 genome editing tools and induced pluripotent stem cell (iPSC) culture and differentiation protocols to model chosen AGS gene defects in a neurological context in vitro. Starting from a well-characterized iPSC clone deriving from healthy donor fibroblasts, we have generated isogenic iPSC clones knock-out (KO) for the cytosolic DNA exonuclease TREX1 or for RNaseH2b, a subunit of the RNase H2 endoribonuclease complex. We have performed a first differentiation experiment towards neural stem cells (NSC) followed by their differentiation towards a mixed population of neural and glial progenitors comparing two established protocols. No phenotypic differences between the AGS and WT cells could be observed at the pluripotent iPSC stage while multipotent AGS KO NSC where more reactive to exogenous NA. A constitutive upregulation of type I IFN, DNA damage and senescence pathways was instead observed in AGS cells compared to WT upon differentiation towards progenitors enriched for pro-inflammatory astrocytes. These responses were more pronounced in the TREX1 KO cells, potentially reflecting worse disease severity in patients harboring mutations in TREX1 as compared to the milder forms associated with RNaseH2B. These preliminary results provide a first temporal indication on the emergence of endogenous triggers leading to AGS-specific phenotypes along the neuronal differentiation in vitro. These in vitro models will allow us to dissect which cellular components and endogenous triggers give rise to aberrant activation of the above mentioned pathways and investigate their functional consequences on neural development.

W-3056

HUMAN IPSC-DERIVED GLOMERULI PROVIDE AN ADVANCED MODEL TO INTERROGATE PODOCYTE BIOLOGY AND PODOCYTE DISORDER IN VITRO

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Numerous kidney diseases leading to proteinuria result from alterations to the podocyte which leads to foot process effacement and loss of slit diaphragms. Immortalised cell lines have been the gold standard in podocyte biology, however due to their inherent limitations, validation of novel disease-associated mutations is most often performed in animal models which may not replicate the human condition. The advent of iPSC organoids now provides an avenue for the study of human podocyte disease ex utero. A number of studies have attempted to generate podocytes from iPSC. These methods produce podocyte-like cells, however their phenotypic appearance is inappropriate alongside elevated expression of genes that do not associate with the kidney. In this study,

sieved whole glomeruli were isolated from human iPSC kidney organoids and characterised by comparing to both organoid-derived podocytes and conditionally immortalised human podocytes by RNA-sequencing. Organoid-derived glomeruli in 3D showed superior podocyte-specific gene expression when compared to 2D podocytes in culture. GO terms associated with slit diaphragm development, and renal filtration cell differentiation were significantly enriched. Proteomic analysis of the matrisome within sieved glomeruli found enrichment of core glomerular basement membrane proteins and structural ECM proteins, including collagen 4 isoforms, mature laminin isoforms and heparan sulfate proteoglycans. Using a CRISPR-derived MAFB reporter iPSC line, functional utility of organoid glomeruli was confirmed by development of a medium-throughput assay. Glomeruli exposed to doxorubicin showed a dose-dependent reduction in both reporter fluorescence and glomerular size, with an associated increase in Caspase-3 activity. In summary, organoid podocytes which are allowed to form in 3D glomeruli via the patterning and segmentation seen during normal nephrogenesis are superior to the current gold standard immortalised podocyte cell system. The capacity to readily generate such an accurate model of the human glomerulus from iPSC will facilitate patient-specific functional genomics to validate novel podocytopathy genes and further interrogate the pathogenic mechanisms of existing podocytopathies in vitro, with an application for drug screening.

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W-3058

STEM CELL-DERIVED NEURONS FOR HIGH THROUGHPUT FINDING AND PROFILING OF TROPOMYOSIN RECEPTOR KINASE B MODULATORS

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Human induced pluripotent stem (hiPS) cell-derived neurons promise to provide better model cells for drug discovery in the context of the central nervous system. Using GRowth factor-driven expansion and INhibition of NotCH (GRINCH) during maturation, sufficient numbers of hiPS cell-derived GRINCH neurons were generated to accommodate for the immense material needs of a high-throughput screening (HTS) approach. GRINCH cells displayed neuronal markers, and their functional activity could be demonstrated by electrophysiological recordings. The GRINCH neurons were employed as model cells to search for and to profile positive modulators of tropomyosin receptor kinase (TrkB), a promising drug target to treat synaptic dysfunctions. TrkB modulation in GRINCH cells was monitored by direct measurement of TrkB phosphorylation and by quantification of TrkB-driven downstream signaling. Both small molecule activators of TrkB and two TrkB-agonistic antibodies were investigated. This pharmacological characterization in GRINCH neurons paves the way for a new generation of predictive cell-based drug discovery and development.

W-3060

PCDH19 REGULATION OF NEURAL STEM CELL DIFFERENTIATION SUGGESTS ASYNCHRONY OF NEUROGENESIS AS A MECHANISM UNDERPINNING PCDH19 GIRLS CLUSTERING EPILEPSY.

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PCDH19-GCE is a female specific epilepsy associated with a spectrum of neurodevelopmental and behavioural problems. It is caused by a variety of loss of function mutations in an X-chromosome gene, Protocadherin19 (PCDH19), with more than 271 cases reported to date. PCDH19-GCE is a disorder of cellular mosaics, that is females who undergo X-inactivation and males with somatic mosaicism. We show that PCDH19 is highly expressed in human neural stem and progenitor cells (NSPCs) and investigate its function in vitro in these cells of both mouse and human origin. Transcriptomic analysis of mouse NSPCs lacking Pcdh19 revealed changes to genes involved in regulation of neuronal differentiation, and we subsequently show that loss of Pcdh19 causes increased NSPC neurogenesis. We reprogramed human skin fibroblast cells harbouring a pathogenic PCDH19 mutation into human induced pluripotent stem cells (hiPSC) and employed neural differentiation of these to extend our studies into human NSPCs. During

neural differentiation, PCDH19 loss-of-function cultures were able to form polarised NSPC structures termed "neural rosettes", but unable to properly maintain these structures. This was evidenced by a 46% decrease in lumen size and a 63% decrease in the number of polarised structures/rosette area, compared to wildtype (WT). A significant increase in the number of neurons at the edge of the rosettes was also observed, associated with a 60% increase in the primary neurite length of loss-of-function PCDH19 neurons, compared to WT suggesting increased neuronal differentiation. Taken together as in mouse, loss of PCDH19 function caused increased neurogenesis, and furthermore, we show this is associated with a loss of NSPC polarity in human. Overall our data suggest a conserved role for PCDH19 in regulating mammalian cortical neurogenesis and has implications for the pathogenesis of PCDH19-GCE. We propose that the difference in timing or 'heterochrony' of neuronal cell production originating from PCDH19 wildtype and mutant NSPCs within the same individual leads to downstream asynchronies and abnormalities in neuronal network formation, predisposing the individual to network dysfunction and epileptic activity.

W-3062

COPY NUMBER VARIANT HOTSPOTS IN HAN TAIWANESE INDUCED PLURIPOTENT STEM CELL LINES - LESSONS FROM ESTABLISHING THE TAIWAN HUMAN DISEASE INDUCED PLURIPOTENT STEM CELL SERVICE CONSORTIUM IPSC BANK

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In 2015, the first and only Taiwan induced pluripotent stem cell (iPSC) core facility, named the Taiwan Human Disease iPSC Service Consortium, was formed to accelerate stem cell research and development in Taiwan by providing a platform for researchers interested in utilizing iPSC technology. Since conception, we have generated and fully characterized 15 iPSC lines from normal Taiwanese donors and 54 disease iPSC lines covering more than 19 diseases, with one to three patients representing each disease type. Our disease iPSC lines cover monogenic, polygenic and chromosomally-inherited diseases, with several carrying mutations that are of high incidence in Taiwan such as α-galactosidase c.936+919G>A in Fabry disease patients. We performed genome-wide copy number variant (CNV) screening of 52 Han Taiwanese iPSC lines and compared them with 1093 control subjects using an Affymetrix genome-wide human SNP array. We identified seven CNV loci (amplification) strongly associated with the reprogramming process, indicating that these recombination regions are hotspots (frequency >5%) during iPSC generation. We have additionally established several differentiation protocols for our iPSC lines including cardiac, neuronal, hepatocyte, beta-cell, retinal pigment epithelium, endothelial, and granulosa cell differentiation. To assess whether differentiated cells are capable of modeling drug response and being used for cell therapy, we showed that our iPSC derived cardiomyocytes respond to doxorubicin and could engraft into mouse myocardium. Thus, we aim to share our experiences and successful outcome in forming a national iPSC resource center as a small country with limited resources capable of producing high quality iPSCs. This was made possible through the establishment of efficient Standard Operating Procedures (SOPs) for management of the consortium and partner sites, and crucial elements of iPSC handling such as cell generation, expansion, characterization, cryopreservation and distribution. Our lines will be banked and made publicly available to researchers in Taiwan and aboard, and we hope our experiences and iPSC lines can benefit the stem cell community in Taiwan and internationally.

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W-3064

ESTABLISHMENT OF IPSC-DERIVED NEUROMUSCULAR MODELS FOR THE ANALYSIS OF NON-CELL AUTONOMOUS NEURODEGENERATION IN MOTOR NEURON DISEASE

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So far, most of the motor neuron diseases, including amyotrophic lateral sclerosis (ALS), spinal bulbar muscular atrophy (SBMA), and spinal muscular atrophy (SMA) have been considered to be caused by cell-autonomous neurodegeneration. However, recent analyses have shown non-cell autonomous neurodegeneration by skeletal muscles and neuro-muscular interaction, though detailed molecular mechanisms have not been fully elucidated. Thus, in this study, for the pathophysiological analysis of neuro-muscular pathology in motor neuron diseases, we established an iPSC based neuro-muscular co-culture system, in which functional neuromuscular junctions (NMJs) could be formed and visualized by fluorescent reporter. We first established a stable human myoblast cell line Hu5/E18-AE by introducing AChR (Acetylcholine receptor)ε-EGFP (AE) that visualizes NMJs. When we co-cultured Hu5/E18-AE with iPSC-derived motor neurons, we could detect clustering of AE in an axon projection site. Furthermore, using this reporter system, we screened for the factor which promoted NMJ formation and identified NMJ facilitation factors (NFFs). We next established co-culture system of iPSC-derived skeletal muscles and motor neurons. At first, we modified previously reported methods and established methods to efficiently derive skeletal muscles from human iPSCs by DOX inducible MyoD1 expression. We, then, co-cultured AE introduced iPSC-derived skeletal muscle with HB9e438-mRFP labeled iPSC-derived motor neurons in the presence of NFFs, and observed significant clustering of AE signals detected in mRFP-labeled motor neuronal projection site, which were well co-localized with the staining of α-Bungarotoxin (α-BTX), a ligand for AChR. Using this reporter system, we successfully performed real-time quantitative analysis of NMJs by the time-lapse imaging. We are also establishing evaluation system for the functionality of NMJs by opto-genetics, calcium imagings, and multi-electrode arrays (MEA). Taking advantage of these systems, we are currently differentiating disease specific iPSCs established from patients of motor neuron diseases into motor neurons and skeletal muscles, and co-culturing them to elucidate neuromuscular pathology of motor neuron diseases, and to identify novel therapeutic targets.

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REPROGRAMMING

W-3068

NOVEL APPROACH OF CELL CONVERSION TOWARDS PANCREATIC PROGENITORS

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Direct in vivo lineage reprogramming has emerged as a potential alternative approach to replenish lost cells in degenerative diseases, such as beta-cells in Diabetics. Recent works have successfully generated these cells in vivo from mouse liver and alpha cells, which are developmentally related cells of the same germ layer but with the caveat of coming from already compromised organs in Diabetic patients. Our lab aims to target abundant, and importantly non-vital cells amenable for in vivo lineage conversion to replenish the lost population of beta cells. Tissues such as muscle, skin, fat, and vasculature are abundant and non-vital, and may be ideal sources of cells for beta-cell conversion. Using the zebrafish as an in vivo vertebrate model, our lab has identified two transcription factors (endoderm like factor 1 and 2 - elif1, elif2) that can reprogram differentiated muscle cells (from mesoderm origin), into early endoderm cells. In addition, because pancreas development is well conserved across species, we explored capability of elif1 and elif2 to undergo lineage conversion of several mouse and human cell lines, in vitro. Elif1 and elif2 were cloned into plasmid DNA under a constitutive CMV promoter. On one hand murine C2C12 muscle cell line and mouse embryonic fibroblasts (MEFs) were electroporated with plasmids expressing our factors. In both cell lines, gene expression was analyzed both at RNA levels by real-time quantitative PCR (qPCR) and protein levels by immunofluorescence (IF). On the other hand, a similar approach was taken using the human embryonic kidney cell line (HEKs). Our data shows induction of early key endoderm markers, such as sox17 and foxA2, in both C2C12 and MEFs murine cell lines at RNA levels. Induction of sox17 was further confirmed by IF. In HEKs, a time course shows initial expression of sox17 followed by foxA3 and hnf1b at mRNA levels. Altogether, our data supports that overexpression of elif1 and elif2 can initiate lineage conversion of several mammalian cell lines. Future studies will focus on further differentiating these induced endoderm cells into pancreatic progenitors, and ultimately, into replacement beta-cells.

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W-3070

RAPID AND EFFICIENT GENERATION OF NEURONS AND ASTROCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Transcription factor-based reprogramming of human pluripotent stem cells (hPSCs) is about to revolutionise the way mature cells are generated. Direct conversion of hPSCs has the potential of being faster, more efficient and more reproducible than conventional directed differentiation. Recently, our lab developed OPTi-OX (optimised inducible overexpression system), which has overcome gene silencing of transgenes in hiPSCs by targeting an efficient overexpression system into two

separate genomic safe-harbour sites. Using OPTi-OX, we overexpressed the transcription factor NGN2 to generate iNeurons (iNs) and the three transcription factors NFIA, NFIB and SOX9 to generate iAstrocyes (iAs). This results in the generation of cells resembling mature neurons with strong expression of pan-neuronal and glutamatergic markers and in a high proportion of cells with a strong expression of astrocytic markers such as GFAP and S100B, after only 1 week of induction. In addition, the iNs demonstrated spontaneous electrophysiological activity within two weeks' post-induction. This activity was blocked with tetrodotoxin, confirming that the recorded activity relies on the activity of voltage-gated sodium channels. By around day 20, iNs developed synchronous burst firing patterns across multiple electrodes, which demonstrate that iNs develop functional networks within three weeks. Thus, these cells can be used as an in vitro model to study neuronal networks. Ongoing work are aimed at evaluating the functionality of our iAs. One of our future goals with the present work is to develop an entirely human in vitro co-culture system for the study of neurons and astrocytes and potentially a simplistic and powerful platform for disease modelling and drug discovery.

W-3072

FUNCTIONAL MATURATION OF LONG TERM CULTURED HUMAN INDUCED NEURONS

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Induced neurons (iNs) are somatic cells that are reprogrammed to change their fate without passing through a pluripotent state, shortening the process of neuronal generation compared to the use of human pluripotent stem cells. The scientific interest in these cells is due to their suitable use for disease modelling, drug screening and cell therapy for diseases such as Parkinson Disease (PD). In order to determine neuronal identity, function and diversity, we have studied the electrical activity of iNs using the whole cell patch-clamp technique. We evaluate how different combinations of transcription factors (TFs) affects neuronal maturation. More specifically, we compare the electrophysiological profile of the iNs generated by a combination of Ascl1, Brain2, Myt1L, NeuroD1, Lmx1, Foxa2, Otx2, Nurr1 and REST inhibition at different time points. The result shows active iNs after 50 days of culture, with presence of immature, intermediate and mature action potentials induced by steps of current depolarisation. The physiological parameters analysed shows a progressive maturation of the iNs that become spontaneously active after 90 days of culture. This result is an important step in the use of the iNs both for disease modelling and cell transplantation.

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W-3074

BRAIN REPAIR VIA IN SITU ASTROCYTE-TO-NEURON CONVERSION

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Glial scar is widely associated with brain and spinal cord injury, stroke, glioma, and neurodegenerative disorders such as Alzheimer's disease. Reactive glia initially exert neuroprotective role but later form glial scar to inhibit neuroregeneration. Currently, there is no effective way to reverse glial scar back to neural tissue. We have recently developed an innovative *in vivo* cell conversion technology to directly convert reactive glial cells into functional neurons inside the mouse brain This is achieved through *in vivo* expression of a single neural transcription factor NeuroD1 in the reactive astrocytes in injured mouse brain or Alzheimer's disease mouse model. Our *in vivo* cell conversion technology makes use of internal glial cells to regenerate new neurons with 90% conversion efficiency, making it possible for the first time in history to reverse glial scar back to neural tissue. Such internal cell conversion method will avoid cell transplantation and immune rejection. More importantly, our recent study in a stroke model demonstrated that in vivo neuroregeneration efficiency can be as high as 100 times of the internal regeneration capability in the cerebral cortex after injury, an unprecedented efficiency in regenerative medicine. We have further discovered a cocktail of small molecules that can directly convert cultured human astrocytes into functional neurons paving the way for a potential drug therapy for human brain repair. We have also successfully converted reactive glial cells into neurons using NeuroD1 in non-human primate brains, making an important step toward future clinical trials.

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W-3076

REPROGRAMMING OF MOUSE FIBROBLASTS INTO SKELETAL MUSCLE WITH CHEMICAL COCKTAIL

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Use of stem cells in regenerative medicine holds a great promise in treating people suffering from various otherwise incurable ailments. Direct transdifferentiation of somatic cell to other lineages bypassing the intermediate pluripotent state has enormous applicability with respect to time requirement for conversion as well as safety issues. Of all the modes for direct reprogramming, chemical conversion is safe yet effective way and use of small molecules has been in tremendous rise in recent years in regenerative field due to the easy applicability and efficient scalability along with rigorous reproducibility. Several lineage specific cells generated by direct conversion of terminally differentiated somatic cells have been tried in recent years. In these efforts, cardiac cell generation has been studied extensively. Many other muscle related diseases like Duchenne muscular dystrophy (DMD), and other age related muscle wasting can be target of skeletal muscle based regeneration therapy. Here we report chemical based direct conversion of mouse fibroblasts to physiologically functional skeletal muscle. This study not only provides the first ever attempt in generating skeletal muscle from somatic cells but also underlines the key signaling pathways that are needed to modulate muscle induction.

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W-3078

MOLECULAR INTERACTIONS AND FUNCTION OF LNCRNAS IN DEFINING PLURIPOTENT STATES

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Throughout mammalian development, controlled gene regulatory networks play an essential role in maintaining stable cellular identities. Misregulation of gene networks in pluripotent cells is a major cause of many human diseases, such as developmental disorders and cancer. A new class of RNAs, long non-coding RNAs (lncRNAs), have been shown to be implicated in defining gene networks and several are linked to the progression of these diseases. LncRNAs do not have a coding region, they are more than 200 nucleotides long, and like mRNAs, they are transcribed by RNA polymerase II and are often capped, spliced and polyadenylated. A well-accepted role of lncRNAs is in interacting with chromatin regulators that establish epigenetic marks to stabilize cell state. However, the mechanisms behind how they coordinate cell state specific interactions between transcription factors and chromatin regulators is not well-characterized. Here we aim to study such potential lncRNA-protein-DNA interactions responsible for controlling cell fate in the context of pluripotent states. We focused particularly on one pluripotency associated lncRNA, G22, that was selected based on its expression in ESCs and during reprogramming. We demonstrate that G22 is localized within the nucleus of ESCs and iPSCs, and that it accelerates reprogramming towards iPSCs, suggesting a role in pluripotency gene network regulation. We also use several CRISPR-Cas approaches to further elucidate G22 function and its effects on mouse ESCs maintenance/differentiation. Overall, our studies will identify the mechanisms of how lncRNAs control transcriptional interactions during the acquisition of pluripotency, but most importantly, they will pave the way towards understanding how such interactions may be implicated in pathological conditions.

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W-3080

INHIBITION OF ABERRANT DNA RE-METHYLATION IMPROVES THE DEVELOPMENT OF NUCLEAR TRANSFER EMBRYOS

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Somatic cell nuclear transfer (SCNT) enables the genome of a differentiated somatic cell to be reprogrammed to totipotency. However, this process is extremely inefficient, and the underlying mechanism of the epigenetic rearrangements following SCNT remains largely unknown. Here, we generated a global DNA methylome of mouse SCNT preimplantation embryos. Surprisingly, we identified widespread re-methylated regions (rDMRs) in 2- to 4-cell stage cloned embryos, which caused mis-expression of genes and retrotransposons important for zygotic genome activation. Overexpression of histone demethylases only alleviated the persistent methylation loci of SCNT embryos, with less impact on these rDMRs. We further demonstrated that knocking-down DNA methyltransferases can partially rescue the re-methylation defects of SCNT embryos and markedly improve the developmental capacity of cloned embryos. Therefore, our study reveals that aberrant re-methylation functions as another barrier for SCNT embryo development, suggesting that removal of hierarchical epigenetic barriers would be a promising approach to improve reprogramming efficiency.

W-3084

TRANSCRIPTOME ANALYSIS OF THE PHASES IN HUMAN SOMATIC CELL REPROGRAMMING

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The derivation of induced pluripotent stem (iPS) cells has presented the opportunity to model human disease in vitro with patient-derived cells, as well as the potential for personalized cell therapies. Understanding how the process of reprogramming somatic cells works at the cellular and molecular level is therefore critical to harness iPS cell technology. In order to study mechanisms underlying the induction of pluripotency, we developed a high efficiency mRNA-based strategy for reprogramming human somatic cells. With this system, we performed temporal transcriptome analysis by RNA-Seq, and characterized distinct phases based on changes in gene expression during reprogramming. Our findings reveal that the previously established phases of reprogramming - initiation, maturation and stabilization - are temporally altered in humans compared to mouse. The mesenchymal-to-epithelial transition (MET) and cell cycle progression, which are hallmarks of the initiation phase in mouse, occur after upregulation of pluripotency markers in human cells. Furthermore, we used a CRISPR/Cas9-mediated screening method to functionally interrogate pathways implicated by these expression profiles at different stages of reprogramming. This approach identified new roles for a set of pathways during early reprogramming of human cells. It is our aim to define the function of these pathways and their influence on cellular plasticity and major cell fate transitions.

W-3086

SINGLE CELL LEVEL ANLAYSIS OF HUMAN REPROGRAMMING PROCESS AND PLURIPOTENCY USING MASS CYTOMETRY

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The detailed process acquiring pluripotency, namely reprogramming, remains veiled despite many efforts. Recently, analysis on individual single cells such as single cell RNA-sequencing and mass cytometry have been employed to understand the reprogramming process in mouse. However, because human pluripotent stem cells (PSCs) are thought to be different from mouse PSCs, in this study, we sought to analyze human reprogramming at single cell level using mass cytometry. We examined ten markers representing cell cycle and pluripotency in the cells of reprogramming process and pluripotent state. We first analyze overall population changes during reprogramming using pluripotency markers and fibroblast marker CD44 and found the main population was shifted to a new population which didn't belong to either fibroblasts or human induced pluripotent stem cells (hiPSCs). PhenoGraph and metaclustering analysis revealed the reprogramming aspects through the several distinct cell clusters representing fibroblasts, human PSCs, loss of fibroblast identity, and the cells on the reprogramming route. We also questioned how PSCs are homogeneous in terms of the cell cycle. Thus, we comparatively analyzed each pluripotency marker expressing cells and their cell cycle status. OCT4 and NANOG expressing cells showed a positive correlation with the cells in typical cell cycle signature of PSCs. However, a surface marker expressing cells showed a different pattern. In summary, we first analyzed human reprogramming process in single cell-level details and found several cell clusters representing intermediate cells of reprogramming and a PSC marker representing alternative cell cycle pattern. These results demonstrate that mass cytometry and following computational analysis are invaluable tools for unveiling hidden phenomena in complicated processes such as reprogramming.

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W-3088

CELL FUSION-MEDIATED TRANSDIFFERENTIATION AND REPROGRAMMING THROUGH MICROFLUDICS AND BIOMATERIALS

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One of the ultimate goals of regenerative medicine is to substitute damaged cells and/or tissues. To realize this, the processes of transdifferentiation or reprogramming have been accomplished by cell fusion or iPS cell method. In this study, we investigated the possibility of transdifferentiation of human cell through cell fusion with mouse cell. Because we aimed to develop a novel way of cell fusion and to identify the characteristics of fused cells such as transdifferentiation or reprogramming.

Particularly, we used a microfluidic device which enables to make cell fusion without nucleus mixing because several issues like pluripotency which would be occurred after cytosol transfer are still subject to attractive and undiscovered field for regenerative medicine. We furthermore aim to investigate reprogramming using embryonic stem and somatic cells such as fibroblasts with this technology. It is expected that we may induce direct reprogramming of somatic cells through the cytosol exchange with stem cells by our microfluidic system. This approach will be useful in a variety of ways to induce direct transdifferentiation or reprogramming and to study the process of those.

LATE BREAKING ABSTRACTS

W-4002

ISOLATION AND IDENTIFICATION OF HUMAN LUNG CANCER STEM CELLS FOR SUBSEQUENT TREATMENT WITH PHOTODYNAMIC THERAPY

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Lung cancer is the principal cause of cancer mortality among males in both more and less developed countries, and has surpassed breast cancer as the main cause of cancer death among females in more advanced countries. Cancer stem cells (CSCs) are a small subsection of neoplastic cells capable of tumour genesis, they have characteristics representing normal stem cells (SCs) which include, self-renewal and pluripotency through which the generation of heterogeneous cells can construct the entire tumour. Due to CSC resistance to conventional therapies and therapeutic incapacity in complete eradication of cancer, development for novel therapeutic strategies for a more effective reduction in the risk of tumour metastasis and cancer recurrence is critical. This project focused on isolating and characterising CSCs from a commercially obtained human lung cancer cell line by identifying their specific cell surface markers and using standardized isolation techniques. Isolated CSCs were characterized using protein detection methods including Immunofluorescence and Flow Cytometry. Photodynamic therapy (PDT) is a clinically approved, minimally invasive, therapeutic procedure that can exert a selective cytotoxic activity toward cancerous cells. It uses a photosensitizer (PS) that becomes excited when subjected to laser light at a specific wavelength, the PS forms reactive oxygen species (ROS) killing malignant cells. In this study PDT was investigated as a potential treatment for CSCs. Cellular localization of the PS was identified using fluorescent microscopy. Followed by determining and documenting the concentration and effect of PDT using various biochemical assays: viability, proliferation, cell death and cytotoxicity, whereas changes in cellular morphology was observed by inverted light microscopy. All cellular responses was observed 24 hours post treatment.

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W-4004

NUCLEAR NESTIN DEFICIENCY DRIVES TUMOR SENESCENCE VIA LAMIN A/C-DEPENDENT NUCLEAR DEFORMATION

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Emerging evidence has revealed that the class VI intermediate filament (IF) protein, Nestin, not only serves as a biomarker for multipotent stem cells, but also regulates cell proliferation, invasion, and stemness in various tumors. However, the mechanistic contributions of Nestin to cancer pathogenesis still need to be clarified. In the present study, previously thought to reside exclusively in the cytoplasm, Nestin can also be found in the nucleus and participate in protecting tumor cell against cellular senescence. Specifically, we revealed that Nestin has a classical nuclear localization signal (NLS, aa318-aa347) at the downstream of Nestin rod domain. We then show nuclear Nestin, especially the rod domain, can interact with lamin A/C (nuclear IF protein). Mechanistic investigations demonstrated that loss of Nestin results in the activation of Cdk5, which causes the phosphorylation of lamin A/C (mainly at S392 site) and its subsequent translocation from the nucleus to the cytoplasm for degradation. The findings establish a new role for Nestin in tumor senescence, which involves its nucleus-localized form and interaction with lamin A/C.

W-4006

EXPLORING THE USE OF POTASSIUM CHANNEL ACTIVATORS TO CORRECT REPOLARIZATION ABNORMALITIES IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE MODELS OF CONGENITAL LONG QT SYNDROME

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Congenital Long QT syndrome type 2 (LQTS2) is an electrical disorder of the heart that predisposes otherwise healthy individuals to life threatening cardiac arrhythmias. Current treatment options for LQTS2 either reduce the incidence of triggers of arrhythmia (beta-blockers) or terminate the arrhythmia after onset (implantable cardioverter defibrillator). While effective at reducing the incidence of sudden cardiac death, these treatments are associated with significant side effects. One alternative strategy is to target the underlying disease mechanism, which is a loss of function of the human ether-a-go-go related gene (hERG) potassium channel that plays a crucial role in repolarisation of the cardiac action potential. Small molecule activators of hERG have been identified but the extent to which these can restore normal cardiac signaling in LQTS2 remains unclear. Here, we characterize a human induced pluripotent stem cell derived cardiomyocyte (iPSC-CM) model of congenital LQTS2 containing an expression defective hERG mutant A422T. Using a Kinetic Imaging Cytometer (KIC) platform that records optical signals for both membrane voltage and intracellular calcium for hundreds of cardiomyocytes per experiment, we show that mutant A422T dramatically prolongs ventricular action potential duration, as well as altering calcium handling properties. We also examined the emergent electrical phenotype of a monolayer (pseudo-tissue) of A422T iPSC-CMs using an automated microelectrode array (MEA) system. Mutant A422T dramatically prolonged the rate corrected field potential duration, a surrogate of QT interval on an electrocardiogram. Application of the hERG activator ICA-105574 was able to reverse the field potential prolongation associated with A422T iPSC-CMs in a concentration dependent manner. However, at higher doses ICA-105574 produced a profound shortening of the field potential duration compared to controls. We conclude that hERG activators could provide a possible treatment option for LQTS2 that targets the primary disease mechanism, with the caveat that there may be a risk of over-correction that could itself be pro-arrhythmic.

W-4008

HYDROXYMETHYLATION PROFILING THROUGHOUT NEURONAL DIFFERENTIATION OF IPSCS DERIVED FROM BIPOLAR DISORDER PATIENTS AND THEIR SIBLINGS

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The epigenetic landscape during early embryogenesis is critical to human neurodevelopment, and is thought to contribute to neuropsychiatric disease risk. In particular, 5-hydroxymethylation is an understudied epigenetic mechanism, for which there is evidence of involvement in behavioral adaptation, memory and neuropsychiatric disease. 5-Hydroxymethylcytosine (5hmC) levels are high in embryonic stem cells, and diminished upon differentiation, except in neuronal cells. Expression in the adult brain is higher than in stem cells, and varies across neuronal subtypes, suggesting a dynamic landscape. Genome-wide studies of 5hmC changes during neuronal differentiation are limited, and have not been investigated in the context of human disease. We therefore conducted a pilot study to generate genome-wide 5hmC profiles during neuronal differentiation in patients with bipolar disorder (BD) and their unaffected

siblings. We established induced pluripotent stem cell (iPSC) lines from two adolescents with BD and each of their unaffected siblings (one male and one female sib-pair). These were subsequently differentiated into neuronal stem cells (NSCs). We performed Reduced Representation Hydroxymethylcytosine Profiling (RRHP) in each cell line to determine genome-wide 5hmC profiles. We identified ~2 million 5hmC sites, and observed increased 5hmC levels in NSCs compared to iPSCs ($p<2.2x10-16$). Site-specific analysis identified differential hydroxymethylation patterns between iPSCs and NSCs within important neuropsychiatric candidate genes, such as GRIN2C, WDR45B, HDAC4, BRD1, and SLC6A1 (all p-values ≤1.40x10-4). Pathways and gene ontology (GO) analysis indicated enrichment for cerebral cortex development, GTPase activity, and postsynaptic density (all p-values ≤1.80x 10-2). Paired t-tests also identified nominally significant associations between NSCs derived from BD patients and their unaffected siblings, including 5hmC differences in SRRM4 and VIPR2 (p-values ≤1.14x10-4), genes which have previously been implicated in mood disorders, autism, and schizophrenia. Our study indicates an important role for 5hmC within neuropsychiatric-related pathways during early neuronal differentiation, and suggests that differential 5hmC expression may be a hallmark of bipolar disorder.

W-4010

STEM CELL TRACKING, LOSS AND RECOVERY IN THE CORNEAL EPITHELIUM

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The corneal epithelium serves as an excellent model for stem cell (SC) research. Nevertheless, fundamental feature of SC that regenerate the corneal epithelium remain under debate or unknown. For example, SC location, prevalence and self-renewal mechanisms were under debate or elusive. Consequently, the mechanisms that involve SC failure in pathology are not clear. Recently, we established a multi-color "Confetti" lineage tracing system which proved that the murine limbus is the major site of bona fide SCs. Additionally, K15-GFP transgene labeled the limbal SC/boundary compartment. In agreement, K15-GFP+ basal limbal epithelial cells expressed SC markers and were located at the margin site of corneal regeneration, as evident by lineage tracing. Surprisingly, however, surgical depletion of the limbal epithelium and K15-GFP+ SC pool was restored by corneal committed cells which underwent centrifugal migration and dedifferentiation into bona fide SCs. The recovered corneas were transparent for many months, displayed normal marker expression and appropriate dynamic of SC regeneration. By contrast, damage to the limbal stromal niche abolished K15-GFP recovery, and led typical limbal SC deficiency phenotype. Altogether, this study reconciles major inconsistencies in the field and suggests that the while limbus is the major site of SCs, the cornea has an extremely efficient mechanism of self-repair, even from exhaustive SC loss.

W-4012

THE HUMAN PLURIPOTENT STEM CELL REGISTRY (HPSCREG)

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In order to assess suitability of pluripotent stem cells (PSC) for clinical and research use, key specific information is required about the donor, the manufacturing process and the cell line characterization. Since PSC lines are being generated at multiple sites, many different means of donor characterization, cell manufacturing and characterization could be used. To allow for comparison between different lines from different sources and to evaluate and select suitable PSC-lines, data standards are required as well as a platform where the data are collected, validated and made comparatively available to users for review. The EU-funded registry for human PSC lines (https://hpscreg.eu) collects a wide range of PSC-related data in standard formats, including ethical provenance, evidence of pluripotency, and genetic constitution. hPSCreg provides a unique cell line identifier based on an internationally accepted standard nomenclature to reduce the risk of misidentification and to allow traceability. Furthermore, hPSCreg validates the provided data and certifies lines for use in EU-funded research. Current developments of hPSCreg include the collection, validation and certification of PSC-lines for clinical use. As an extension of the hPSC project register, a clinical trial register focused on PSC will be established.

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W-4014

DEFINING THE SYSTEMS BIOLOGY OF LENS AND CATARACT DEVELOPMENT USING LIGHT-FOCUSING MICRO-LENSES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Vision impairment caused by opacification of the eye's lens (i.e., cataract) can have profound emotional, social and financial impacts on both childhood and adult cataract patients. Cataract is the leading causes of blindness worldwide (affecting 12.6 million people) and the second leading cause of low vision (affecting 52.6 million people). Moreover, the number of people affected by cataract is increasing due to population ageing, and also the 314,000 new cases of childhood cataract estimated to arise each year. This increase in worldwide cataract burden is occurring in spite of cataract surgery being the most commonly performed ophthalmic procedure; millions of cataract operations are performed annually costing billions of dollars. Notably, laser-based treatment of secondary cataract - a common side-effect of cataract surgery - is the second most commonly performed ophthalmic procedure. A pharmacological treatment capable of inhibiting, delaying or reversing cataract formation is needed. However, cataract is not a single condition, and it likely has different mechanisms of formation depending on the cause. For example, a range of genetic factors are known to cause childhood cataract, while others have been associated with higher risk of age-related cataract. Environmental risk factors (such as smoking and UV light) and diseases (e.g., diabetes) have also been associated with cataract formation. The molecular mechanisms initiated by these factors that lead to cataract formation are poorly understood. This is largely due to difficulties accessing normal or diseased lens tissue at different stages of cataract development, particularly initiating stages. To address this we have established simple and reliable methods for producing large numbers of human lens epithelial cells and light-focusing micro-lenses from pluripotent stem cells. We have also shown these functional human micro-lenses can be used to study clinically-relevant, drug-induced cataract. We have complemented these micro-lens studies with unpublished bioinformatics approaches that predict signaling pathways and gene regulatory networks operating in the lens. This information is enabling us to generate and test molecular hypotheses for risk factor-associated cataract, and to identify and test candidate anti-cataract drugs.

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W-4016

HISTONE DEACETYLASES LIMITS MULTIPOTENCY FOR T CELL LINEAGE COMMITMENT AND MATURATION

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The T cell lineage arises from hematopoietic stem cells and progresses through CD4- CD8- double negative (DN) T cell progenitors to mature CD4+ or CD8+ single positive T cells. During differentiation of T cell progenitors, the potential for non-T cell lineages is sequentially lost from DN1 (CD44+, CD25-) to DN2 (CD44+, CD25+) stage, which facilitates the commitment toward T-cell lineage in DN2 and DN3 (CD44-, CD25+) stage. Here, we assess the role of histone deacetylases (HDAC) in the T cell differentiation process, using an in vitro model of T cell differentiation that relies on culture with OP9 stromal cells expressing ligands for the Notch receptor. Treatment of early T cell progenitors with HDAC inhibitors (HDACi) shows CD11b-positive population, suggesting a role of HDAC to maintain differentiation in T cell lineage. Moreover, T cell progenitors treated with HDACi show limited transition from DN2 to DN3. DN2-to-DN3 transition, marked by losing stem cell marker CD44, is a critical commitment gate that depends upon the appropriate recombination and expression of the T Cell Receptor (TCR). Here we show the TCR surface phenotype is dysregulated in HDACi-treated DN cells, indicating HDAC mediates a TCR-dependent commitment. More than commitment, the T-lineage committed DN3b cells (CD44-, CD25+, CD28+) reduce progression into CD4+CD8+ double positive and CD4+, CD8+ single positive cells upon HDAC inhibition. These data together highlight the importance of HDAC in regulating T-lineage commitment and maturation.

W-4018

HIGHLY-BRANCHED N-GLYCANS GENERATED BY MGAT5 CONTROL NEURAL STEM CELL DIFFERENTIATION AND CELL SURFACE PROTEIN EXPRESSION

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Neural stem cells have the potential to treat many neurological diseases and injuries due to their ability to secrete beneficial factors and form the major cell types of the central nervous system. A better understanding of the mechanisms controlling their differentiation will improve their use as therapeutics. We found previously that N-glycosylation patterns correlate with fate potential of NSPCs; specifically, populations of NSPCs expressing more highly branched N-glycans generate more astrocytes and fewer neurons. Glycosylation can modulate the expression and function of almost all proteins on the cell surface, thus impacting cellular interactions with extracellular cues. Despite its importance, glycosylation's role in NSPC fate had not been described. We found increasing expression of highly-branched N-glycans on NSPCs by N-acetylglucosamine (GlcNAc) treatment enhanced astrocyte formation at the expense of neurogenesis. This effect was specifically due to GlcNAc incorporation in the N-glycan branching pathway since blocking an early enzyme in the pathway with kifunensine abrogated the GlcNAc effect on NSPC differentiation. We hypothesized that NSPC fate might be regulated by downstream enzymes in the branching pathway rather than the presence or absence of all N-glycan branching. We therefore measured levels of N-glycan branching enzymes in astrocyte-biased NSPCs and found higher expression of MGAT5, a late-stage branching enzyme necessary for the formation of tri- or tetra-branched N-glycans. Compared to control cells, NSPCs derived from cerebral cortices of MGAT5 deficient mice generated more neurons and fewer astrocytes upon differentiation. We analyzed neurogenesis in vivo and found elevated expression of neuronal markers NeuN and TuJ1 in the cerebral cortices of MGAT5 deficient mice. Thus, the loss of MGAT5 and lack of highly branched N-glycans impaired astrocyte generation and favored neurogenesis. Mass spectrometry analysis of plasma membrane proteins from control and GlcNAc-treated NSPCs identified changes in the levels of several cell-cell and cell-ECM adhesion proteins. Taken together, these data show MGAT5-mediated N-glycan branching significantly impacts NSPC differentiation, potentially by modifying cell surface proteins that respond to extracellular cues.

W-4020

CONSTRUCTION A NT-3 DELIVERY TISSUE ENGINEERING NEURAL NETWORK FOR THE TREATMENT OF COMPLETELY TRANSECTED SPINAL CORD OF RAT

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Yuan-shan Zeng - *Sun Yat-sen University, Guangzhou, China*

One of the greatest challenges in recovery of spinal cord injury is that there are no enough neurotrophic factors and neurons to support nerve regeneration and target contact of neuronal axons. Our previous study successfully fabricated a novel neurotrophin-3 (NT-3)/fibroin (NF) delivery gelatin sponge (GS) scaffold (NF-GS). In the present study, we used the NF-GS scaffold to construct an NT-3 delivery neural network for the treatment of rat spinal cord transected completely. Firstly, six groups of different scaffolds were designed in vitro. TrkC gene-modified neural stem cells (NSCs) or NSCs were respectively seeded into the scaffolds. The differentiation and synaptogenesis of the neural network were tested by morphology and electrophysiology. Then, the ideal neural network was implanted into the injury site of spinal cord to directly investigate the therapeutical effects in vivo. In vitro, TrkC overexpressing NSCs (TrkC-NSCs) in the NF-GS scaffold group could differentiate into more NSC-derived neurons, form myelin sheath and generate a neural network. The synaptophysin and PSD95 were highly expressed in the NSC-derived neurons, which indicated excellent potential of synapse formation in the same group. In vivo, it was showed that the neural network transplantation improved the motor function of paralysis limbs, and increased amplitude and shortened latency of cortical motor evoked potential. The cells remained in the network maintained the characteristic of NSC-derived neurons or NSC-derived astrocytes and contacted with each other by synapse-like structure in the injury/graft site of spinal cord. The results suggest that the NT-3 delivery neural network graft may maintain its structure in the injured spinal cord and integrate into the host neural network for repairing neural conduction of spinal cord transected completely.

W-4022

USING HUMAN IPSC COLLECTIONS TO GENERATE FUNCTIONAL LIVER LIBRARIES

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Human induced pluripotent stem cells (hiPSC) provide a promising opportunity to establish human-specific models of various diseases for drug development. Population-scale iPSC reprogramming initiatives will allow for the study of any imaginable population cohort for disease phenotypes, drug safety, and efficacy. However, a one to one comparison of each individual stem cell line is inefficient and impractical due to both time and cost considerations. Therefore, we developed a method to pool together and culture iPSC from a diverse group of unrelated individuals. These pooled stem cells are maintained in a poly-clonal manner and are fully capable of differentiating into endoderm, ectoderm and mesoderm. In addition, the pooled iPSC collection is capable of producing human hepatocyte-like cells and throughout the differentiation process the population is maintained. Furthermore, we demonstrate that the iPSC collection can generate liver organoids, and that single cells can successfully make clonal organoids. These capabilities enable forward cellomics experiments that allow pooled cells from unrelated individuals to

differentiate and generate varying phenotypes at a much higher throughput than standard workflows. We are also developing a method to detect the donor ratio in the collection from cell free environmental DNA (eDNA) in the culture supernatant. Remarkably, the supernatant harbors a reasonable amount of eDNA, and we found that the fractional distribution of donor-specific eDNA was proportional to actual donor ratios. We developed a dropout-based screening assay to identify liver toxicity sensitive clones by combining our eDNA based donor detection method with an iPSC hepatocyte library. We performed a dropout screening on the pooled iPSC population with fatty acid and troglitazone treatments, and the results indicated that the hepatocyte iPSC populations derived from donors having the high-risk Wolman disease were decreased, and in contrast, the population from the low risk control group was increased. Thus, this stem cell pooling approach provides a novel method to evaluate a population phenotype in a pooled culture.These higher throughput experiments make progress towards conducting clinical trials in a dish and will help promote drug development as well as precision medicine.

W-4024

IMPROVEMENT OF LOW-COST AND HIGH EFFICIENT METHOD FOR PANCREATIC BETA CELL DIFFERENTIATION IN HUMAN IPS CELLS

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For the medical treatment of type I diabetes, transplantation of pancreatic islets is an effective strategy. A number of methods for beta-cell induction have been reported so far. We have also established beta-cell induction method, but there are some problems including differentiation efficiency and immaturity. Moreover, large amounts of βcells are necessary for transplantation, thus it is thought to cost too high for an actual treatment. For these reasons, it is indispensable to establish a method that can efficiently differentiate at the low cost. In this study, we examined how both medium and additives affected efficiency of pancreatic cell induction. When the medium was altered, induction efficiency as well as the ratio of the types of differentiated cell was changed. Especially, low-cost medium enhanced the expression of maturation marker genes. Furthermore, differentiation efficiency of beta cells was increased by combinational usage of several chemical reagents. We think that our results will provide some information for the improvement of beta cell differentiation.

Funding Source: Research Center Network for Realization of Regenerative Medicine, AMED (Japan)

W-4026

ROAD TO CLINIC: FIRST CGMP STEMCELL FACILITY IN INDONESIA FOR MANUFACTURING XENO-FREE WHARTON'S JELLY-DERIVED MESENCHYMAL STEMCELLS (WJ-MSCS)

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Wharton's jelly-derived stem cells (WJ-MSCs) are predominantly good source of cells because it is maintained in an early embryologic phase, therefore have retained some of the primitive stemness properties. Recent studies describe a hypoimmunogenic phenotype, multipotent differentiation potential, and trophic support function for WJ-MSCs, with variable clinical benefit in degenerative disease models such as stroke, myocardial infarction, Parkinson's disease and etc. Similar to others countries, manufacturing of cell therapy products for clinical application in the Indonesia Community must be conducted in compliance with Good Manufacturing Practice (cGMP) and requires a manufacturing license. Safety product are remains one of the main concerns and refers to donor validation, choice of starting material, processes, and the controls used, not only at the batch release level but also during the development of processes. Here we report for the first time the first manufacturing of clinical-grade WJ-MSC developed in GMP-compliant culture system in Indonesia. We established in house xeno-free expansion protocols using composition of several growth factor including PDGF, IGF, EGF and etc as medium supplement and confirmed its safety and feasibility for manufacturing. WJ-MSCs was isolated from umbilical cord and expanded to produce Master Cell Banks (MCB), Working Cell Bank (WCB) and Finished Product (FP) with average population doubling time (PDT) 1. 46 days. The expanded cells were complied to ISCT standard criteria for MSC such as more than 95% positively expressed for CD 73, CD 90, CD 105, while less than 2% of expressing marker for CD 14, CD 19, CD 34, CD 45, and HLA-DR. Sterility tested MSC; proved by less than 0. 25 EU in endotoxin test and negative in mycoplasma, can also be differentiated into osteoblast, chondrocytes, and adipocytes. All of aspects including culturing, homogeneous product, cryopreservation, and characterization were strictly monitored and documented for quality practices and to ensure safety and product quality.

Funding Source: PT Kalbe Farma TbK

W-4028

REPROGRAMMING P53-DEFICIENT GERMLINE STEM CELLS INTO PLURIPOTENT STATE

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Cultured mouse spermatogonial stem cells (GSCs), revert back to pluripotent state either spontaneously or upon being modified genetically. However, the reprogramming efficiencies are low, and the underlying mechanism remains poorly understood. In the present study, we conducted transcriptomic analysis and found that many transcription factors and epigenetic modifiers were differentially expressed between GSCs and ESCs. We failed in reprogramming GSCs to pluripotent state using the Yamanaka 4 Factors but succeeded when Nanog and Tet1 were included. More importantly, reprogramming was also achieved with Nanog alone in a p53-deficient GSC line with an efficiency of 0.02‰. These GSC-derived-iPSCs possessed in vitro and in vivo differentiation abilities despite of low rate of chimera formation, which might be caused by abnormal methylation in certain paternally imprinted genes. Together, these results show that GSCs can be reprogramed to pluripotent state via multiple avenues and contribute to our understanding of the mechanisms of GSC reprogramming.

W-4030

IMMUNOMODULATORY AND ANTI-INFLAMMATORY CAPABILITIES OF CANINE MESENCHYMAL STEM CELLS AND THEIR CLINICAL APPLICATION

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With a view towards harnessing the therapeutic potential of canine mesenchymal stem cells (MSCs) as modulators of inflammation and the immune response, and to avoid the issues of the variable quality and quantity of harvested MSCs, we have examined the immunomodulatory and anti-inflammatory properties of MSCs produced from canine induced pluripotent stem cells (ciPSC.MSCs), and compared them to MSCs harvested from adipose tissue (cAT.MSC) and bone marrow (cBM.MSC). When stimulated with the pro-inflammatory cytokines, canine tumor necrosis factor-α (cTNF-α), canine interferon-γ (cIFN-γ) or a combination of both (cTNF-α/IFN-γ), ciPSC.MSCs expressed inducible nitric oxide synthase (iNOS), indoleamine 2, 3 dioxygenase (IDO), galectin-9 (GAL-9), interleukin-8 (IL-8), interleukin-1β (IL-1β) and prostaglandin receptor-2α (PTGER-2α) at higher levels, and transforming growth factor-β1 (TGF-β1) and cyclooxygenase-2 (COX-2) at comparable levels, compared to cAT.MSCs and cBM.MSCs. Expression of IDO, an anti-inflammatory cytokine, was significantly upregulated in both ciPSC.MSCs and cAT.MSCs when co-cultured with mitogen-stimulated canine leukocytes. Stimulated canine lymphocytes downregulated their expression of GAL-9, vascular endothelial growth factor (VEGF), PTGER2α and TGFβ1 when cultured with either ciPSC.MSCs or cAT.MSCs. In conclusion, ciPSC.MSCs may be a candidate for clinical application in canine immune-mediated and inflammatory disorders.

W-4032

EFFECT OF BMP4 TREATMENT DURING EMBRYOID BODY FORMATION FOR FACILITATING DIFFERENTIATION OF DIFFERENT HUMAN IPS CELL LINES INTO A CARDIAC LINAGE

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Accurate pre-clinical evaluation of cardiac toxicity risk such as QT prolongation and arrhythmia is still a major challenge in the pharmaceutical industry. Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) have attracted attention as novel tools for drug safety screening and iPS-CMs derived from different donors can recapitulate individuals' different susceptibilities to a drug-induced QT prolongation response by using multiple iPS cell lines. However, cardiac differentiation efficiency is highly variable depending on the cell line used. In this study, we investigated the effect of BMP4 (0.1, 1, 10, 50, and 100 ng/ml), which was reported as an important factor for differentiation into mesoderm, on differentiation into cardiomyocytes during embryoid body (EB) development. We found that treatment with 10 ng/ml of BMP4 during EB formation promoted differentiation into cardiomyocytes. The other concentrations (0.1, 1, 50, and 100 ng/ml) of BMP4 were less efficient in cardiac differentiation, and BMP4 treatment after EB formation did not contribute to the differentiation into CMs in our condition. At the optimal BMP4 concentration, we observed increases of gene expression levels of T (a mesoderm marker) and GATA6 (an endoderm marker), while decreasing gene expression of PAX6 (a neuroectoderm marker) in EBs. Single cell gene expression analysis revealed the presence of mesendoderm cell population in addition to mesoderm cell population in the EB treated with BMP4. To investigate the feasibility for multiple hiPSC lines, we demonstrated that the effective concentration of BMP4 differed between different hiPSC lines in culture condition for differentiation into CMs and the presence of mesoderm and mesendoderm cell population. Thus, our results suggest that the timing and concentration of BMP4 treatment are important factors for differentiation

into a cardiac linage and BMP4 treatment during EB formation is expected to become a useful step for suppressing variability in cardiac differentiation efficiency in hiPSC lines derived from different donors.

W-4034

ENTEROENDOCRINE REGULATION OF NUTRIENT ABSORPTION

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Enteroendocrine cells (EECs), which are nutrient-sensing cells found throughout the gastrointestinal tract, secrete more than 20 hormones which individually and collectively regulate many physiologic processes in response to nutrient ingestion. While EECs only comprise 1-2% of the intestinal epithelium, mice and humans without EECs suffer from severe malabsorptive diarrhea, demonstrating that EECs are essential regulators of nutrient absorption. Surprisingly, it is not known how EECs control nutrient absorption. As EEC differentiation depends on the bHLH-containing transcription factor Neurogenin 3 (NEUROG3), we used CRISPR/Cas9 to induce a null mutation in NEUROG3 in pluripotent stem cells (PSCs) and directed their differentiation into human intestinal organoids (HIOs) which lack EECs. We observed that loss of NEUROG3 resulted in complete absence of EECs and increased goblet cells in human intestinal epithelium, suggesting that EECs exert local juxtacrine or paracrine effects on the differentiation of intestinal cells. We found no obvious defects in the expression or localization of glucose transporters, peptide transporters or lipid transporters in HIOs lacking EECs. However, in vivo transcellular transport of nutrients from the luminal brush border to the basal lamina propria depends on electrochemical gradients of sodium, potassium, chloride, and other electrolytes. These electrochemical gradients are sensitive to inputs from the enteric nervous system, such as vasoactive intestinal peptide (VIP) as well as to EEC hormones, such as peptide YY (PYY). We hypothesized that the absence of EECs would disrupt normal enterocyte electrochemical gradients and thus impair electrogenic nutrient absorption. In support of this, we detected enhanced epithelial anion secretion in response to VIP in NEUROG3-null intestinal epithelium that could be tempered with addition of exogenous PYY. Moreover, we observed an enhanced electrogenic response to luminal glucose in Neurog3-null mouse intestine that could be partially rescued by exogenous addition of PYY. Taken together, these results support a central role for EECs in controlling nutrient absorption by coupling an epithelial-neurohormonal signal with nutrient and ion transport.

Funding Source: Funded in part by the American Diabetes Association, 1-17-PDF-102.

W-4036

DIRECTED EVOLUTION OF REPROGRAMMING FACTORS AND POOLED LIBRARY SCREENING METHOD IN MAMMALIAN CELLS TO ENHANCE CELLULAR REPROGRAMMING

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Directed evolution is widely used to method to enhance enzymes, fluorescent proteins and antibodies but it has not been applied in transcription factors to reprogram mammalian cells. Here we describe a novel method termed directed evolution of reprogramming factors by cell selection and sequencing (DERBY-seq) to identify artificially enhanced and evolved reprogramming transcription factors. DERBY-seq entails pooled screens with randomised gene libraries, cell selection based on phenotypic marker and genotyping by amplicon sequencing for candidate identification. We used benchmark this approach by using pluripotency reprogramming with libraries based on the reprogramming factor Sox2 and the reprogramming incompetent endodermal gene Sox17. We identified several Sox2 variants outperforming the wild-type protein under three and four factor cocktails conditions. Surprisingly, the most potent variants were discovered from the Sox17 library demonstrating that this factor can be converted into a highly potent inducer of pluripotency with a range of highly diverse modifications. We propose DERBY-seq as a broad-based approach to discover vigorous reprogramming factors for any donor/target cell combination applicable to direct lineage reprogramming in vitro.

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W-4038

GENERATION OF GENETICALLY STABLE HUMAN DIRECT CONVERSION DERIVED NEURAL STEM CELLS USING QUANTITY CONTROL OF PROTO-ONCOGENE OVEREXPRESSION

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As the human lifespan has increased due to developments in medical technology, the number of patients with neurological diseases has rapidly increased. Therefore, studies on effective treatments for neurological diseases are becoming increasingly important. To perform these studies, it is essential to obtain a large number of patient-derived neural cells. The purpose of the present study was to establish a technology that allows the high-efficiency generation of genetically stable direct conversion derived neural stem cells (dcNSCs) through expression of a new combination of reprogramming factors, including a proto-oncogene. Specifically, human c-Myc proto-oncogene and the human Sox2 gene were overexpressed in a precisely controlled manner in human dermal fibroblasts, human umbilical cord blood-derived mesenchymal stem cells, and human Niemann-Pick type C disease-derived dermal fibroblasts. As a result, the direct conversion into multipotent dcNSCs occurred only when the cells were treated with a multiplicity of infection (MOI) of 1 of hc-Myc proto-oncogene and hSox2 retrovirus. When MOIs of 5 or 10 were utilized, distinct results were obtained. In addition, the pluripotency was bypassed during this process. Notably, as the MOI used to treat the cells increased, expression of the p53 tumor suppressor gene, which is typically a reprogramming hurdle, increased proportionately. Interestingly, p53 was genetically stable in dcNSCs generated through direct conversion into a low p53 expression state. In the present study, generation of genetically stable dcNSCs using direct conversion was optimized by precisely controlling the overexpression of a proto-oncogene. This method could be utilized in future studies, such as in vitro drug screening using generated dcNSCs. In addition, this method could be effectively utilized in studies on direct conversion into other types of target cells.

Funding Source: This project has been supported by the Korea National Research Foundation grant (2017K1A3A1A61091969 and 2016R1C1B1010742).

W-4040

EVALUATION OF GENOME ABERRATION IN HUMAN IPS CELLS USING NON-INVASIVE LC-MS/MS ANALYSIS APPROACH

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Pluripotent stem cells (PSCs) have an unlimited self-renewal capacity, and can differentiate into any cell type in the body. They consequently hold great promise as a source of cells for applications in regenerative medicine and drug discovery. For these applications, development of technology for the mass production of high quality pluripotent stem cells is essential. But these cells are susceptible to genome aberration during long time culture or reprogramming process. Karyotyping and genome sequencing are widely used to check genome aberration, but these methodologies are generally applicable at the end of culture because they are invasive to cells. We have aimed to establish a method for evaluation of the cell normality without cell disruption. We focused on culture supernatant and have developed a novel cell culture media analysis platform, C2MAP, that can carry out automated sample pretreatment and simultaneous analysis of up to 95 compounds found in basal media and secreted metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS). In this study, we tested applicability of our non-invasive LC-MS/MS approach for evaluation of genome aberration occurred in human iPS cells. We used the CGT-RCIB10 iPS cell line established by the Cell and Gene Therapy Catapult. As the model of abnormal cells, we used the same cell lines carrying aberration on chromosome 18. Cell culture supernatant was collected every 24 hours and analyzed time course changes of each culture supernatant component by the C2MAP system. No significant differences in proliferation curve between normal and abnormal karyotype did not observed. Moreover, these cells consumed nutrients such as glucose and some amino acids, and secreted lactate at similar rate. On the other hand, we could found significant differences in temporal changes in some metabolites. Our non-invasive LC/MS/MS method has the potential to be an effective means to evaluate the genome aberration.

W-4042

COPINE 7 DETERMINES ODONTOGENIC DIFFERENTIATION AND DENTINAL REGENERATION OF HUMAN BONE MARROW STEM CELLS

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You-Mi Seo - *Hysensbio, Seoul, Korea*
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The importance of preameloblast-derived factor in odontogenic differentiation of dental stem cells has been reported in our initial studies. Through additional experimentation, we determined that a dental epithelium-derived protein, Copine 7 (CPNE7) was the causal factor for regulation of odontoblast differentiation in human dental pulp stem cells. Other experiments in vivo were supported that CPNE7 promoted dentin-like tissue formation. In dentin defect models, we observed the effect of CPNE7 on regeneration of physiologic reactionary dentin. However, it is not clearly discovered if CPNE7 interacts with non-dental stem cells as a signaling molecule causing odontogenesis and dental tissue formation. Accordingly, our latest experiment involved the hypothesis of whether exposing human bone marrow-derived mesenchymal stem cells (hBMSCs) to CPNE7 would result in increased odontogenic differentiation and induced dentin regeneration. To investigate the odontoblastic differentiation capability of hBMSCs, we utilized recombinant CPNE7 protein (rCPNE7) and analyzed by quantitative PCR and western blotting for odontoblast markers, dentin sialophospho protein (DSPP) and dentin matrix protein-1 (DMP-1). The results in vitro showed that hBMSCs exposed to rCPNE7 expressed comparatively higher mRNA and protein levels for DSPP and DMP-1 than the control group. To further investigate these results in vivo, we transplanted hBMSCs with rCPNE7 or rBMP2 in HA/TCP scaffold into immunocompromised mice subcutaneously. Because we had previously observed that bone morphogenetic protein 2 (BMP2), a well-known osteogenic differentiation factor, has no effect on hBMSCs to generate dentin structure in vivo, hBMSCs treated with rBMP2 was expected to have different results as opposed to hBMSCs with rCPNE7. After 12 week transplantation, we performed histological analysis of tissue formation by H&E staining. We found out hBMSCs exposed to rCPNE7 induced dentin-like tissue formation whereas hBMSCs exposed to rBMP2 presented bone-like tissue. This result demonstrates that CPNE7 has a direct impact on dentin regeneration. From these experiments we have concluded that CPNE7 is the key variable resulting in induced odontoblast differentiation and dentin formation in non-dental stem cell such as hBMSCs.

Funding Source: This work was supported by the Technology Innovation Program (10078369, "Development of original technology for the treatment of tooth hypersensitivity using dentin regenerating functional peptides") funded by MOTIE, Korea.

W-4044

SAFETY OF METABOLIC LABELING USING AC4MANNAZ FOR TRACKING OF ENDOTHELIAL PROGENITOR CELLS

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Metabolic labeling is one of the most powerful methods to label the live cell for in vitro and in vivo tracking. However, the cellular mechanisms by modified glycosylation due to metabolic agents are not fully understood. Therefore, metabolic labeling has not yet been widely used in EPC tracking and labeling. In this study, cell functional properties such as proliferation, migration and permeability and gene expression patterns of metabolic labeling agent-treated hUCB-EPCs were analyzed to demonstrate cellular effects of metabolic labeling agents. As the results, Ac4ManNAz treatment (> 20 μM) led to the inhibition of functional properties, and down-regulation of genes related to cell adhesion, PI3K/AKT, FGF, and EGFR signaling pathways. Interestingly, the new blood vessel formation and angiogenic potential of hUCB-EPCs were not affected by Ac4ManNAz concentration. Based on our results, we suggest 10 μM as the optimal concentration of Ac4ManNAz for in vivo hUCB-EPC labeling and tracking. Additionally, we expect that our approach can be used for understanding the efficacy and safety of stem cell-based therapy in vivo.

Funding Source: This study was supported by the INNOPOLIS Foundation grant (2016-02-DD-016) funded by the Korean government.

W-4046

AN ARRAY OF PLANT-DERIVED HYDROGELS SUITABLE FOR STEM CELL CULTURE AND 3D BIOFABRICATION

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Molly Patton - *St. Vincent's Hospital Melbourne, Fitzroy, Victoria, Australia*
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The future of bioprinting in the context of regenerative medicine is dependent upon the use of clinically-appropriate, sustainable materials. Most hydrogels are comprised of either naturally-sourced or synthetically-manufactured analogs of materials found in animal or human tissues. This study proffers an array of plant-derived hydrogels for use within the context of tissue engineering, specifically their suitability for 3D printing. A comparative study of the physical characteristics of each hydrogel and behaviour in physiological conditions will be presented. Their suitability for the culture of stem cells (both animal and human) will be discussed, as will guidance for use in 3D printing.

W-4048

PROLIFERATION TENDENCY OF NEURAL CELLS WITH ONE-STEP GRADIENT SCAFFOLD FABRICATION

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Mira Cho - *Yonsei University, Seoul, Korea*
Jae-Hyung Jang - *Yonsei University, Seoul, Korea*

This work focuses on the fabrication of 3D scaffolds composed of multi-layered fibrous sheets having features of microchannels. Previous studies have reported the spontaneous formation of multi-layered scaffolds by the single-jet electrospinning of a polymeric solution. The simple addition of additives, vanadium(III) chloride with polycaprolactone (PCL) solution, forms multi-layered structures. Vanadium-doped PCL fibers can have a different morphology for each sheet as the pore size changes. Every layer of the sheets allows the diffusion of drugs and other soluble factors to target tissues or organs. Under dynamic conditions, medium flow through the channels enhances nutrient availability to the cells on the multiple layers, increasing cell proliferation on all layers. We applied one-step gradient scaffold fabrication to neural cells, including dorsal root ganglion (DRG) and human neural stem cells, and evaluated its potential as a guidance template for neural tissue generation.

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W-4050

TRANSDIFFERENTIATION USING ADIPOSE DERIVED MESENCHYMAL STEM CELLS

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Transdifferentiation process is the transformation of one cell type to another, also called metaplasia. The pancreas is a multifaceted organ, and there are several illnesses that demand its substitution. Developing methods in bioengineering and regenerative medicine, generate new procedures to secure pancreas generation. We used 80 renal tissue samples using GFR Matrix + AHEGF + ADSC to complete the transdifferentiation into pancreatic tissue. The therapeutic and regenerative capacities of adipose derived stem cells (ADSC) and growth factors were evaluated histologically (n=40). We established two study groups; the first treated with GFR Matrix + AHEGF + ADSC (n=40), and the control group (n=40). Based on the circumstances, we would apply one-way repeated-measures ANOVAs. Results. 2 weeks after the GFR Matrix + AHEGF + ADSC, we observed the presence of various islets of Langerhans in the samples (n=38) in the first group. While in control group (n=40), 100% of samples had not transdifferentiation process (p=0,001). These transdifferentiation mechanisms could have potential application for several diseases, i.e. cancer and diabetes.

THURSDAY, 21 JUNE, 2018

POSTER SESSION II-ODD
18:00 – 19:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

T-1001

FUNCTIONAL ANALYSIS OF TET2 IN TROPHOBLAST STEM CELLS

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Previous works have indicated critical roles of TET2 both in reprogramming and in pluripotent stem cells. However, less is known about its role in trophoblast stem cells (TSCs) and during trophoblast development. Here, we show that TET2 is upregulated during both human and mouse placenta development. Interestingly, preeclampsia shows abnormal TET2 and 5hmC levels as well as anomalous structures in human placenta. By using CRISPR/Cas9 system, we generated catalytic domain specific deficient Tet2 knockout mice. Further investigation indicated that Tet2 knockout pups showed certain variations in placenta size and structure. Tet2 knockout TSCs were then successfully derived from Tet2 deficient E3.5 blastocysts with typical morphology. Although these Tet2-/- TSCs retained a relative normal

expression level of key markers as wide-type TSCs did, certain differentiation related genes as well as imprinted genes were mis-regulated. Besides, deficient in Tet2 dramatically reduced the genomic 5hmC content of TSCs. Moreover, both in vivo and in vitro differentiation ability of TSCs were greatly impaired. Importantly, mRNA-seq and (h)MeDIP-seq analysis further indicated the essential role of Tet2 in both transcription and 5mC/5hmC regulation in TSCs.

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T-1003

EVALUATION OF DEGRADABLE NANOFIBROUS MESHES WITH HUMAN ENDOMETRIAL MESENCHYMAL STEM CELLS FOR PELVIC ORGAN PROLAPSE REPAIR IN AN IMMUNOCOMPROMISED MOUSE

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Pelvic organ prolapse (POP) is a debilitating gynaecological condition caused by the herniation of pelvic organs into the vaginal wall. POP is mainly caused by vaginal childbirth injury and affects 1 in 2 parous women aged 50+ years. Until recently, polypropylene meshes were often used as a surgical treatment option. However, they have been associated with serious complications such as inflammation, pain and erosion. As a result, transvaginal meshes were banned in Australia and New Zealand in 2017. At present, there is no optimal therapy for treatment of chronic POP leaving millions of women in despair. In order to provide a biomimetic character into the mesh materials, we fabricated nanostructured biomaterials by electrospinning poly (L-lactic acid)-co-poly (ε-caprolactone) (PLACL) polymer inspired by atomic force microscopic analysis of the native vaginal tissue. Addition of gelatin (G) significantly affected hydrophilicity, pore size and fiber size without any changes in biomechanical properties. Previously, our team discovered SUSD2+ Mesenchymal stem cells in the endometrium (eMSCs) and showed they can even be collected from post-menopausal women in a phase IV clinical trial. Herein, we show significant differences in cell-cell and cell-material communication of SUSD2+ eMSCs on PLACL+G in vitro by SEM micrograph and expression of F-actin and vinculin. Our in vivo analysis of the foreign body response highlights that eMSCs play a significant role in controlling nanofiber degradation, cellular infiltration into the mesh and tissue integration over 6 weeks. Furthermore, eMSC/PLACL+G promoted significant macrophage polarization characterized by changes in F4/80 (macrophage marker), CCR7 (M1) and CD206 (M2) macrophages improving biocompatibility compared with PLACL alone at 1 and 6 weeks. This study shows that degradable nanofiber meshes have a significant potential as alternative surgical constructs whose overall integration and performance may be improved by combining with eMSCs for an effective long-term treatment of chronic POP.

Funding Source: Funding Acknowledgement: NHMRC Australia, Science and Industry Endowment Fund, Rebecca L Cooper Foundation and Monash University

T-1005

EFFECTIVE CRYOPRESERVATION OF WHOLE UMBILICAL CORD TISSUE - THE INDUSTRIALISATION SOLUTION

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Umbilical cord stroma has been widely accepted as an easily available, non-ethical concerned, and promising source of multipotent stem cells for regenerative medicine. The low cost effective cryogenic storage of umbilical cord tissue for banking will have a broad commercialising potential for autologous transplantation or scientific purposes. The umbilical cord tissue was sliced into unified 1mm thick fragments, then incubated in a cocktail cryopreservation solution, followed a programmed loading and cooling procedure and stored in liquid nitrogen for up to 6 months. The cryopreserved cord tissues were thawed by a three-step protocol to remove the Cryopreservation agents. Viability test showed 60% of enzymatically isolated cells are alive. Confocal flourescent microscpe image disclosed the spreading of live and dead cells within the cryopreserved cord tissue. The earliest outgrowth cells were observed from day 5 after plating and incubating in 37°C incubator. These cells displayed the classic spindle shape and similar proliferation rate as the cells derived from fresh tissue, expressed typical MSC surface markers, including: CD73, CD90 and CD105 but negative for endothelial marker CD31. They showed satisfied adipogenic and osteogenic differentiation ability. The whole process was repeated in a GMP lab at clinical grade, to test the feasibility of carrying out the procedure at clinical product manufacture level. This study proved that reserving the viability of cells in cryopreserve whole umbilical cord tissue could be effectively achieved by

using a proper penetrating CPA in well osmotically balanced solution. The outcome gives the stem cell storage companies an alternative more cost-effective way in commercial service.

Funding Source: China Regenerative Medicine International Limited

T-1007

GATA4 REGULATES THE SECRETORY PHENOTYPE IN THE DEFECTIVE LAMIN A-MEDIATED HUMAN MESENCHYMAL STEM CELL AGING THROUGH MCP-1

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Defects in the nuclear lamina occur during physiological aging and premature aging disorders. Aging is also accompanied with an increase in the transcription of genes encoding cytokines and chemokines, a phenomenon known as the senescence-associated secretory phenotype (SASP). Progerin and prelamin A trigger premature senescence and loss of function of human mesenchymal stem cells (hMSCs), but little is known about how defects in nuclear lamin A regulate SASP. Here we showed that both progerin overexpression and ZMPSTE24 depletion induce paracrine senescence, especially through the expression of monocyte chemoattractant protein-1 (MCP-1) in hMSCs. Importantly, we identified that GATA4 is a mediator regulating MCP-1 expression in response to prelamin A or progerin in hMSCs. Co-immunoprecipitation revealed that GATA4 expression is maintained due to impaired p62-mediated degradation in progerin-expressing hMSCs. Furthermore, depletion of GATA4 abrogated SASP-dependent senescence through the suppression of NF-κB and MCP-1 in hMSCs with progerin or prelamin A. Thus, our findings indicate that abnormal lamin A proteins trigger paracrine senescence through a GATA4-dependent pathway in hMSCs. This molecular link between defective lamin A and GATA4 can provide insights into physiological aging and pathological aging disorders.

T-1009

DIFFERENT CHARACTERISTICS OF MSC ISOLATED FROM DIFFERENTIATED LAYERS OF CHORIONIC MEMBRANE & TROPHOBLASTIC LAYERS OF TERM PLACENTA

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Human placenta-derived mesenchymal stem cells (PD-MSCs) are very attractive candidates for clinical application in regenerative medicine due to readily availability, non-invasive acquisition, and avoidance of ethical issues. Mesenchymal stem cells isolated from all layers of full term placenta and these MSCs may differentially express some functions, and hence have different therapeutic potentials. The Aim of this study is to isolate MSCs from different 4 parts (CM, tCT, bCT, uCT) of CMT layers and whole CMT layers and compare their characteristics. Chorionic membrane and chorionic trophoblast (CMT) layers divided into chorionic tissue (chrionic membrane; CM), trophoblast entire floor (total chorionic trophoblast layer; tCT), trophoblast basal (basal portion of chorionic trophoblast layer; bCT), and trophoblast upper part (upper portion of chorionic trophoblast layer; uCT). Cells isolated from 5 layer were subjected to analyses for their morphology, proliferation ability, surface markers, and multi-lineage differentiation potential. MSCs were isolated from all placental layers and their characteristics were compared. Morphology, proliferation ability, surface markers, and differentiation characteristics of cells from 5 different layers indicated that they exhibited properties of MSCs. MSCs from different placental layers had different proliferation rates and differentiation potentials. MSCs from bCT MSCs had better population doubling time and multi-lineage differentiation potentials compared to those from other layers. Our results demonstrate that it is necessary to appropriately select MSCs from different placental layers for successful and consistent outcomes in clinical applications. Our findings suggest that the bCT MCSs is a good source for clinical applications.

T-1011

ETP8, A NOVEL COMPOUND, ENHANCES PROLIFERATION AND MIGRATION OF HUMAN MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are a useful source for cell therapy in numerous degenerative diseases. In the past, studies on MSCs therapies have focused on cell replacement through transdifferentiation. However,

recent studies have shown that damaged cells can be treated by the paracrine action of MSCs. MSCs secrete various cytokines which promote anti-inflammation, anti-oxidant activities, neuroprotection, and neurogenesis in the brain. In spite of these effects, limitations are present due to the poor survival rate of MSCs and their migration towards injury sites in-vivo. In this study, we screened a library of FDA-approved compounds by using the high-throughput screening system. We identified ETP8, a potential therapeutic compound which accelerated the proliferation of human MSCs. After ETP8 priming, MSCs exhibited a significant increase in proliferation and expression of cell cycle-related genes. The expressions of phospho-Akt and phospho-ERK were also enhanced after ETP8 treatment. Furthermore, ETP8-primed MSCs showed enhanced migration abilities and expressions of migration related chemokine receptors and their ligands, such as CXC chemokine receptor4, CXC chemokine receptor7 and C-X-C motif chemokine 12. ETP8-primed MSCs also promoted the secretion of paracrine factors such as neurotrophic factors and anti-oxidants. These results suggest that enhancing MSC function by ETP8 treatment represents a promising strategy to maximize the effectiveness of MSC-based therapies.

ADIPOSE AND CONNECTIVE TISSUE

T-1013

ISOLATION AND DIFFERENTIATION OF HUMAN ADIPOSE- DERIVED STEM CELLS CULTURED ON BIOMATERIALS IMMOBILIZED WITH ECM AND ECM-DERIVED OLIGOPEPTIDE

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Human adipose-derived stem cells (hADSCs) exhibit heterogeneous characteristics, indi-cating various genotypes and differentiation abilities. The isolated hADSCs can possess dif-ferent purity levels and divergent properties depending on the purification methods used. Microenvironment plays an important role on gene expression and differentiation of hADSCs. Previously, we developed purification of hADSCs from adipose (fat) tissue by (a) the cul-ture method on specific culture materials and (b) the membrane migration method through Nylon mesh filter.1 In this study, we developed a new hADSC line from adipose tissue by culturing on the optimal cell culture condition, which can purify hADSCs having high plu-ripotency and high differentiation ability into chondrocytes, osteoblasts and adipocytes. We isolated hADSCs from adipose tissue by the culture method where different substrates were used; (a) tissue culture polystyrene (TCPS) dishes and TCPS dishes coated with Matrigel (b), collagen type I (c), Synthemax II (oligo-vitronectin based substrate) (d), human recombi-nant-vitronectin (e), or human fibronectin (f) where Matrigel and collagen type I are xeno-contained materials and another extracellular matrices (ECMs) are xeno-free materials. Among these substrates, hADSCs cultured on Matrigel-coated dishes presented the high-est purity of hADSCs, the highest pluripotent gene expression, and the highest differentiation abilities. Despite hADSCs cultured on TCPS dishes coated with xeno-free ECMs showed lower pluripotency and differentiation ability compared to those on Matrigel-coated TCPS dishes. However, TCPS dishes coated with human recombinant-vitronectin and Syn-themax II were found to be the best dishes for hADSCs isolation and culture among the TCPS dishes coated with xeno-free ECMs. It should be necessary to develop optimal xeno-free culture dishes and conditions of hADSCs for clinical application of hADSCs in future. Furthermore, we are now developing a new culture medium by replacing the fetal bovine serum (FBS) with human platelet lysate (hPL) as the supplement to improve the purity of hADSCs and build up more complete xeno-free culture conditions of hADSCs for clinical applications.

T-1015

IN VITRO CULTURE OPTIMIZATION OF ISOLATED ADIPOSE-DERIVED STEM CELLS FROM BREAST CANCER PATIENTS

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Fat implant has been long introduced to oncoplastic breast surgery and adipose-derived stem cells (ADSCs) play important role in the final outcomes. The desirable outcomes may rely on the quantity of ADSCs in which different amounts were obtained from each individual. To achieve sufficient number, cell-assisted lipotransfer including stromal vascular fraction (SVF) cells and in vitro cultured ADSCs was introduced to increase the amount of transferred ADSCs. There are several developing methods available to improve the yield by optimizing tissue digestion processes, however, there is no study concerning the amount of cells used at the initiation of cell culture. In this study, an optimized isolation and cell culture protocol based on cell seeding density for expansion of ADSCs was determined. Lipoaspirate from 6 breast cancer patients aged 31-57 years were isolated to obtain SVF cells which were then incubated overnight to acquire processed-lipoaspirate (PLA) cells. After that, PLA cells were cultured with different amount of cells per culture area (i.e., 1,000, 2,000, 5,000, 10,000, 20,000, 50,000 cells/cm2) for ADSC expansion until completing at passage 2. Phenotypic characters, fold expansion, cell viability and growth kinetic were observed. A flow cytometric analysis of SVF cells showed heterogeneous cell population in which ADSCs were determined as

CD45-CD31-CD34+. Results show that our isolation protocol provided average ADSCs quantity of 14,000 cells per milliliter lipoaspirate. The culture with the lowest cell seeding density (1,000 cells/cm2) exhibited the highest growth kinetic and fold expansion followed by 2,000, 5,000, 10,000, 20,000, and 50,000 cells/cm2 (10-fold, 6-fold, 3-fold, 2-fold, 1-fold and 0.5-fold in average, respectively). Moreover, the lower amount of cells used in the culture, the longer culture period used for cell growth to reach 80% confluency. At the end of passage 2, ADSCs expanded in the culture with 1,000 cells/cm2 required 51 days as compared to 13 days when expanding at 50,000 cells/cm2. However, the 39-day culture with 2,000 cells/cm2 providing a good yield of 6-fold and 93% viability is thus considering as the most suitable condition for ADSC expansion. It is then suggested that our optimized protocol can provide sufficient quantity for breast reconstruction procedure.

Funding Source: This project is supported by Siriraj Research Fund, Faculty of Medicine Siriraj Hospital, Mahidol University, Grant number: R016033006.

T-1017

ROLE OF TALE TRANSCRIPTION FACTORS IN CHROMATIN REMODELING DURING ADIPOGENIC DIFFERENTIATION

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During the process of cell differentiation an extensive chromatin remodeling occurs. It was previously shown in detail for adipogenic differentiation. Binding of many transcription factors to chromatin well controlled in time and space is required. We have shown that the homeodomain-containing transcription factor Prep1 is a repressor of adipogenic differentiation. Its down-regulation in both ex vivo bone marrow-derived mesenchymal stromal cells (MSC) and in vitro 3T3-L1 pre-adipocytes significantly increases adipogenic differentiation potential. In order to discover a mechanism for this phenomenon we explored binding of TALE transcription factors (Prep, Pbx and Meis) to chromatin during adipogenic differentiation. We performed ChIP-seq and ATAC-seq analysis before and after the differentiation induction and found that Prep1 is pre-bound to chromatin in "closed" conformation before differentiation. The differentiation induction stimulated chromatin remodeling and binding of Meis1 to many positions where Prep1 was pre-bound. The down-regulation of Prep1 expression using siRNA resulted in chromatin opening even before the differentiation induction. Thus, we concluded that precise cross-talk between TALE transcription factors is required for the proper adipogenic differentiation to proceed.

Funding Source: Russian Foundation for Basic Research Grant #18-015-00465.

MUSCULOSKELETAL TISSUE

T-1019

SINGLE-CELL RNA SEQUENCING ANALYSIS OF FRESHLY ISOLATED HUMAN BONE MARROW STROMAL CELLS/SKELETAL STEM CELLS

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Human bone marrow stromal cells (BMSCs) include a subset of multipotent skeletal stem cells (SSC), and are considered to be central mediators of skeletal homeostasis and support of hematopoiesis. In order to better understand BMSC/SSC biology, analysis of freshly isolated uncultured cells is needed. However, the use of single-cell analysis techniques is seriously hampered by red blood cell (RBC) contamination of marrow cell suspensions. In an attempt to solve this problem, we first compared four standard methods of RBC depletion: ACK lysis, elutriation, a commercial RBC depletion reagent, and density gradient separation. Using all approaches, 30-80% of the colony forming units-fibroblasts (CFU-Fs, 1:5 of which are SSCs), were lost. Subsequently, RBC depletion was followed by magnetic separation for depletion of $CD45^+/CD31^+$ cells, followed by FACS to isolate $CD146^+/CD271^{\pm}$ cells. Few CFU-Fs were found among the $CD146^+/CD271^-$ cells, but were abundant in both $CD146^-/CD271^+$ and $CD146^+/CD271^+$ fractions. In the latter two, CFU-Fs were enriched 140-170- and 220-720-fold, respectively, compared to unpurified cells. Single-cell RNA sequencing analysis (10X Genomics Chromium) revealed that both of these fractions had two distinct osteogenic populations: one subpopulation with over-representation of mature osteogenic markers (*RUNX2, SPP1, IBSP, BGLAP, COL1A1, IFITM5*), and another with over-representation of pericyte/BMSC markers (*CXCL12, LEPR, FRZB, VCAN, IGFBP4, TAGLN, FRZB*). Interestingly, the $CD146^-/CD271^+$ fraction also included transcripts associated with primitive cells (*KLF2, JUN, JUNB, SOCS3, FOSB*). All genes indicated are in the top 20 for each group (P-Value-6). Seurat, an R toolkit for single cell genomics was used to further confirm the transcriptional profile described above. Further single-cell analysis will provide a better understanding of the hierarchy, biological nature and function of BMSCs/SSCs, cells of importance in the bone/marrow microenvironment.

Funding Source: This research was supported by the DIR, (funding to the SBS and the CTRC) of the NIDCR, a part of the IRP, NIH, HHS.

T-1021

HMGB1 ACCELERATES REGENERATION OF MULTIPLE TISSUES BY TRANSITIONING STEM CELLS TO G(ALERT)

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Whilst stem cell therapy has been successful for some haematological disorders, considerable challenges remain for enhancing the repair of solid organs. Targeting endogenous cells overcomes many of the hurdles associated with exogenous stem cell therapy. For over 40 years it has been observed that prior injury accelerates subsequent wound healing. Recently it was described that this was due to stem cells transitioning within the quiescence cycle from G(0) to G(Alert); this primes them for cell cycle entry, thereby promoting tissue repair. However, the identity of the crucial transitioning factor(s) remain to be clarified. Alarmins are endogenous molecules released upon tissue damage and are major triggers of the immune response. We hypothesised they may have a role in tissue repair, and found the alarmin HMGB1 accelerated tissue regeneration in certain tissues by transitioning particular stem cell types to G(Alert). Alarmins were elevated post-fracture in both human and murine blood samples. In-vitro screening of candidate alarmins with human bone marrow-derived mesenchymal stromal cells (MSCs) showed that in response to osteogenic media, only pre-treatment with the native fully reduced form of HMGB1 improved osteogenesis. Next, using in vivo microCT and biomechanical analysis, we found that addition of HMGB1 accelerated fracture healing in wild type C57BL/6J mice via the CXCL12-CXCR4 axis. We confirmed our findings using small molecule inhibitors and conditional HMGB1-/- mice. Analysis of cell cycle kinetics, cell size, ATP levels, mitochondrial DNA, and mTORC1 dependency revealed this was due to HMGB1 transitioning the murine skeletal stem cell to G(Alert). This effect also extended to murine muscle and haematopoietic stem cells, as well as human haematopoietic stem and progenitor cells and MSCs. Treatment with HMGB1 accelerated recovery in muscle and haematopoietic in-vivo injury models. Finally, we found that a single dose of HMGB1 administered systemically two weeks before injury also accelerated bone, muscle and haematopoietic tissue regeneration. In summary we have identified HMGB1 as a crucial stem cell 'alerting' factor and highlight its therapeutic potential to accelerate the regeneration of multiple tissues following trauma, chemotherapy or elective surgery.

T-1023

GENERATION OF A HUMAN INDUCED PLURIPOTENT STEM CELL LINE WITH A PATIENT OSTEOGENESIS IMPERFECTA COL1A1 MISFOLDING MUTATION BY GENOME EDITING FOR IN VITRO DISEASE MODELING

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While the genes underlying the genetic brittle bone disease, osteogenesis imperfecta (OI), are well established, the precise pathological mechanisms are unclear. In particular recent studies have suggested that ER-stress resulting from mutations in COL1A1 or COL1A2 that cause protein misfolding may be a significant contributor to the pathology. If this proves correct then the ER-stress pathways may offer new drug targets to modify disease severity. However, to understand these pathological pathways in the correct cellular context we need to study patient bone cells (osteoblasts). Since these are unavailable we have developed a human iPSC line with a severe OI collagen I (COL1A1) C-propeptide misfolding mutation introduced by genome editing to produce osteoblast disease-in-a-dish models. To introduce this mutation (c.3969_3970insT) in exon 49 of COL1A1 gene, we used a CRISPR-Cas9 system where Cas9 is fused to a human Geminin peptide (SpCas9-Gem) to facilitate transient Cas9 expression and reduce NHEJ-mediated indels. Cas9-Gem mRNA was transfected into control human iPSCs and after single cell sorting, the heterozygous iPSC clones were identified by PCR screening using primers specific for wild-type and mutant alleles. Sequencing confirmed targeting in one allele without an indel mutation in the other allele, and SNP arrays demonstrated chromosomal integrity. The colonies of targeted iPSCs have normal stem cell morphology. Immunocytochemical and flow cytometry

confirmed the expression of pluripotency markers. The ability of this OI mutant iPSC line to differentiate into three main germ layers was confirmed. Osteogenic differentiation was achieved via initial differentiation via the paraxial mesoderm-sclerotome pathway followed by culture in osteogenic medium for 2-3 weeks. These cultures express sentinel osteoblast markers including RUNX2, SP7, BGLAP, IBSP, COL1A1 and produce an extensive alkaline phosphatase positive and mineralized matrix. Importantly, protein analysis demonstrated the synthesis and deposition of mutant and normal collagen I in the extracellular matrix. Comparing these OI iPS cell-derived mutant osteoblasts with isogenic controls will allow us to dissect disease pathways and screen ER-stress modifying drugs.

T-1025

PHENOTYPIC DRUG SCREENING FOR DYSFERLINOPATHY USING PATIENT DERIVED iPS CELLS

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Dysferlinopathy is the progressive muscle disorder which includes limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM). Currently, there is no effective treatment for this disease. Both muscular disorders (LGMD2B and MM) are caused by mutations in DYSFERLIN gene, and it is reported that the main function of DYSFERLIN protein is related to the resealing of muscular membrane when the membrane was broken by such as exercise. For the particular series of mutations, called 'mis-sense mutation', it was reported that the treatment of proteasome inhibitor MG-132 increased the protein level of misfolded DYSFERLIN in the cells and recovered the function of membrane resealing in the mutant fibroblast. Based on that information, we hypothesized that the forced elevation of misfolded DYSFERLIN protein in the muscle cells would be the effective treatment for Dysferlinopathy. We established human iPS cells derived from MM patient and induced skeletal myocyte by the forced expression of MyoD, a skeletal muscle specific transcription factor. We also established 384 multi-well based screening system using MM myocytes. As the result of chemical library screening, one of the hit compound, nocodazole, effectively increased the protein level of DYSFERLIN in the myocytes. In addition, increased DYSFERLIN protein effectively enhanced the function of membrane resealing injured by the laser irradiation. It is known that nocodazole has the toxicity to the cells, however, these data suggested the increment of the misfolded DYSFERLIN protein in the muscle cells by chemical treatment would be the effective clinical treatment for Dysferlinopathy in the future.

Funding Source: This research is being conducted at T-CiRA, the collaboration program of Takeda pharmaceutical company and Center for iPS Cell Research and Application (CiRA), Kyoto University, and founded by Takeda.

T-1027

EFFICIENT MINERALIZATION OF HYDROGEL ENCAPSULATED HUMAN ADIPOSE STEM CELLS BY DISSOLVED BIOACTIVE GLASS IONS

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Miina Ojansivu - *University of Tampere, Tampere, Finland*
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Toni Montonen - *Tampere University of Technology, Tampere, Finland*
Heini Huhtala - *University of Tampere, Tampere, Finland*
Leena Hupa - *Åbo Akademi University, Tampere, Finland*
Minna Kellomäki - *Tampere University of Technology, Tampere, Finland*
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To address the urgent demand for bone grafts, our aim was to promote robust osteogenic differentiation of human adipose stem cells (hASCs) with bioactive glass (BaG) ionic dissolution products in 3D hydrogel culture with gellan gum (GG) or collagen type I (COL) hydrogels to mimic cell microenvironment for bone tissue engineering applications. Hydrogel mechanical properties were evaluated with compression testing. GG compositions crosslinked with either bioamine or divalent cation were used in the study. We analyzed hASC viability, adhesion, and osteogenic differentiation embedded in GG and COL. Cells were viable in both hydrogels but there was a notable difference in cell

morphology between the hydrogels. In GG, there were no visible cellular extensions in contrast to COL, where hASCs were more spindle shaped. We measured cell number and gene expression of osteogenic marker genes ALP, OSX, DLX5, and RUNX2A of the hASCs embedded in GG and COL hydrogel cultured with or without soluble BaG ionic products. Late osteogenic differentiation of hASCs was confirmed with analysis of mineralization and immunostaining of bone marker osteocalcin. Transparent GG hydrogels with encapsulated hASCs were imaged with Optical Projection Tomography (OPT) and Selective Plane Illumination Microscopy (SPIM) to analyze cell and mineralized matrix distribution inside 3D hydrogels in high resolution. Both hASC-laden GG and COL hydrogels were analyzed by Raman spectroscopic measurement of the matrix calcium phosphate content. Our results showed enhanced mechanical properties and compressive modulus of cell-free hydrogels with incubation in BaG ionic dissolution products. OPT and SPIM imaging showed homogeneous cell distribution of GG-encapsulated hASCs in 3D. Raman spectroscopy verified hASC mineralization as hydroxyapatite. Our results attested to robust osteogenic differentiation of hASCs and novel 3D hydrogel stem cell culture methods potential for future engineered bone-like tissue constructs.

CARDIAC TISSUE AND DISEASE

T-1029

CHARACTERIZATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTE ELECTROPHYSIOLOGY WITH THE LOCAL EXTRACELLULAR ACTION POTENTIAL ASSAY

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Human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) technology has progressed rapidly in the last five years, leading to applications in drug safety and disease-in-a-dish modeling based on assays of the depolarization and repolarization of a functional cardiomyocyte syncytium. As such, multiple commercial sources exist for hiPSC-CMs, with each displaying distinct electrophysiologic properties. Multiwell microelectrode array (MEA) technology is commonly used to evaluate cardiomyocyte electrophysiology with label-free measurements of depolarization and repolarization derived from the field potential signal acquired from microelectrodes embedded in the substrate of each well in a tissue culture plate. Here, we describe a new assay for evaluating cardiomyocyte action potential morphology with the Maestro Pro multiwell MEA platform by combining the LEAP signal and optogenetic pacing. Following a brief induction phase that strengthens the attachment of the functional syncytium to the embedded microelectrodes, the signal acquired at the electrode becomes the local extracellular action potential (LEAP) signal, which resembles the transmembrane cardiomyocyte action potential and enables the automated detection and quantification of repolarization timing, triangulation, and repolarization instabilities. We used this label-free technology to compare the action potential morphology of commercial hiPSC-CMs in baseline and dosed conditions. To facilitate comparisons across conditions, we paced the cardiomyocyte beat rate using optogenetics and precisely controlled light delivery with the Lumos multiwell optical stimulator.

T-1031

NKX2-5 REGULATES HUMAN CARDIAC DIFFERENTIATION VIA A HEY2-DEPENDENT TRANSCRIPTIONAL PATHWAY

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The differentiation of human pluripotent stem cells (hPSCs) to the cardiac lineage can be used to model human heart development and, in turn, to analyze the developmental consequences of genetic abnormalities. *NKX2-5* encodes a highly conserved homeobox transcription factor that is an ancient and critical component of the vertebrate cardiac gene regulatory network. We have genetically modified human embryonic stem cell (hESC) lines to elucidate *NKX2-5* function in early heart development. Cardiomyocytes derived from NKX2-5 null hPSCs fail to up-regulate VCAM1, a marker of cardiac differentiation, and maintain expression of PDGFRA, indicating a failure to complete cardiomyogenesis. Furthermore, NKX2-5 null cardiac monolayers display asynchronous contraction and individual mutant cardiomyocytes have altered electrophysiology. Molecular profiling by RNA-seq and ChIP-seq demonstrates that NKX2-5 has an important role in regulating the maturation of cardiomyocytes and expression of key calcium handling, ion channel and gap junction associated genes. Furthermore, genetic rescue experiments demonstrate that the bHLH protein HEY2 is a key mediator of NKX2-5 function during human cardiomyogenesis. These findings identify HEY2 as a novel component of the NKX2-5 cardiac transcriptional network, providing tangible evidence that hESC models can decipher the complex pathways that regulate early stage human heart development. These data provide a human context for the evaluation of pathogenic mutations in congenital heart disease.

T-1033

ANISOTROPIC CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES LEADS TO CELLULAR FEATURES OF ENHANCED PHYSIOLOGICAL RELEVANCE

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Cardiac liability is an important cause of drug failure in late stages of clinical trials, as well as in the removal from the market. Part of these failures have been attributed to the deployment of non-human and non-cardiac systems in cardiac safety assessments. More recently human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) have increasingly become a complementary tool for the study of acute cardiac toxicity and safety, providing a human and tissue-specific model to deconvolute potential cardiac liabilities. However, hiPSC-CMs typically display an abnormal sub-cellular structural organization and unsatisfactory electro physiological maturity. When cultured in standard cell cultureware, hiPSC-CMs often display undefined or disarrayed sarcomeric organization. Here we describe and characterize a novel micro-engineered high-density screening platform for hiPSC-CMs that emulates correct cardiac muscle fiber organization through passive self-alignment, generating anisotropic cultures. In agreement with previous reports utilizing anisotropic cardiomyocte cultures, the platform employed here improved sarcomeric organization, as seen by readily identifiable, correctly patterned myofibrils along the cell body, while nuclear size and shape were significantly changed. Contraction patterns of hiPSC-CM preparations were observed to become markedly unidirectional in this platform. Increased gene expression of ryr2, atp2a2, and pln, key components of cardiomyocyte calcium handling pathways, which are crucial for cardiac physiology, were also observed. The expression levels of cardiac ion channel genes such as cacnac1c, scn5a, kcne1, kcnq1 as well as cardiac cell junction components gja1, gja5 and dsp also showed an increase. High throughput kinetic fluorescence analysis of calcium flux in hiPSC-CMs indicated that the anisotropic hiPSC-CM cultures presented significant changes in cardiomyocyte physiology, highlighting that anisotropic hiPSC-CM cultures may present an attractive platform for cardiac safety and toxicity studies. In summary, high throughput anisotropic hiPSC-CM cultures displayed several physiologically-relevant changes which may have a significant impact in the investigation of cardiac liabilities of developing drugs.

T-1035

TOWARD IN VITRO CONSTRUCTION OF IPSC-DERIVED HUMAN HEART-LIKE TISSUE

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To date, research on heart development has focused on generating cardiomyocytes (CMs) with the goal of cell transplantation to cure myocardial infarction. In recent years, engineering of myocardial tissue rather than a pure CM population to study cardiac function and transplantation potential has become more prominent. Myocardial-like tissue has been generated using mainly primary tissue or human iPSC-derived CMs in combination with HUVEC and/or fibroblasts, but these models do not represent the human physiological system well. Here, we aim to generate human heart-like tissue by combining a series of iPSC-derived cells including CMs, endothelial cells, epicardial-like cells, and/or cardiac fibroblasts. Directed differentiation of human iPSC enables cardiac cell generation from a common progenitor pool (pre-cardiac mesoderm), which represents human heart development further increasing the correlation with human tissue. We generated various reporter cell lines to characterize

each cell type. CMs could be easily identified by MYH6 promotor-driven eGFP expression and sorted cardiac endothelial cells or epicardial-like cells were derived from iPSCs constitutively expressing different fluorescent proteins (i.e. mCherry, BFP). Because 3D cell culture systems permit us to mimic the physiological state more closely than common 2D-monolayer culture systems, we cultured cardiac cells in 3D as spheroid formed by cell aggregation. Self-patterning of cells can be tracked by fluorescent proteins, and cellular behaviour at the transcriptional, translational and epigenetic levels will be analysed. 3D bioprinting is a technique that allows scaffold-free controlled arrangement of spheroids. During human development, the heart forms from a tube structure. Mimicking this state using 3D bioprinting is expected to aid in comprehending human heart developmental stages better. Other tissues generated by 3D bioprinting have been shown to engraft and vascularize after transplantation showing the potential of such engineered tissues. In the future, research on spheroids representing other layers of the heart will enable us to gain more insight into unexplored heart tissues. Finally, the possibility of genetically engineering iPSCs easily can be used to generate novel disease models.

T-1037

THERAPEUTIC POTENTIAL OF A NOVEL NECROSIS INHIBITOR, 7-AMINO-INDOLE, IN MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

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Opening of mitochondrial permeability transition pore (mPTP) and Ca2+ overload are main contributors to myocardial ischemia-reperfusion (I/R) injury, which paradoxically causes a wide variety of myocardial damage. We investigated the protective role of a novel necrosis inhibitor (NecroX-7; NecX) against myocardial I/R injury, using in vitro and in vivo models. H9C2 rat cardiomyoblasts and neonatal cardiomyocytes were exposed to hypoxia-reoxygenation stress, after pretreatment with NecX, vitamin C, a combination of vitamin C and E, N-acetylcysteine, an apoptosis inhibitor (Z-VAD-fmk), or cyclosporine A (CsA). The main mechanism of cell death after hypoxia-reoxygenation stress was not apoptosis but necrosis, which was prevented by NecX. Protective effect of NecX was based on its potent reactive oxygen species (ROS) scavenging activity, especially on mitochondrial ROS. NecX preserved mitochondrial membrane potential through prevention of Ca2+ influx and inhibition of mPTP opening, which was more potent than that by CsA. Using Sprague-Dawley rats exposed to myocardial ischemia for 45 minutes followed by reperfusion, we compared therapeutic efficacies of NecX with CsA, vitamin C, a combination of vitamin C and E, and 5% dextrose, each administered 5 minutes before reperfusion. NecX markedly inhibited myocardial necrosis and reduced fibrotic area, to a greater extent than did CsA and other treated groups. Additionally, NecX preserved systolic function and prevented pathologic dilatory remodeling of left ventricle. The novel necrosis inhibitor has a significant protective effect against myocardial I/R injury through inhibition of mPTP opening, indicating that it is a promising candidate for cardioprotective adjunctive measure on top of reperfusion therapy.

T-1039

POTENT IMMUNOMODULATION AND ANGIOGENIC EFFECTS OF MESENCHYMAL STEM CELLS VERSUS CARDIOMYOCYTES DERIVED FROM PLURIPOTENT STEM CELLS FOR TREATMENT OF HEART FAILURE

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Heart failure (HF) following myocardial infarction (MI) is the leading cause of mortality and morbidity worldwide. Cell-based therapeutics has been explored as a potential approach to replenish the lost cardiomyocyte and restore cardiac function in HF. But the optimal cell type remains unclear. We sought to compare the safety and efficacy of direct intramyocardial transplantation of human embryonic stem cell-derived cardiomyocytes(hESC-CMs) and human induced pluripotent stem cell-derived mesenchymal stem cells(hiPSC-MSCs) in a porcine model of HF. Eight weeks after induction of MI, animals with left ventricular ejection fraction(LVEF) <40% were randomly assigned to receive direct intramyocardial injection of saline; 2×108 hESC-CMs or 2×108 hiPSC-

MSCs. All animals underwent serial echocardiography and hemodynamic evaluation to assess LV function after cell transplantation. The hearts were harvested for immunohistochemical evaluation after assessment of ventricular tachyarrhythmia (VT) induced by in-vivo programmed electrical stimulation.At 8-weeks post-transplantation, LVEF, maximal positive pressure derivative and end systolic pressure-volume relationship were significantly higher in the hiPSC-MSC group but not in the hESC-CM group compared with the MI group. The incidence of early spontaneous VT episodes was higher in the hESC-CM group but the incidence of inducible VT was similar among the different groups. Histological examination showed no tumor formation but hiPSC-MSCs exhibited a stronger survival capacity by activating regulatory T cells and reducing the inflammatory cells. In-vitro study showed that hiPSC-MSCs were insensitive to pro-inflammatory Interferon-gamma induced Human Leukocyte Antigen Class-II expression compared with hESC-CMs. Moreover, hiPSC-MSCs also significantly enhanced angiogenesis compared with other groups via increasing expression of distinct angiogenic factors. Our results demonstrate that transplantation of hiPSC-MSCs does not increase proarrhythmia or tumor formation; and superior to hESC-CMs for improvement of cardiac function in HF due to their immunomodulation that improves in-vivo survival and enhanced angiogenesis via paracrine effects.

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T-1041

SUSTAINED DELIVERY OF SECRETOME OF HUMAN CARDIAC STEM CELLS FOR EFFECTIVE CARDIAC REPAIR

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Therapeutic strategies for protecting the ischemic heart are an important unmet medical need and stem cell therapy has great potential for repair of ischemic hearts. The cardioreparative effects of adult stem cells have been attributed to their salutary paracrine activity. Human W8B2$^+$ cardiac stem cells (CSCs) isolated from adult atrial biopsies have been shown to exert powerful paracrine effects on endothelial cells and cardiomyocytes that are indicative of cardiac repair and regeneration. The secretome constituents (both soluble proteins and extracellular vesicles) of human W8B2$^+$ CSCs have recently been characterized using proteomic and transcriptomic approaches. Here, we present an innovative methodology to deliver the secretome of CSCs in a sustained manner using TheraCyte devices. These devices consist of a bilaminar membrane system: an inner membrane to protect encapsulated cells from the host's immune system while allowing nutritional inputs and therapeutic outputs, and an outer membrane that promotes neovascularisation. Using the TheraCyte device, allogeneic cells can be delivered without risk of rejection. In a rat model of myocardial infarction, subcutaneous transplantation of human W8B2$^+$ CSCs (1x10^6 cells) encapsulated within the TheraCyte device significantly improved cardiac function (ejection fraction: 49.835.1% vs. 40.430.7% in control, p+ CSC within the devices, and the outer membrane was highly vascularized by host blood vessels. Our study indicates that W8B2$^+$ CSC secretomes contain bioactive paracrine factors that can be harnessed for therapeutic use in cardiac repair and regeneration. Moreover, the TheraCyte device can be employed to deliver allogeneic stem cells for effective secretome-based cardiac therapy.

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T-1043

ERR GAMMA AGONIST AND SKP2 INHIBITOR ARE NOVEL MATURATION FACTORS IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Although human pluripotent stem cells (hPSC)-derived cardiomyocytes (CMs) are potential cell sources for in vitro disease modelling and cardiotoxicity screening, the resulting CMs are immature and show phenotypes consistent of CMs in fetal heart. In this study, we screened about 10,000 chemical compounds to find the cardiac maturation stimulators observing the expression of Troponin I1 (TNNI1) and Troponin I3 (TNNI3), because switch of TNNI isoform expression is a hallmark of cardiac development from fetal to adult. To this end, we prepared TNNI1, TNNI3, double reporter human induced pluripotent stem cell (hiPSC)-derived CMs. We identified 8 compounds that stimulated TNNI3 expression. One compound is an estrogen-related receptor gamma (ERRγ) agonist and four are cell-cycle related inhibitors including S-phase kinase-associated protein 2 (SKP2) and tubulin polymerization inhibitors. Treatment with the ERRγ agonist or SKP2 inhibitor resulted in hiPSC-derived CMs highly expressing TNNI3 reporter (60.63%33.16% and 42.93%35.29%, respectively) compared to vehicle-treated hiPSC-derived CMs (e.g. 10.30%32.16%). Moreover, the effect of two compounds was additive in terms of the TNNI3 reporter expression (86.53%31.75%). In addition, the expression level of other maturation-related genes (e.g., MYH7, MYL2, S100A1, RYR2) in combination-treated hiPSC-derived CMs exceeded the expression level of that in fetal heart tissue. Next we generated ERRγ KO iPSCs and investigated the mechanism of action of the ERRγ agonist in hiPSC-CMs. ERRγ agonist treatment in ERRγ KO hiPSC-CMs did not increase the expression of the TNNI3 reporter (13.43%34.18%), but SKP2 inhibitor treatment had the same effect as it did in wild-type hiPSC-CMs. The hits of ERRγ agonist and SKP2 inhibitor from our high throughput screening are consistent with studies that have shown that ERRγ is essential for a metabolic switch in postnatal mouse heart and that CMs after birth exit the cell cycle. In summary we have demonstrated that an ERRγ agonist and SKP2 inhibitor significantly promote the maturation of hiPSC-derived CMs and can be used for the in vitro generation of hiPSC-derived matured CMs applicable to biomedical fields.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

T-1045

FUNCTIONAL HIERARCHY FROM PROGENITOR TO MATURE CELLS IN MURINE AND HUMAN VASCULAR ENDOTHELIUM

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The existence of endothelial progenitors and their contribution to vasculature in adults has been controversial. Our aim was to define vessel-resident endothelial progenitors *in vivo* at the functional and molecular level. Using common endothelial markers (CD34, CD31, VEGFR2) after exclusion of hematopoietic cells (Lin-) with flow cytometry in Cdh5cre ^{ERt2}/Rosa-YFP reporter mice, three sub-populations of endothelial cells could be identified among YFP+ and Lin- cells. These were termed endovascular progenitor (EVP, CD34+CD31loVEGFR2lo), transit amplifying (TA, CD34loCD31intVEGFR2lo) and differentiated (D, CD34+CD31hiVEGFR2hi). Only EVP cells and not TA and D cells had self-renewal capacity as demonstrated by in TM plugs in recipient mice. Importantly, EVP cells arose from vascular resident beds that could not be transferred by bone marrow transplantation. In lineage tracing studies in wounds, EVP cells gave rise to TA and D cells. This same hierarchy was recapitulated in the human vasculature in the term placenta. Among CD45-CD34+ cells only CD31low/int cells could give rise to endothelial colonies. CD31high cells only gave rise to small clusters with no self-renewal. RNA sequencing on flow sorted populations revealed that EVP cells highly expressed genes related to progenitor function such as Sox9, Il33 whereas D cells highly expressed genes related to differentiated endothelium such as Cd31, Vwf and NOTCH pathway target genes. Sox18 transcription factor had a significant role in EVP to D differentiation, as determined by lineage-tracing using Sox18Cre^{ERt2}/Rosa-YFP mice. In the absence of functional SOX18/SOXF, in a mutant model of SoxF, EVP progenitors were still present, but TA and D populations were significantly reduced. However, conditional deletion of RBPJ or Sox9 abrogating notch signaling in vivo or DAPT inhibition of this pathway in vitro resulted in loss of quiescence of progenitors and loss of colony formation and therefore a depletion of the EVP pool. EVP cells is a functionally and molecularly defined resident progenitor in vivo. This is a paradigm shift in our understanding of vascular resident endothelial progenitors in adult endothelial regeneration.

T-1047

LONG-TERM PRIMING BY 3-MODULATING FACTORS IS A PROMISING STRATEGY FOR ENHANCING LATE ENDOTHELIAL PROGENITOR CELL (EPC) 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 factors (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 factors significantly induced the differentiation potential of CD34+ stem cells into EPC lineage cells. Notably, long-term priming of OECs with the three factors (OEC-3Fs) increased the proliferation potential of EPCs via ERK activation. The migration, invasion, and tube-forming capacities were also significantly enhanced in OEC-3Fs compared with unprimed OECs. Further, the cell survival ratio was dramatically increased in OEC-3Fs against H2O2-induced oxidative stress via the augmented expression of Bcl-2, a pro-survival protein. In conclusion, we defined three modulating factors for enhancing the bioactivities of ex vivo-expanded OECs for vascular repair. Long-term 3F priming might be a promising methodology for EPC-based therapy against ischemic diseases.

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HEMATOPOIESIS/IMMUNOLOGY

T-1049

CLONAL STEM AND PROGENITOR CELL TRACKING IN EMERGENCY HEMATOPOIESIS USING CELLULAR BARCODING

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Single adult hematopoietic stem and progenitor cells (HSPCs) are demonstrated to be lineage-primed in the steady state using clonal fate tracking and single cell RNA profiling. However, how single cell fate is regulated during emergency conditions that lead to skewing of lineage production is largely unknown. One possibility is that environmental cues such as excess cytokines enhance clonal expansion of pre-existing lineage-primed progenitors. Alternatively, cytokines might provide signals to divert progenitors from an otherwise different fate. Here, we tag individual progenitors with unique and heritable DNA barcodes and track their fate in vivo under Fms-like tyrosine kinase 3 ligand (Flt3L) induced emergency state, which mediates an increase in dendritic cell (DC) number. Our results indicate that enhanced clonal expansion of pre-existing progenitors accounts for the majority of emergency production of different DC subtypes (cDC1, cDC2 and pDC). In particular, we observe more dramatic increases in DC number by multipotent progenitors than progenitors with more restricted fate. We observe minimal emergency recruitment of DC-generating clones and find little evidence of lineage divergence in single HSPCs by splitting siblings derived from the same clone into two recipients, one with and the other without Flt3L administration and comparing their fate. Collectively, our results demonstrate that while greater clonal expansion can occur upon exogenous Flt3L stimulation, lineage-primed programs within single HSPCs are relatively stable and cannot be diverged. These findings provide new insight into the control and regulation of fate decision during hematopoiesis.

T-1051

SINGLE CELL RNA-SEQ REVEALS DACH1 EXPRESSION SEGREGATES LYMPHOID AND MYELOID FATE IN EARLY HAEMATOPOIESIS

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The classical view of blood cell development is of multipotent haematopoietic stem and progenitor cells (HSPCs) that become lineage-restricted at defined stages. The Lin-c-kit+Sca1+Flt3+ stage (MPPs - also known as MPP4), is still purported to contain multipotent B-cell and myeloid cell progenitors despite single cell level studies that reveal a more complex picture of gene expression and fate heterogeneity. Markers that definitively separate these lineages are lacking but would help bring clarity to the process of haematopoiesis. Through single cell RNA-sequencing, we identify heterogeneous expression of Dach1 in HSPCs, where it co-expresses with myeloid/stem genes Gata2, Mdga1, Csgalnact1, Muc13, Pafah2, Cdkn1b, and inversely correlates with lymphoid genes such as Dntt, Lck, Il7r. Using generated Dach1-GFP reporter mice and cellular barcoding, we demonstrate that the 'MPP4' fraction is, unequivocally, not multipotent, and that Dach1 expression separates myeloid and lymphoid cell fate.

T-1053

ZBTB11 IS REQUIRED FOR HEMATOPOIETIC STEM CELL FUNCTION IN MOUSE

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Hematopoiesis manages and executes the daily requirement of producing and maintaining the various blood cell lineages in the right quantities. It must also rapidly generate vast numbers of specific lineages in response to injury and infection. Understanding the sophisticated mechanisms regulating this balancing act can uncover therapeutic targets for manipulating blood cell production in disease treatment. In a forward genetic screen for zebrafish myeloid mutants, we identified a requirement for ZBTB11, a ZBTB (BTB/POZ) transcriptional repressor, in definitive hematopoiesis. To understand its relevance to mammalian systems, we extended these studies to the mouse. Because the zebrafish Zbtb11 mutant is embryonic lethal we designed a conditional knockout strategy that enables deletion of a floxed Zbtb11 allele in vav-cre expressing cells when crossed with a vav-cre mouse. In the absence of the Zbtb11 transcriptional repressor in this compartmental deletion model, phenotypic HSCs (SLAM) are specified in over-abundance at E14.5 through E17.5 compared to controls. Functional analysis shows that despite their cell surface phenotypic identity they are unable to effectively differentiate into committed progenitors and mature cell populations in the fetal liver, and hematopoietic content in the fetal bone marrow is largely absent at E16.5 - E17.5 pointing to bone marrow failure. Embryos do not survive past E18.5 likely due to insufficient hematopoiesis. Further analysis showed that Zbtb11-/- HSCs fail to proliferate in an in vitro proliferation assay, and Ki67/Hoechst profiling indicates accumulation in G1 (LSK) or G0 (Gr1+) with limited progression through the cell cycle. These data demonstrate a role for Zbtb11 in proliferation and cell cycle regulation in mammalian hematopoiesis. We are currently undertaking scRNAseq in Zbtb11-/- and control HSC populations to understand heterogeneity within and between these populations, and what genetic programs have been altered. In summary, our findings now establish a conserved cell autonomous role for Zbtb11 in mammalian definitive hematopoiesis, specifically in HSC function, opening up new avenues for understanding and manipulating HSCs.

T-1055

AN ENDOGENOUS TRANSMEMBRANE INHIBITOR CONTROLS DISTINCT MTORC2 FUNCTIONS IN NORMAL AND MALIGNANT HEMATOPOIESIS

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mTOR/AKT are the Ser/Thr kinases, central to cellular proliferation, survival and metabolic responses to cytokine signals, activated in many human cancers. mTOR exists as two distinct multi-subunit protein complexes, mTORC1 and mTORC2. Activation of AKT in hematopoiesis leads to a myeloproliferative syndrome and eventual loss of hematopoietic stem cells. mTORC1 deletion lead to hematopoietic failure during the stress condition. Moreover, pharmacologically inhibition of mTOR resulted in anti-leukemia effects in a mouse model of acute myeloid leukemia (AML). Further, Acute lymphocytic leukemia (ALL) is more clearly driven by activation of the AKT/mTOR pathway. More than ~ 50% of the human T-ALL cells depend on ongoing NOTCH-initiated signals for their growth. And enforced NOTCH signaling is a potent inducer of T-ALL in the mouse model. Here, we report an endogenous transmembrane protein in hematopoietic progenitor cells that inhibits the activation of pAKT, and interacts with and inhibits RICTOR which is a component of mTORC2, identified through the analysis of a mouse model of microenvironment-induced AML. 'Upstream-of-mTORC2 (UT2)' interacts with the RICTOR/mTORC2 negatively regulating mTORC2 activity. Modulation of UT2 altered pAKT activation in NOTCH-induced T-cell acute lymphoid leukemia (T-ALL), which is established murine model of T-ALL and influenced their survival. Mice transplanted with UT2 overexpressing NOTCH+ cells displayed significantly prolonged disease survival while those animals with knocked down UT2 died significantly more rapidly. These studies identify an unrecognized inhibitory component of the mTORC2/AKT signaling network in hematopoietic cells and uncover a means by which environmental cues may down-modulate the activity of this critical cell-regulatory pathway. UT2 is as both a new participant in the key pathway of mTORC2/AKT and a possible contributor to viable therapeutic strategy. Therefore, our preliminary studies identify a possible therapeutic opportunity in which suppression of mTORC2 preferentially impedes leukemia progression without harming normal hematopoietic stem and progenitor cells.

T-1057

MECHANO STIMULATION PROMOTES THE PRODUCTION OF MULTIPOTENT BLOOD PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Definitive hematopoietic stem cells (HSC) arise from haemogenic endothelial (HE) cells lining the ventral domain of the dorsal aorta (AoV) in the AGM region. Fluid shear stress generated by blood flow has been shown to promote production of definitive haematopoiesis in the mouse and zebrafish. However, the effect of fluid shear stress on the development of definitive haematopoiesis in the human has not been studied. A human PSC differentiation system that generates blood from HE with an aortic identity was adapted to shear culture systems. Blood formation was tracked by time-lapse fluorescence microscopic imaging using a dual fluorescent reporter ESC line (SOX17mCHERRY/wRUNX1CGFP/W). Fluid shear forces were applied to cells either by microfluidic pulsatile shear flow or orbital shaking. Shear treatment promoted production of SOX17+arterial endothelium as well as SOX17+ RUNX1C+ haematopoietic progenitors. RT-PCR analysis of day18 cultures revealed that shear also upregulated the expression level of HOXA10, NOTCH1, NOTCH2, GATA1, SPI1 and HES1 but not HOXA9, SOX17 and RUNX1. CD34+ cells isolated from Day 18 PSC differentiation cultures were grown for 2 weeks in suspension culture with haematopoietic cytokines to determine their multilineage proliferative potential by immunophenotyping. Orbital shear-induced cultures generated highly proliferative CD34+ multipotent blood progenitor cells (MPC) when compared to static cultures. Each shear-induced CD34+ cell generated on average 210 haematopoietic progeny while only 5.4 progeny were generated from static-cultured CD34+ cell. In comparison to static culture, shear increased the production of lymphoid progenitors (CD7, x1.33 shear, x0.07 static), leukocytes (CD45, x22 shear, x0.81 static), megakaryocytes/MPC (CD41, x5.9 shear, x0.54 static) and red blood cells (GYPA, x149 shear, x2.3 static), while there was no expansion of CD34+ cell numbers (x0.18 shear, 0.84 static). This is the first study showing the potent effect of fluid shear stress on production of MPC derived from HE with an aortic identity. Further study should examine safety, potency and cost of manufacture in comparison to other sources of MPC.

T-1059

CHARACTERIZATION OF PRIMITIVE HSC SUBSETS IN UMBILICAL CORD BLOOD SAMPLES:INFERENCES FOR CELL THERAPY

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The most primitive HSCs in mammals, including mice and humans, have long been believed to be CD34 antigen positive (CD34+) cells. However, several studies reported that murine and human long term repopulating (LTR) exist in lineage-negative (Lin-CD34low/-). Thus, understanding the biology and the molecular signature of the different subsets of primitive HSC and their early development is crucial and has important implications for clinical transplantation as well as for basic research. UCB has been accepted as an alternative safe source for HSC other than adult BM; however, the small amount limited its utilization mostly for children with obstinate malignancies Therefore, characterization of the early HSC in cord blood and identification of an unequivocal marker will facilitate their selection; proliferation and propagation in large scale for clinical usage. So, in this study Lin−CD34−CD38Low/− (+/-) and Lin−CD34+CD38Low/− (-/-) HSC, were sorted and their molecular and cellular profiling was compared by morphological, gene expression profile, and proteomics analysis. Around 17 UCB units were collected from women going on full delivery in our local hospitals. The occurrences of both -/- and +/- subsets phenotypes were measured by flow Cytometry. The expression of ALDH was analyzed. Additionally, multi differential ability was weighed up by colony forming unit (CFU). Gene expression and proteomics technology were applied on very few samples to compare similarities and differences in protein expressions profiles. Our results showed that (+/-) and (-/-) HPSCs subsets were present in cord blood samples in variable proportions and could be identified based on the ALDH expression and their ratios estimated to be 1:2. Furthermore, colonogenic ability of +/- and -/- populations as demonstrated by CFU assay was found to be reduced in -/-. Additionally, both HPSC subsets expressed pluripotency/stemmness genes i.e. Oct4, Nanong, TERT as quantified by real time PCR. Protein profiling showed that similar distinct spots were aberrant in both HPSCs compartments and currently, more analysis is being conducted to reveal this

spots. Finally, this approach may lead to the discovery of universal specific marker(s) on HSCs subsets and hence facilitate their selection and evaluation before transplantation

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T-1061

SCRIBBLE CONTRIBUTES TO THE REGULATION OF MULTIPLE LINEAGES DURING STEADY-STATE HAEMATOPOIESIS

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The evolutionarily conserved scaffolding protein, Scribble (SCRIB), acts as a tumour suppressor in multiple epithelial cancers. However, SCRIB's role in haematopoiesis and haematopoietic malignancies is largely unknown. As SCRIB knockout mice are embryonically lethal, we utilised conditional knockout mouse models driven by the Mx1 or hScl promoter in conjunction with extensive phenotypic analyses to elucidate SCRIB's role in haematopoiesis. Our comprehensive analyses revealed loss of SCRIB produced subtle but significant defects in lymphoid and myeloid progenitor fractions although the specific fraction affected varied between the conditional knockout mouse models. As these two models have different kinetics we utilised a reverse transplant assay, with similar kinetics to the Mx1-Cre model, to examine the cell intrinsic effect of SCRIB loss in haematopoiesis. Similar to our previous results, this assay revealed a significant role for SCRIB in early lymphoid and myeloid development. The subtlety of phenotype produced by deletion of SCRIB is most likely due to compensatory mechanisms so we induced stress haematopoiesis through irradiation to mitigate the effect of compensatory mechanisms. Lethally irradiated recipients receiving SCRIB knockout bone marrow revealed a more severe phenotype compared to previous assays with defects in erythropoiesis, myelopoiesis and lymphopoiesis. Taken together, our data reveals a role for SCRIB in multiple haematopoietic lineages during steady-state haematopoiesis thereby suggesting SCRIB may be important during leukemogenesis. Studies are currently underway to investigate how SCRIB impacts on the onset and severity of acute myeloid leukaemia and acute lymphocytic leukaemia.

T-1063

PROFILING MESENCHYMAL STEM CELL IMMUNOMODULATION IN VITRO

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Mesenchymal stem cells (MSCs) are multipotent adult stem cells found in a variety of tissues, including but not limited to bone marrow, adipose, and umbilical cord. Although MSCs have the ability to differentiate into cell types useful for regenerative medicine, their ability to modulate the immune system has been at the forefront of clinical studies. The mechanisms by which MSCs affect the immune system are not fully understood. This makes it difficult to design specific quality control measures for MSCs used in cell therapy as well as to understand what parameters are the most critical for their immunomodulatory abilities. Defining specific analytical tests and parameters is important for monitoring batch-to-batch variability and to better forecast patient-to-patient efficacy of MSCs used for cell therapy. In this study, we present a workflow to investigate the effects of MSCs on immune cell subset populations. Activated human MSCs were co-cultured with human T cell subtypes (Th1, Th2, Th17, and Treg) that had been differentiated via CellXVivo™ Differentiation Kits. Co-culture supernates were then analyzed via Proteome Profiler™ Antibody Arrays to screen for changes in cytokine levels, relative to T cell subsets cultured in the presence of non-activated MSCs. Analytes identified from the screening arrays were then quantitated using Luminex® and Simple Plex™ Assays. Additionally, changes in immune cell phenotype were analyzed via flow cytometry. We hypothesize that these tools and

techniques allow for a better understanding of the mechanisms behind MSC-mediated immunomodulation and provide methods for routine quality control testing of MSC populations prior to clinical use.

PANCREAS, LIVER, KIDNEY

T-1065

SINGLE-CELL TRANSCRIPTOME ANALYSIS OF HESC PANCREATIC DIFFERENTIATION REVEALS PRODUCTIVE AND NON-PRODUCTIVE PATHS OF DIFFERENTIATION

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Ever since human embryonic stem cells (hESCs) were first successfully cultured, it has been appreciated that hESCs differentiated to specific cell types have tremendous potential for use in cell replacement therapy for a variety of developmental, traumatic, and degenerative 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 due to the inability to achieve full maturation and functionality *in vitro*, and the inability to definitively ensure that tumorigenic cells are absent. Single-cell RNAseq (scRNAseq) enables detailed characterization of the relationships among cells in heterogeneous populations. We performed scRNASeq 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 and identify 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 pancreatic progenitor stage of differentiation, which deviates from the expected direct path between the preceding posterior foregut and following endocrine pancreas stages. In addition, some individual cells appear to have departed from path taken by the majority of the differentiating cells, and may be trapped in an unproductive state. These results point to transcriptional networks that may be aberrantly 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

Funding Source: UCSD Department of Reproductive Medicine

T-1067

REPOPULATION OF TERT+ ACINAR CELLS BY YAP/TAZ ARE INDISPENSABLE FOR MOUSE PANCREATIC REGENERATION

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Stem cells play crucial roles in tissue homeostasis and regeneration. However, it remains elusive how the self-renewing cells contribute to pancreatic regeneration. Employing Tert knock-in (tdTomato-CreERT2) genetically engineered mouse models, we located the rare population of Tert+ acinar cells. Tert+ acinar cells express various pancreatic lineage markers. Tert+ acinar cells are quiescent but acutely become proliferative upon pancreatic injury. Lineage-tracing assays showed that Tert+ cells are repopulated into the progenitor and differentiated acinar cells. Importantly, Tert+ cell ablation or conditional double knockout of YAP and TAZ in Tert+ cells impairs pancreatic regeneration. Our results identify Tert+ self-renewing cells as the cells-of-origin in the pancreas and unveil the crucial roles of YAP/TAZ signaling axis in activating Tert+ cells for pancreatic regeneration.

T-1069

ARID1A-DEPENDENT CHROMATIN "PRE-OPEN" ORCHESTRATES HEPATOCYTE PLASTICITY COMPETENCE IN LIVER REGENERATION

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The liver possesses a remarkable capability to regenerate after injuries. To understand cell sources for newly regenerated hepatocytes and the molecular mechanisms underlying is in the center of liver regeneration. Hepatic progenitor cell (HPC) differentiation and hepatocyte self-replication are the classic paradigm in liver regeneration. Recent studies uncovered a new mechanism that hepatocytes undergo reprogramming (aka "metaplasia") to a bi-potential state expressing both mature hepatocyte genes and HPC genes, implying a potential strategy to promote liver regeneration. Yet, the physiological significance and molecular regulations of hepatocyte reprogramming remain poorly defined. Here, we focused on epigenetic factors and identified that Arid1a, a key regulator of the SWI/SNF chromatin remodeling complex, specifically controls hepatocyte reprogramming. Arid1a ablation results in blockage of hepatocyte reprogramming, which leads to impaired liver regeneration. Mechanistically, Arid1a is required for chromatin accessibility at reprogramming genes. Surprisingly, Arid1a opens reprogramming genes even prior to injury in association with the transcription factor Tead. By preparing such pre-open states, Arid1a gears Yap to activate reprogramming upon liver injury. Our findings indicate the important role of hepatocyte reprogramming in liver regeneration, and Arid1a-dependent chromatin "pre-open" in orchestrating competence of hepatocyte plasticity.

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T-1071

PHARMACOLOGICAL ACTIVATION OF THE NRF2 PATHWAY PROTECTS HEPATOCYTES FROM DRUG INDUCED LIVER INJURY

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Liver disease is a major cause of mortality and morbidity. There are many etiologies, with drug-induced liver injury (DILI) representing the most common cause of acute liver failure. Anti-inflammatory and anti-oxidative stress therapies have been proposed as powerful approaches to reduce organ injury and to enhance regeneration. A key transcription factor which drives the anti-oxidant response is 'nuclear factor erythroid-derived 2-like 2' (NRF2). Therefore, the activation of the NRF2 pathway offers the potential to exert a cytoprotective effect and promote cell survival and tissue integrity. Dimethyl fumarate (DMF) is an approved drug used to treat patients with multiple sclerosis. DMF's protective effect is mediated in part by activation of the NRF2 pathway. We hypothesize that DMF could be used to reduce the severity of DILI. To study the protective effect of DMF in hepatocytes, we developed an automated system to produce hepatocytes-like cells (HLCs) from human pluripotent stem cells. Using this platform, the optimal dose of DMF was determined. Following this, single cell-high content image analysis was used to determine the dynamics of NRF2 nuclear translocation following DMF administration and challenge with paracetamol. In summary, we have developed an automated platform to study NRF2 biology, with a focus on developing new clinical strategies to reduce DILI and promote tissue repair.

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T-1073

ANEUPLOIDY AND POLYPLOIDIZATION OF INDUCED HEPATOCYTE LIKE CELLS (IHEP) DERIVED FROM MOUSE EMBRYONIC FIBROBLAST (MEF)

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In normal liver, up to ~ 90% and ~ 50% of mouse and human hepatocytes are polyploid, respectively. The cause has been known as the diploid hepatocytes undergo cytokines failer to generate polyploid cells. Proliferating polyploid hepatocytes, which form multipolar spindles during cell division, generate reduced ploidy progeny and/or aneuploid daughters. They can be contributed to promote adaptation to liver injury. In recent era of stem cells, there has been a growing interest in generation of stem cell based hepatocyte like cells for treatment of liver diseases or development of new drug. In this study we analyzed, polyploidization and aneuploidy in induced hepatocyte like cells (miHep) using male or female mouse embryonic fibroblast (MEF). For generation of miHep, male and female MEF were co-transfected with 3 lentiviral vectors (MOI=1) inserted each liver transcription factors (hHnf1a, hHnf4a, hFox) for 2 days and cultured in hepatocyte induction medium, continuously. After one month, clusters of hepatocyte like cells with polygonal cytoplasm and bipolar nucleic acid were clearly observed and continually cultured to passage10. At passage 9, male and female iHeps were analyzed for karyotyping by GTG banding staining while, fixed cells were subjected to flowcytometry analysis after 1 ug/ml PI solution treatment. The results of karyotyping revealed that more than 60 % of iHeps

were tetraploid as well as anuploid, as the number of chromosomes was higher or lower, regardless of gender. The result of a flowcytometry analysis also proved polyploidization of iHep, especially in male iHeps. Contrary to MEF cells, iHeps showed higher 4n ratio, ~ 63 % and ~50% more than 4n in male and female iHeps, respectively. In conclusion, mouse iHeps produced by direct conversion with 3 hepatic transcription factors could restore polyploidization characterization of normal mouse hepatocyte.

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T-1075

THERAPEUTIC POTENTIAL OF ACTIVATED MESENCHYMAL STEM CELLS IN NON-ALCOHOLIC FATTY LIVER DISEASE -AS SECOND GENERATION CELLULAR THERAPY

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Nonalcoholic fatty liver disease (NAFLD) is an increasing cause of chronic liver disease and broadly defined by the presence of steatosis with inflammation and progressive fibrosis. We have reported the therapeutic potential of human adipose tissue-derived multi-lineage progenitor cells (hADMPCs) in liver fibrosis but not NAFLD activity scores (NAS) of model mice in the past ISSCR meeting. These results evoke us whether the activation of hADMPCs could improve both of them. In this study, we propose the novel strategy to identify the bio-active, which could activate hADMPCs as the representatives of mesenchymal stem cells (MSCs), and show the selected bio-active-primed MSCs could improve the NAFLD. hADMPCs were isolated from subcutaneous adipose tissues of volunteers and expanded. To select the activating bioactive, hADMPCs were challenged to 160 kinds of bio-active proteins, and the cells were applied for DNA microarray analysis. Principal component analysis showed 7 bio-actives could significantly prime hADMPCs. Of these bio-actives, IL-1βwas selected as the candidates for the activating reagent for hADMPCs, because IL-1β-activated hADMPCs could show 1200 fold or more-augmented mRNA-expressions of G-CSF, which is known to be hepato-protective, but less augmented probes with harm. To confirm the IL-1β-activated ADMPCs could improve NAFLD, the cells were applied for the NAFLD model mice. The model was induced by a single subcutaneous injection of 200 μg STZ 2 day-after birth followed by feeding a high fat diet beginning at 4 weeks of age. After randomization of animals, the NAFLD mice received hADMPCs, IL-1β-activated hADMPCs (3 x 10^5 cells/kg, respectively), or placebo control via tail vein injection at an age of 6 weeks and were applied for histological at an age of 9 weeks. NAFLD model mice with hADMPCs or IL-1β-activated hADMPCs but not with placebo exhibited a significant reduction in liver fibrosis, as evidenced by Sirius red staining. IL-1β-activated hADMPCs could improve the NAS more than those of the non-activated ones nor control. We propose the novel strategy to identify the bio-active, which could activate MSCs for therapeutics, and show the selected IL-1β-activated MSCs could be applicable for the diseases.

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T-1077

REGIONAL DIFFERENCES IN HUMAN BILIARY TISSUES AND CORRESPONDING IN VITRO DERIVED ORGANOIDS

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The biliary tree is a series of ductular tissues responsible for the drainage of bile produced by the liver and pancreatic juices from the pancreas. The biliary tree is affected by a diversity of life-threatening diseases collectively called cholangiopathies. Cholangiopathies show regionalization, with some diseases such as biliary atresia predominantly targeting extrahepatic bile ducts (EHBDs) outside of the liver. Despite this, little is known on whether anatomical location within the biliary tree contributes to differences in functionality of biliary epithelium, especially in the EHBD compartment. Additionally, recent reports have demonstrated the possibility for in vitro culture of bile duct stem/progenitor cell organoids from both intrahepatic (IHBD) and EHBD sources. The relation of these organoid systems to each other, and to their tissue of origin, is largely unknown. Here we address these major questions by combining transcriptional analyses and in vitro culture of human bile duct organoids derived from primary IHBD and EHBD epithelium. Primary tissue from human gallbladder, common bile duct, pancreatic duct, and liver were used to generate organoid cultures and obtain primary tissue RNA. Characterization of IHBD and EHBD organoids demonstrated expression of the stem cell markers LGR5 and PROM1 and ductal markers KRT19/7. IHBD organoids appeared distinct from EHBD organoids and required different conditions for sustained growth. Our results also suggest IHBD, but not EHBD organoids, are capable of low-level expression of hepatocyte markers when subjected to a previously published differentiation protocol. RNA-Seq analyses revealed that primary tissues from different regions of the biliary tree display unique expression profiles. Further, a limited number of these differences are maintained in the in vitro organoids. EHBD organoids appear transcriptionally similar to each other regardless of their tissue source, while IHBD organoids are distinct. Taken together, our results uncover regional specific markers for different anatomical regions of the biliary tree. Further, we demonstrate that major differences exist between IHBD organoids and EHBD organoids in vitro. Ultimately, these results may help to identify new targets for therapeutic development for cholangiopathies.

T-1079

IL-8 INDUCING TRANSDIFFERENTIATION OF MATURE HEPATOCYTES TOWARD BILIARY EPITHELIAL CELL PHENOTYPE

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Immature bile ducts emerge in excessively injured liver cases such as with acute liver failure (ALF); these occurrences are known as ductular reactions (DRs). DRs in the ALF liver arise from the portal area and are believed to contain putative liver progenitor cell compartments. Recently, it has been suggested that mature hepatocytes might transdifferentiate into a biliary epithelial cell phenotype followed by formation of the DRs in the severely injured liver. Here, we aimed to investigate whether a cytokine elevated in ALF plasma could induce phenotypic conversion of mature hepatocytes into the biliary epithelial cells. The plasma cytokine levels both in patients with ALF and healthy volunteers were evaluated using a Bio-Plex suspension array system. The interleukin (IL)-8 level was significantly higher in the ALF patients than that of the healthy volunteers. Thus, we focused on the effect of IL-8 on mature hepatocytes in the subsequent analysis. In the MTT assay, the administration of recombinant mouse IL-8 homologue KC significantly suppressed the proliferation of the immortalized mouse mature hepatocyte AML12 cell line. In addition, western blotting analysis revealed that the administration of IL-8 suppressed the expression level of Cyclin D1. AML12 showed branching morphogenesis after the administration of IL-8, suggesting that IL-8 induces transdifferentiation of the mature hepatocytes toward the biliary epithelial cell phenotype. After five days of culture in the presence of KC, the expression levels of Sox9 and AFP mRNA, which are a bile duct-associated gene and progenitor marker, respectively, were significantly upregulated. Under the same culture conditions, Sox9-expressed cells emerged by immunofluorescence staining. Our results suggest that IL-8 induces differentiation of the mature hepatocytes toward the biliary epithelial cell phenotype. As a result, these findings may provide valuable insights for clarifying the cell source of DRs in ALF.

EPITHELIAL TISSUES

T-1081

COMPARISON OF FETAL BOVINE SERUM AND PLTMAX® FOR LONG TERM MAINTENANCE OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED RETINAL PIGMENT EPITHELIAL CELLS

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The differentiation of retinal pigment epithelial (RPE) cells from induced pluripotent stem cells (iPSCs) requires the use of defined media. However, once differentiated, iPSC-derived RPE cells can be maintained in media supplemented with fetal bovine serum (FBS). While readily available, FBS has several shortcomings. There is substantial lot-to-lot variability, and because it does not readily transfer to clinical use. Human platelet lysates have been used in the culture of mesenchymal stem cells. Platelet lysates such as PLTMax® are non-xenogeneic, available in clinical grades, and are currently in use in multiple human clinical trials. Here we sought to determine whether PLTMax® could be used as a replacement for FBS in the long term maintenance of iPSC-derived RPE cells with an eye toward clinical trials. To accomplish this, iPSC-RPE cells were obtained from LAgen Laboratories in T25 flasks and either grown in RPEM (LAgen Laboratories, Rochester, MN) supplemented with 5% FBS, or 4% PLTMax®. In some cases iPSC-RPE were passaged according to the manufacturer's protocols onto Matrigel® coated 1.2 cm diameter permeable supports (Transwells, Corning, USA). In both FBS and PLTMax® the cells maintained their cobblestone appearance and appeared to continue to pigment. Western blots indicated no significant difference in the expression or localization of the RPE marker proteins Bestrophin 1, or expression of RPE65, and CRALBP. Secretion of PEDF and VEGF was similar in both conditions and the polarity of secretion determined on Transwells to be similar under both conditions with PEDF being secreted primarily into the apical media and VEGF the basal media. Interestingly measurement or transepithelial electrical resistance (TER) of iPSC-RPE grown on Transwells® indicated a significant ($p < 0.05$) advantage of PLTMax®. Within 2 weeks of switching the monolayers in PLTMax® demonstrated an increase in TER as high as 69% vs. those in FBS. In summary, we conclude that PLTMax® is superior to FBS in the long term maintenance of iPSC-derived RPE cells. These data should help to facilitate the growth and maintenance of these cells for both academic and therapeutic purposes.

Funding Source: The Gund Fund for Career Development in Retinal Degenerative Disease Research, a VitreoRetinal Surgery Foundation Research Fellowship, & an unrestricted grant from Research to Prevent Blindness.

T-1083

CRYPT STEM CELLS AND ALTERATION OF THE INTESTINAL EPITHELIAL REGENERATION IN ULCERATIVE COLITIS

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Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis (UC), afflict 2.2 million people and their prevalence increase in developed and emerging countries. IBD correspond to life-long chronic inflammatory states affecting the intestine and/or the colon. Continued epithelial injury and impaired epithelial regeneration are key features of these pathologies. To date, no cure exists. Current therapies only aim at regulating the impaired immune system, while they induce severe side effects, and often resection of the inflamed part of the gut remains the only option. Control of the persistent inflammation is not enough to complete the healing process, suggesting the need of strategy for appropriate epithelial repair/regeneration beside the currently used treatments. Hence, the enhancement of the epithelial regeneration process is a promising therapeutic approach. Homeostasis of the intestinal epithelium is achieved by two populations of intestinal stem cells (ISCs) located at the bottom of the colorectal crypt: the crypt base columnar cells (CBCs) and the +4 ISCs located at 4 cells from the base of the crypt. A new murine model deficient for both IL10 and Nox1 (IL10/Nox1 dKO mice) spontaneously develops colonic inflammation with similar complications (colorectal cancer) than human ulcerative colitis. From the eighth month after birth, 30% of mice present dysplasia, 40% of mice develop colonic cancer and 15% of mice exhibit multifocal dysplasia and cancer (15%). Using this model, we investigated the very early alteration in crypt cells that could lead to the tumor initiation in ulcerative colitis. Transcriptomic analysis revealed that at very early age, even before any manifestation of the disease, the main signaling pathways involved in crypt stem cells regulation are altered and plasticity markers are increased in the IL1010/Nox1 mice. Moreover, once the disease is established, the CD133 and CD166 tumor stem cell markers are detected. Testing the stem cell capacity using the 3D organoid model, we observed an impaired stem cell survival and disregulated growth capacities in the UC model. Our preliminary results support the hypothesis in UC, colorectal crypt could be the origin of cancer.

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T-1085

INTEGRATED CYTOPLASMIC REORGANIZATION DURING HUMAN INDUCIBLE PLURIPOTENT STEM CELL MITOSIS

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The Allen Institute for Cell Science is developing high-replicate, dynamic image data on cell organization and activities using endogenous fluorescently tagged human induced pluripotent stem cells (hiPSCs). To date, we have generated a collection of genome-edited hiPS cell lines (WTC line) with ~15+ key GFP-tagged structures (www.allencell.org and other Institute poster abstracts). These include major organelles and structures, including the nucleus, mitochondria, ER, Golgi, microtubules, centrioles, actin bundles, and cell-cell junctions. hiPSC colonies grow with an epithelial sheet-like morphology and several key hallmarks of polarized epithelial cells including apical junctions and a filamentous actin band, apico-basal microtubule arrays and primary cilia. We quantified the relative location and dynamics of major cellular structures and activities as the stem cells traverse the cell cycle. As an internal positional reference, we label the cells with fluorescent dyes that localize to the plasma membrane (CellMask Deep Red) and nucleus (Hoechst DNA staining). We image the cells in 100s-1000s replicates using 3D live-cell spinning disk microscopy. The DNA dye also allows us to identify 8 stages of the cell cycle, using DNA morphology and texture attributes: two stages of prophase, two stages of prometaphase, metaphase, anaphase, telophase/cytokinesis, and interphase. We manually annotated a dataset of 700 mitotic cells. We used a parallel approach to integrate and analyze intracellular localization of cellular organelles and structures over the 8 stages of the cell cycle, permitting us to identify and correlate localization patterns in time for all of the structures studied. For example, we observed very similar localization for both the ER (sec61beta) and the nuclear envelope (laminB1), contrasting an anti-localization pattern for the mitotic spindle (alpha-tubulin) and mitochondria (tom20p) throughout mitosis. We validate our single timepoint-derived observations with live-cell timelapse imaging, which permits us to directly observe transitions between intracellular structure localization patterns. As part of our workflow, we are performing 3D image processing and a statistical analysis of the variation of organelle position and morphology during mitosis.T-1087

REPARATIVE ROLE OF EXOSOMES COLLECTED FROM IPSC-DERIVED MSCS IN SKIN CELLS

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The reparative potential of mesenchymal stem cells (MSCs) is attributed to various routes via cell differentiation, paracrine effects and nano-sized particles such as microvesicles and exosomes. Despite MSCs' therapeutic role in various diseases, the controversy regarding their safety is still unsolved, and their proliferative capacity in vitro is limited. As an alternative strategy, recent studies suggested that exosomes from induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSC-exo) holds regenerative potential in various animal studies. In this study we report that treatment of exosomes collected from the culture of iPSC-MSCs stimulated the proliferation of skin dermal fibroblasts and keratinocytes. Also, such co-incubation accelerated wound healing in scratch assays. Confocal analysis showed that found that iPSC-MSC-exos internalized into the cells and skin tissue. Finally, the expression level of genes related to cell proliferation, wound healing were increased by iPSC-MSC-exo in a dose-dependent manner. Collectively, our findings indicate that iPSC-MSC-exo can facilitate cutaneous wound healing via changing the characteristics of skin fibroblasts and keratinocytes.

T-1089

PRE-DIFFERENTIATED AMNIOTIC FLUID MESENCHYMAL STEM CELLS ENHANCE LUNG ALVEOLAR EPITHELIUM REGENERATION AND REVERSE ELASTASE-INDUCED PULMONARY EMPHYSEMA

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Pulmonary emphysema is a major component of chronic obstructive pulmonary disease (COPD). Emphysema progression attributed not only to alveolar structure loss and pulmonary regeneration impairment, but also to excessive inflammatory response, proteolytic and anti-proteolytic activity imbalance, lung epithelial cells apoptosis and abnormal lung remodeling. To ameliorate lung damage with higher efficiency in lung tissue engineering and cell therapy, pre-differentiating graft cells into more restricted cell types before transplantation

could enhance their ability to anatomically and functionally integrate into damaged lung. In this study, we aimed to evaluate the regenerative and repair ability of lung alveolar epithelium in emphysema model by using lung epithelial progenitors pre-differentiation on amniotic fluid mesenchymal stem cells (AFMSCs). An optimal ling epithelial progenitor-like cells condition has been established in EGFP-expressing AFMSCs, which resulted in a yield of approximately 20% lung epithelial progenitors-like cells (LEPLCs) from AFMSCs in a 7-day period. In porcine pancreatic elastase (PPE)-induced emphysema mice, transplantation of LEPLCs significantly improved regeneration of lung tissues through integrating into the lung alveolar structure, relieved airway inflammation, increased expression of growth factors such as vascular endothelial growth factor (VEGF), and reduced matrix metalloproteinases and lung remodeling factors when compared with mice injected with AFMSCs. Histopathologic examination observed a significant amelioration in DNA damage in alveolar cells, detected by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL), the mean linear intercept, and the collagen deposition in the LEPLCs transplanted groups. We conclude that transplantation of pre-differentiatd AFMSCs showed better regeneration of lung tissue and reverse elastase-induced pulmonary emphysema than AFMSCs.

T-1091

ISOLATION OF FUNCTIONALLY DISTINCT RESIDENT MOUSE LUNG MESENCHYMAL CELL POPULATIONS PROVIDES INSIGHT INTO THE MECHANISMS OF LUNG TISSUE REPAIR AND REGENERATION.

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Tissue repair and regeneration of the lung epithelium is regulated by highly coordinated intracellular signaling between epithelial stem cells and neighbouring mesenchymal cells. However, little is known about the heterogeneity of the mesenchymal compartment in adult lung. In this study, we sought to identify the functionally distinct resident lung mesenchymal cells and determine the cellular relationships that guide cell fate and regulate the wound-repair process. High throughput flow cytometry revealed a panel of distinct cell surface markers that were differentially expressed in the mesenchymal compartment. Subsequent variable reduction through manual correlation and multi-parameter Spanning tree Progression of Density normalized Events (SPADE) analysis of marker expression revealed substantial heterogeneity of mesenchymal cell populations in the adult mouse lung. To establish functional diversity, proliferation and mesenchymal differentiation was assessed, and found to be broad ranging across the populations. Fibroblastic colony-forming mesenchymal progenitor cells were enriched in a very rare cell fraction. Moreover, the epithelial-supportive capacity of the mesenchymal cells differed greatly between populations. Specifically, a subset of highly proliferative cells with multi-lineage differentiation potential support the proliferation of epithelial stem cells, while more differentiated myofibroblasts do not. These functional differences were supported by markedly different transcriptional profiles as determined by population RNASeq of sorted cells. This supports the concept that lung regeneration is tightly controlled by dynamic changes in the mesenchymal niche compartment and provides important insight into how changes in the cellular composition of this compartment in interstitial lung diseases may impede normal epithelial homeostasis.

EYE AND RETINA

T-1093

HUMAN PLACENTA DERIVED MESENCHYMAL STEM CELLS ENHANCED BY HYPOXIC CONDITION ARE EFFECTIVE IN THE OPTIC NERVE COMPRESSION MODEL

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Human placenta derived stem cells (hPMSCs) with a therapeutic potential to recover the optic nerve injury has been reported in the previous studies. We have recently demonstrated that hPMSCs has protective abilities in hypoxic damage. To improve hPMSCs capacity, we established enhanced hPMSCs (EhPMSCs) using hypoxic chamber. We exposed hPMSCs in short-term hypoxic conditions at 2.2 % O2 and 5.5 % CO2 concentration. After exposure, we determined cell viability and candidate protein expression of EhPMSCs. As a result, we could find cell viability of EhPMSCs was more increased and the expression of neurogenic markers, glial fibrillary acidic protein (GFAP), Thy-1 and Vimentin assessed by immunoblot test was up-regulated under 2.2% O2 short-term hypoxic conditions than normoxic conditions. In current study, we performed in vivo experiment to demonstrate recovery effects of EhPMSCs using optic nerve injury animal model. We injected naïve hPMSCs or EhPMSC to optic nerve injury model. After 1, 2, or 4 weeks, we analyzed the changes of target proteins in optic nerve tissues of rats. We observed the Hif-1α level of hPMSCs injection group was significantly decreased at 2 weeks after optic nerve crush. Thy-1 and Nf-1 protein expressions of EhPMSCs injection group were increased. We also investigated enhanced recovery function of EhPMSCs in R28 cells exposed to hypoxic condition. We found cell viability of hypoxic damaged R28 cell treated with EhPMSCs was more increased and the recovery effect treated with

EhPMSCs was more than with naïve PMSCs in ischemic in vitro models. Based on our findings, we could suggest EhPMSCs would be more optimal candidate for cell therapy in the traumatic optic nerve injury.

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T-1095

ESTABLISHMENT OF INDUCED PLURIPOTENT STEM CELL LINE FOR BEST1-ASSOCIATED RETINOPATHY

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Peripheral blood mononuclear cells (PBMCs) were collected from a clinically characterized 41-year-old woman with Best vitelliform macular dystrophy patient (BEST1 mutation, p.Gln96Arg) and a 57-year-old woman with autosomal recessive bestrophinopathy (BEST1 mutation, p.Leu40Pro, p.Ala195Va). The PBMCs were reprogrammed with Yamanaka transcriptional factors (Oct3/4, Sox2, Klf4, c-yc) using episomal plasmids. Normal karyotypes were observed in iPSC lines and the pluripotency was verified by immunocytochemistry, RT-qPCR, and ScoreCard assay that use gene expression signatures to quantify differentiation efficiency.

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T-1097

INVESTIGATION INTO THE MAINTENANCE OF CD34 EXPRESSION IN CORNEAL STROMA-DERIVED STEM CELLS

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The keratocyte is a specialised fibroblast found in the corneal stroma. It can be identified by CD34, a marker typically associated with haematopoietic stem cells but also linked to various progenitor cells. A subpopulation of CD34+ keratocytes in limbal stroma can derive multipotent progenitor cells in vitro, known as corneal stroma-derived stem cells (CSSC). These cells have great potential as a stem cell therapy for the ocular surface. However, extraction and culture in conventional foetal bovine serum (FBS)-containing medium leads to rapid loss of CD34-expression. To investigate whether CD34 expression can be maintained in vitro, CSSC were extracted from human corneoscleral rims and phenotype was compared in 4 different media at early and late passage. Genotype of isolated CD34+ CSSC and the effect of siRNA knockdown of CD34 were also evaluated by RT-qPCR. The media termed Stem Cell Medium (SCM; DMEM-F12 with 20% knockout serum replacement, 4 ng/mL bFGF and 5 ng/mL human LIF) was the only media of the four that maintained CD34 gene and protein expression through passages. This was also associated with increased expression of progenitor cell markers ABCG2, PAX6, THY1, SSEA4 and SOX2 in SCM compared to the other media. CD34+ CSSC had significantly increased expression of pluripotency markers compared to CD34- and siRNA knockdown of CD34 had a significant effect on gene expression. The works demonstrates that media optimisation is integral for inducing an optimal stem cell phenotype and that the expression of CD34 is linked to the stem cell properties of CSSC.

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STEM CELL NICHES

VISUALIZATION OF THE LGR5+ STEM CELL NICHE AND THE IMMUNE RESPONSE DURING PROGRESSION OF INFLAMMATION IN THE MOUSE INTESTINAL EPITHELIUM

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The adult intestinal epithelium is a rapidly self-renewing tissue, maintained by stem cells that reside within the crypts. These stem cells express Lgr5 and require Wnt/β-catenin signaling, which is augmented by R-Spondin. With its rapid turnover and well-defined structure in cellular orientation and differentiation, the intestine provides a great model to understand the mechanisms behind adult stem cell functions and insights towards regenerative medicine. It is also of great interest to understand the relationship between intestinal stem cells (ISCs) and immune cells in the inflammatory environment of gut mucosa during disease progression and therapeutic treatments. However, investigation of the specific functions and mechanisms of these cell types at single cell resolution in intact tissue architecture has been challenged by the lack of reliable and specific antibodies to detect key target receptors, such as Lgr5 and Fzd,

and to identify key functional secreted molecules, such as Wnt, R-Spondin, cytokines, and chemokines. In this study we utilized the single-molecule RNA in situ hybridization (ISH) assay RNAscope to visualize the expression of multiple ISC markers and the immune response in a mouse model of colitis. This assay is a well-suited method for the cellular resolution of resident ISCs and their progenitors in the normal and inflamed intestine because it detects single RNA molecules in individual cells with morphological context. The impact of inflammation on Lgr5+ ISCs and their niche as well as the Wnt/β-catenin pathway was examined over a time course of TNBS-induced colitis. The cellular source and spatial orientation of several cytokines, chemokines, and their receptors was identified within the inflamed intestinal tissue environment. Overall these results demonstrate the utility of the RNAscope technology in elucidating the direct effects of inflammatory cues on Lgr5+ ISCs and their niche during the pathogenesis of colitis and other inflammatory diseases, as well as developing potential therapeutics.

T-1101

HEPATIC THROMBOPOIETIN IS REQUIRED FOR HEMATOPOIETIC STEM CELL MAINTENANCE

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Maintenance of hematopoietic stem cell (HSC) number and function depends on extrinsic regulatory cues. Currently, only local signals from the bone marrow niche have been shown to maintain HSCs. However, systemic factors can modulate HSC behaviors such as proliferation and mobilization. This has raised the question of whether systemic factors may play fundamental roles in HSC maintenance. We assessed the physiological source of thrombopoietin (TPO), a key cytokine required for maintaining HSCs. Human patients with severe nonsense or missense mutations in TPO or its receptor c-MPL develop congenital amegakaryocytic thrombocytopenia and aplastic anemia. It was previously thought that local bone marrow cells were the critical source of TPO for HSCs. We engineered a novel TpoDsRed-CreER knockin mouse, the first genetic tool for in vivo labeling of cells that successfully translate Tpo transcripts. Unexpectedly, we found that under both steady state and stress conditions, TPO protein was expressed in liver hepatocytes but not cells in the bone marrow. To functionally test the findings of our reporter mouse, we used Cre-lox technology to conditionally delete Tpo from candidate cell populations. Deletion of Tpo from hematopoietic cells, osteoblasts, or bone marrow mesenchymal stromal cells did not affect HSC number or function. However, when Tpo was deleted from hepatocytes, HSCs were depleted from the bone marrow. Notably, we found that inducible deletion of hepatic Tpo in adult mice by a hepatocyte-specific Cre-bearing adenovirus had the same effect as deletion by a transgenic Cre line. This suggests a continuous, rather than developmental, dependence on hepatic TPO. Thus a long-range regulatory factor - circulating TPO made in the liver by hepatocytes - is required for bone marrow HSC maintenance.

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T-1105

ID1 IN MOUSE EPIDERMAL PROGENITOR CELLS

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The choice between stem cell self-renewal and differentiation must be tightly controlled since alterations will lead to disease. However, the transcriptional networks orchestrating and maintaining this balance are poorly understood. The developing interfollicular epidermis (IFE) is an ideal model to study stem and progenitor cell fate as progenitors and differentiated cells can be identified by marker expression and distinct spatial distribution. Our work aims at disclosing mechanisms that dictate progenitor cell fate by studying basic helix-loop-helix transcription factors and their modulators the ID proteins (inhibitor of DNA-binding) in the developing IFE. Immunohistochemical analyzes of the four family members of ID proteins revealed ID1 to be highly expressed in interfollicular epidermal progenitor cells, in the placodes of developing hair follicles at embryonic day (E) 14.5 and also the hair peg at E18.5. In contrast, ID1 expression was weaker in differentiated cells of the IFE. To study the role of ID1 in the IFE progenitor cells, we silenced ID1 expression in the embryonic skin using ultrasound-guided in utero microinjections of lentivirus co-expressing an shRNA against ID1 and the reporter H2BGFP. Silencing of ID1 expression resulted in an 8-fold reduced proliferation at E14.5 when compared to control scrambled embryos, corresponding to an overall loss of infected cells over time. However, we failed to detect any differences in apoptosis. At E16.5, the ID1 shRNA targeted epidermis was significantly thinner than normal, lacked hair follicles and displayed an overall disorganized appearance. We found 7% ID1 shRNA targeted EdU+ proliferating progenitors in suprabasal layers. Furthermore, around 30% of infected and 32% of uninfected progenitor cells co-expressed basal progenitor marker K5 and the suprabasal differentiation marker K10, suggesting that ID1 acts non-autonomously. In vivo E-cadherin mRNA levels were down-regulated in ID1 shRNA targeted epidermis, suggesting that ID1 keeps epidermal progenitor cells attached to their niche by enforcing cell adhesion.

T-1107

BONE MARROW MESENCHYMAL STEM CELLS (BM-MSCS) FROM DECOMPENSATED CIRRHOTICS ARE NOT FIT FOR THERAPEUTIC USES

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Autologous BM-MSCs are often used for hepatic regeneration in patients with advanced cirrhosis. Use of autologous BM-MSCs in chronic liver disease (CLD) has shown varied clinical response ranging from negligible to mild improvement in CTP scores. In the current study we aim to investigate the effect of chronic liver damage on tissue repair and regenerative function of BM-MSCs in Decompensated-Liver-Disease (DLD). MSC were isolated from BM of DLD patients(n=10) and their tissue repair and regenerative function were compared with respect to age-matched healthy BM-MSCs(n=8). cells done as per ISCT criteria In-vitro functional efficacy of cells was accessed by studying their potential to suppress the proliferation of T cells, macrophage polarization, Tube formation & secretome study. In-vivo therapeutic potential studied in mice acetaminophen-induced ALF in mice. Global-transcript profiles of cells were done by NGS. Metabolic profiles of BM plasma were done by 1D NMR. Bioenergetics of cells were done by analyzing the oxygen-consumption-rates(OCRs) and Extracellular-Acidification-Rate(ECAR) using the Seahorse. Stamens property of cells was analysed by CFU-F. and senescence property by SA-βGal assay. Similar to HBM-MSCs, DBM-MSCs fulfilled the minimal criteria for MSC; plastic-adherence, spindle shape, inducible-osteo/ adipogenesis. In compression to HMSCs, DMSCs showed significant defect in immunomodulatory functions,Angiogenic support and paracrine support While infusion of HMSC significantly improve the 48 hour survival after APAP induced liver injury,DMSC failed to improve the survival, suggesting the loss of therapeutic potential of DMSCs. NGS analysis showed impeachment of genes associated to insulin-resistance in DMSC. DMSCs were significantly defective in glucose-uptake(and are bio energetically quiescent. NMR analysis showed increased level of glucose in DLD BM-plasma in comparison to non-cirrhotic control. This might be responsible for insulin-resistance and loss of function in DMSCs. Further analysis showed the loss of self-renewal capacity replicative senescence in DMSCs. BM-MSC in DLD patients are defective in their regenerative functions due to premature aging and senescence and are not good candidates for MSCs transplantation.

T-1109

STOCHASTIC MIGRATION EVENTS DRIVE COMMITMENT AND TRANSCRIPTIONAL STATE CHANGE IN THE MOUSE NEPHRON PROGENITOR NICHE

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Nephrons in the mammalian kidney are formed from self-renewing mesenchymal progenitors that reside within a niche defined by the cap mesenchyme and ureteric epithelial tip. Throughout mouse kidney development progenitor cells differentiate in response to inductive cues and exit the niche to form nephrons. The cap mesenchyme population is highly dynamic and cells migrate both within and between domains in response to niche cues. The process by which this motile progenitor population initiates nephrogenesis in a precise, spatially regulated manner is not well understood. We activated a fluorescent reporter using Wnt4-CreERT2, a tamoxifen-inducible marker of terminal commitment, and observed labelling within differentiating cells as well as a proportion of apparently uncommitted progenitors. Whole organ image analysis revealed a Wnt4 lineage that originates at sites of differentiation, but re-enters the niche over time. We observed cells undergoing niche re-entry in live time lapse imaging, and an increase in migration speed in the Wnt4-Cre labelled population compared to cells labelled with progenitor marker Six2-Cre. Faster moving nephron progenitors appear to escape commitment by limiting their exposure to inductive signals. Whole kidney single cell transcriptional profiling identified Wnt4-Cre labelled cells with both committed and progenitor-like profiles. Thus Wnt4 lineage cells appear to be able to move both toward a nephron state or back into the progenitor pool. We propose a model whereby fate depends on stochastic movement events that determine the extent of exposure to spatially defined inductive cues, enabling commitment and renewal to be regulated as niche and organ size change.

Funding Source: National Health and Medical Research Council, Australian Research Council

T-1111

MURINE SPLEEN STROMAL NICHES WHICH SUPPORT MYELOID RESTRICTED HEMATOPOIESIS

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Hematopoiesis occurs throughout the lifespan of an organism. This process involves the formation of blood cells from self-renewing hematopoietic stem cells (HSC). The hematopoietic niche environment, which comprises non-hematopoietic stromal cells, regulates the quiescence, dormancy, self-renewal and differentiation of HSC. It is known that multiple HSC niches including endosteal, vascular and perivascular exist in the bone marrow, although niches in other sites like spleen are only starting to be defined. Previous studies in this lab have described unique splenic stromal cell lines 5G3 and 3B5 which can support restricted hematopoiesis in vitro reflecting HSC niches. Inhibition assays were also used to identify the important role of SCF and Notch but not C-XC motif ligand 12 (CXCL12) in supporting in vitro hematopoiesis. Transcriptome data previously showed that 5G3 and 3B5 express many genes in parallel with perivascular cells described in bone marrow. The study here shows that both 5G3 and 3B5 stroma share lineage origin with perivascular cells in bone marrow. 5G3 and 3B5 express cell surface markers in common with these cells including CD105, CD29, VCAM1, Sca-1, CD51, CD140a and Thy1.2. In addition, freshly isolated splenic stromal cells with the Sca-1+gp38+Thy1.2+CD29+CD51+ phenotype, reflecting cells of mesenchymal lineage were found to be important for in vitro hematopoiesis. Stromal cells expressing gp38 or Thy1.2 are also associated with HSC in spleen identified through section staining. Restricted hematopoiesis giving rise to specific types of myeloid cells was replicated in vivo following grafting of splenic stromal cell lines 5G3, 10C9 and 3B5 under the kidney capsule. This study has improved our understanding of HSC niches in spleen and identified several molecular regulators of hematopoiesis. Information obtained will be important for development therapies involving splenic or ectopic niches to enhance hematopoietic output, in immunocompromised patients or following HSC transplantation.

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T-1113

INTERROGATING THE MECHANISMS UNDERLYING COGNITIVE IMPAIRMENTS IN TYPE 1 DIABETES USING HUMAN EMBRYONIC STEM CELLS

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Type 1 diabetes mellitus (T1D) is an early onset childhood disease with increasing incidence worldwide. Children and adolescents with T1D are more likely to suffer from neurocognitive deficits, including poor attention, language, memory, information processing speed and executive skills compared with their non-diabetic peers. The severity of the problem demands a better understanding of the mechanisms underlying the long-term consequences of T1D on cognitive functions. The first step is to increase our understanding of the direct effects of hyperglycemia on neuronal structure and function in the brain. Cortical neurospheres created from human embryonic stem cells (hESC), offer a powerful and amenable in vitro model of cellular self-organisation of the forebrain cerebral cortex in the developing brain, allowing practicable manipulation of the external environment, in the absence of other complicating factors. There for the aim of this study was to expose hESC-derived cortical neurospheres to a T1D-like microenvironment and investigate how altered glucose\insulin levels may impact neurogenesis and neuronal differentiation. Culture conditions for hESC neural induction were established to mimic the physiological levels of glucose and insulin within the brain. To assess the impact of glucose and insulin on neural differentiation, cell viability assays were used to measure cell death, and quantitative gene expression analysis of neuronal and glial markers associated with neuronal differentiation, cell fate, maturation and connectivity, and the expression of glucose transporters and insulin receptors, were performed on resultant neurospheres. Data revealed a decrease in neuronal and cell viability as a result of lower insulin or glucose, as well as changes in expression of glial and neuronal markers, synaptic connectivity markers, early cortical progenitor and glucose transporter markers in 2 week old neurospheres exposed to different glucose conditions. These preliminary findings suggest that altered glucose

levels may affect neuronal differentiation. Further analyses will determine whether these changes are reversible, including on downstream factors regulating glucose uptake. Such analyses are significant for developing therapeutic interventions to treat cognitive dysfunction in TD1.

Funding Source: The Melbourne International Research Scholarship; The Murdoch Children's Research Institute; Stem Cell Australia

NEURAL DEVELOPMENT AND REGENERATION

T-2001

MODIFICATIONS OF MSCS WITH HGF AND GDNF FOR NERVE REGENERATION

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Ischemia is known to trigger pathological changes resulting in neuropathies, which accompany limb ischemia and leading to multifocal sensory and motor axonal loss. In our study we examined effect of neurotrophic and angiogenic cytokines on vessel and axonal growth. This can facilitate the reparative potential of the cells via their paracrine effect on blood vessel growth and induction of nerve recuperation. We introduced genetic modifications of human adipose-derived mesenchymal stem cells (ASCs) using hGDNF- and hHGF-containing AAV virus 2 serotype. Conditioned medium (CM) of HGF-expressing ASCs significantly stimulated angiogenesis in in vitro model of HUVEC tubulogenesis on Matrigel. In contrast, in GDNF-expressing cells these effects were comparable to the effect of unmodified cells CM. The addition of HGF-expressing ASCs to endothelial cells immobilized on dextran beads in fibrin gel also stimulated growth of vessel-like structures from the beads, increasing the sprouts length and number by 2.5-fold at the day 3 of co-culture. However, CM of GDNF-expressing ASCs showed significantly lower effect than HGF in this model. Using the model of neurite outgrowth from spinal ganglia of neonatal mice we have shown the cooperative effect that combined CM of modified ASC producing mHGF and mGDNF on cell migration from the ganglion and the rate of neurite growth. Experiments with recombinant proteins confirmed the cooperative effect of GDNF/HGF combination primarily on the growth rate of neurites (24 hours). It was found that CM from unmodified ASCs did not induce differentiation of the neuroblastoma cells, while CM from HGF - ASC, GDNF-ASC and HGF/GDNF-ASC stimulated neuroblastoma cell differentiation and neurite outgrowth with the greatest effect of CMs from GDNF-ASCs and HGF/GDNF-ASCs. CM from HGF - ASC or GDNF-ASC increased the length of neurite growth almost twice as compared to the control (CM of ASC), however while in combination the effect of HGF/GDNF-ASCs CM was almost threefold greater. We have found that both factors activated C-met in neuroblastoma cells, but only GDNF can stably activate Erk1/2 signaling. The combination of HGF and GDNF demonstrated a cooperative effect on the activation of the C-met and the Erk1/2 signaling pathway.

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T-2003

CLONAL LINEAGE TRACING AND SINGLE CELL ANALYSES OF NEURAL STEM AND PROGENITOR CELLS IN THE MOUSE EMBRYONIC VENTRAL FOREBRAIN GERMINAL ZONE

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We define two distinct types of neural stem cells (NSCs) 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 embryonic-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 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 many more unipotent neuronal progenitor cells that were surprisingly 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 gene expression differences between these NPCs, we performed Drop-seq on E17.5 neural stem and progenitors cells from neurospheres grown in either LIF or FGF2/EGF. 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.

T-2005

NOVEL HUMAN GAPTRAP HPSC LINES FOR TRACTABLE AND MODULATORY IN VIVO ANALYSIS IN RODENTS

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The directed differentiation of human pluripotent stem cells (hPSCs) to neuronal subtypes represents a valuable source for cell replacement therapies to treat diseases. Integral to the progression and realisation of neuronal cell therapy is a detailed understanding of the in vivo properties of stem cell-derived neurons. To assess hPSC-derived neurons in vivo we utilized novel GAPTrap lines that have been genetically modified to constitutively express transgenes encoding fluorescent proteins; green fluorescent protein (GFP), tandem tomato (Tdt), or blue fluorescent protein (BFP) to track and characterise in detail the anatomy of human grafted cells in vivo. GAPTrap reporter hPSCs were differentiated efficiently to neural progenitors in vitro and transplanted into rodent brains. GAPTrap grafts maintained robust reporter expression over long periods in vivo and analysis of graft fiber patterns revealed extensive axonal growth in the host brain. Grafts were composed of a mix of neural cell types including differentiated neurons and glia, but also dividing neural progenitors and migrating neuroblasts. Patch-clamp recordings at 10, 26 and 50 weeks post-transplantation showed progressive maturation of neurons, with the majority of cells firing spontaneous action potentials by the later time point. To assess the functional integration of human grafts we have generated modulatory hPSC lines that constitutively express either channelrhodopsin (ChR2) or DREADD receptors (hM4Di) that allow us to switch 'on' or 'off' neuronal firing. The use of these reporter and modulatory hPSC lines are allowing us to dissect the mechanisms through which human grafted neurons elicit therapeutic benefits in rodent models of neurodegeneration.

Funding Source: NHMRC, Australia

T-2007

CO-ORDINATE SIGNALING GRADIENTS REGULATE DEVELOPMENT OF VENTRAL FOREBRAIN NEURAL SUBTYPES IN VITRO

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The basal ganglia is a collection of neurons in the ventral forebrain and plays a critical role in controlling movement and emotion. Movement disorders such as Huntington's disease largely affects a population of GABA-producing, inhibitory neurons termed medium spiny neurons (MSNs). These neurons reside in the striatum and send efferent projections to distinct neuroanatomical structures. During development, MSN progenitors are generated in close proximity to interneuron progenitors within the basal ganglia where morphogen signalling gradients influence intrinsic molecular program to differentially pattern neural subtypes. The purpose of this study was to optimize current differentiation trends of human pluripotent stem cells using extracellular matrices (ECMs), growth factor signaling gradients and genetic approaches. These modifications allowed the generation, identification and isolation of in vitro-derived (IVD) MSN progenitors for developmental profiling & limit development of unwanted interneuron cell types. In vitro differentiation toward striatal progenitors was achieved using a 2D-based adherent protocol with key MSN progenitor markers including MEIS2, ISL1 & CTIP2 enriched in growth factor-optimized conditions. Extensive molecular profiling of these cultures at multiple time points throughout differentiation, revealed stage-specific determinants of striatal/MSN identity. Expression of the dopamine-and-cAMP regulated phosphoprotein (DARPP-32) was observed only after terminal differentiation revealing an extensive

network of GAD65/67+DARPP-32+/CTIP2+ neurons. Using genetic & immunocytochemistry end points, we demonstrate that fine-tuning growth factor signaling gradients and ECMs during in vitro differentiation of striatal progenitors, is sufficient to enrich for MSNs.

T-2009

USP9X-NULL MICE SHOW CORPUS CALLOSUM DYSGENESIS AND CORTICAL DYSPLASIA

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Autism spectrum disorder (ASD) is a form of neurodevelopmental disorder that manifests in three major diagnostic features: deficits in social interactions, verbal and non-verbal communication, and restricted repetitive behavior. Interestingly, the comorbidity of Intellectual Disability (ID) and ASD sits at around 60%. One of the genes that is implicated in ID and ASD, is the deubiquitylating enzyme ubiquitin specific peptidase 9, X-linked (Usp9x). Using a conditional knock-out (cKO) mouse model, current study investigates the role of Usp9x in the pathophysiology associated with ID and ASD. Adult Usp9x cKO brains were stained using hematoxylin and cortical lamination markers. The thickness of the corpus callosum was measured from the rostral to caudal aspect of the brain at the midsagittal line on coronal sections. A significantly thinner corpus callosum was observed in the cKO brains. The length of the corpus callosum was measured on midsagittal sections from the rostral-most point to the caudal-most. Little to no visible corpus callosum was observed in the cKO brains midsagitally. Hematoxylin staining revealed corpus callosum dysgenesis of the cKO brain. Axonal tracts failed to project across the midline. The thickness of somatosensory, somatomotor and cingulate cortices were measured from the first cortical layer to the corpus callosum. Despite the unaltered overall cortical thickness, a significant decrease in the cellular density in the somatosensory and somatomotor cortices were observed in the cKO brains compared to controls. A significant increase in the height of the cingulate gyrus and volume of the lateral ventricle were also observed in the cKO brains. These findings show that the deletion of Usp9x in the cortex leads to corpus callosum dysgenesis and cortical dysplasia. Further experiments are warranted to confirm the ASD-like behaviours in Usp9x KO mice and link them to the underlying mechanisms, which may help to identify novel therapeutic targets for ASD.

T-2011

THE HISTONE DEMETHYLASE KDM5A REPRESSES ASTROCYTOGENESIS IN NEURAL STEM CELLS AND IS REGULATED BY THE TRANSLATIONAL MACHINERY

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Histone demethylases are known to play important roles in stem cell fate determination and in cancer progression. In this study, we show that the lysine 4 of histone H3 (H3K4), lysine-specific demethylase 5A (KDM5A) is essential for the repression of astrocyte differentiation in neural progenitor cells (NPCs), and its expression is regulated by translational machinery. Knockdown of KDM5A in NPCs increased astrocytogenesis, and conversely, KDM5A overexpression reduced the transcriptional activity of the *Gfap* promoter. Induction of astrocytogenesis by ciliary neurotrophic factor (CNTF) or small interfering RNA-induced knockdown of KDM5A decreased KDM5A recruitment to the *Gfap* promoter and increased H3K4 methylation. The transcript level of *Kdm5a* was high, whereas KDM5A protein level was low in CNTF induced astrocytes. During astroglial differentiation, translational activity indicated by the phosphorylation of eukaryotic translation initiation factor (eIF)4E was decreased. Treatment of NPCs with the cercosporamide, a MAPK-interacting kinases inhibitor, reduced eIF4E phosphorylation and KDM5A protein expression, increased GFAP levels, and enhanced astrocytogenesis. These data suggest that KDM5A is a key regulator that maintains NPCs in an undifferentiated state by repressing astrocytogenesis and that its expression is translationally controlled during astrocyte differentiation. Thus, KDM5A is a promising target for the modulation of NPC fate. The histone demethylase KDM5A is required for the repression of astrocytogenesis and regulated by the translational machinery in neural progenitor cells.

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T-2013

TRANSPLANTATION OF DIRECTLY REPROGRAMMED OLIGODENDROCYTE PRECURSOR CELLS ENHANCE FUNCTIONAL RESTORATION AFTER SPINAL CORD INJURY

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Spinal cord injury (SCI) generally results in neuron loss and demyelination of the nerve axons at and near the site of injury. Demyelination causes immediate cell damage and subsequently leads to sensory and motor dysfunctions that may ultimately produce a fatal paralysis. Since there are no full restorative treatments yet available, cell replacement therapy by transplanting directly reprogrammed oligodendrocyte precursor cells (iOPCs) for restoration of demyelinated lesions is a promising treatment option promotes axonal remyelination and contributes to functional restoration following SCI without tumor formation. In this study, we transplanted iOPCs into contused spinal cord of rats and found that transplanted iOPCs were survived, migrated adjacent to the injured spinal cord, and successfully differentiated into NG2+, APC-CC1+, O4+, and MBP+ cells at 8 weeks after transplantation. The electrophysiological study by motor evoked potentials (MEPs) revealed the majority of motor nerve conduction velocities and onset latency were improved, and the bladder and locomotor dysfunctions were decreased than control at 8 weeks after transplantation. The present data establish the importance of iOPCs in the functional recovery of the injured spinal cord after transplantation. Therefore, we conclude that transplantation of iOPCs play a key role in promoting functional restoration following SCI in rats.

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T-2015

DYNAMIC ALTERATION OF IMPRINTED GENES DURING NEURAL DIFFERENTIATION OF HUMAN PARTHENOGENETIC PLURIPOTENT STEM CELLS

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Imprinted genes modulate the growth and development in mammalian, and the abnormal expression of them causes genetic disorders. Notably, since most imprinted genes were highly expressed in brain, the loss of the function of imprinted genes in brain can lead to brain genetic disorders. To establish a human in vitro model for investigation of genomic imprinting status in neurodevelopment, we used human uniparental pluripotent stem cells that originated from benign ovarian teratoma and possessed only maternal alleles. We analyzed DNA methylation status and gene expression of imprinted genes associated with brain development and confirmed cell type-specific or parent-origin-specific alterations of DNA methylation and gene expression of imprinted genes during neural differentiation. The results were consistent with previous reports using animal model and human patient samples. Furthermore, we confirmed new aspects of genomic imprinting status throughout neural differentiation from neural stem cells to terminally differentiated cells, neurons and astrocytes. Thus, this study suggests that human parthenogenetic induced pluripotent stem cells and differentiated cells from that can be used for screening of imprinted genes related with neurodevelopment and can be a useful in vitro model to study roles of imprinted genes in neurodevelopment and human neurodevelopmental disorders.

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T-2017

OPTIMISING METHODS FOR MURINE MESENCHYMAL STEM CELL NEURAL INDUCTION

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Mesenchymal stem cells (MSCs) are multipotent stem cells with well-established neurogenic properties. Human MSCs (hMSCs) provide an ideal candidate for in vitro modelling of human neurogenesis, bypassing the ethical considerations and highly technical skills required for other neurogenic stem cells. Unfortunately, MSCs are often unavailable from patients with severe neurological conditions due to the invasive nature of harvest. MSCs isolated from murine models (mMSCs) of neurological disease present an alternative source of disease MSCs; however, methods for mMSC neural induction are not well established. This study aimed to determine an optimum method for mMSC neural induction by comparing the baseline gene expression profiles of mMSCs and hMSCs over the course of neural induction using two different induction methods; a well-established method involving neurogenic priming for seven days before promoting induction with retinoic acid (NP+RA), and a modified method which excluded neurogenic priming (RA). Both methods successfully induced neurogenesis in hMSCs; significant increases in nestin and NCAM gene expression were seen 14 days and 16 days post-induction when using the NP+RA and RA methods respectively. Both methods successfully formed mature, post-mitotic neurons, as evidenced by significant elevations in β-tubulin, Neurofilament-H and Enolase 2 (NSE) expression over the course of induction. Similarly to hMSCs, both methods successfully initiated neural induction in mMSCs; however, neurogenesis was significantly accelerated. Regardless of method, neuron-like morphology was evident within four hours of neural induction, coupled with significant elevations in nestin

expression. Mature neurons were formed within 12 hours of induction, evidenced by significant, concomitant increases in β-tubulin and Neurofilament-M expression. Whilst not dependent on neural priming for successful neural induction, priming enhanced neurogenesis in both human and murine MSCs, with further upregulation of neural genes. Due to the accelerated nature of mMSC neurogenesis, further promotion with RA following neural priming was not required. This study therefore provides a simple method for mMSC neural induction which can be easily transferred to disease mMSCs for in vitro modelling of neurogenesis.

T-2019

NEURAL PROGENITOR CELL DAMAGE LEADS TO LONG-TERM LEARNING AND MEMORY DEFICITS IN A MOUSE MODEL OF PRETERM BIRTH RELATED NEONATAL LUNG INJURY

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Bronchopulmonary dysplasia (BPD), a chronic lung disease, is an independent risk factor for adverse neurodevelopment and the most common complication of preterm birth. Oxygen supplementation, mechanical ventilation, and inflammation are all key factors contributing to BPD; however, the mechanism leading to BPD-associated brain injury remains unclear. Neural progenitor cells (NPCs)—crucial for brain development—remain unexplored in BPD. We hypothesize that NPCs are impaired in experimental BPD, leading to adverse neurodevelopment. To mimic BPD, neonatal mice were exposed to either: 1) hyperoxia (80-85% oxygen) for 10-14 days or 2) postnatal inflammation and mechanical ventilation for 8 hours. Tissue was harvested from P9-P14. The mean linear intercept (MLI) was used to assess lung damage. In vitro NPC function was assessed using clonal neurosphere assays. In vivo neurogenesis was examined using confocal microscopy. Long-term follow-up at 4.5 months and 1 year included behavioural tests to evaluate neurodevelopment. Mice from both experimental BPD models displayed the characteristic alveolar enlargement and simplification compared to control mice. This was quantitatively confirmed by the MLI. NPCs from the subventricular zone (SVZ) of experimental BPD mice formed significantly fewer secondary neurospheres compared to control mice. Confocal microscopy revealed significantly fewer newborn neurons in the SVZ and dentate gyrus of hyperoxia-exposed mice. At 4.5 months, fear conditioning demonstrated that hyperoxia-exposed mice have significant learning and memory deficits. At 1 year, fear conditioning and the Morris Water Maze tests further confirmed this finding. These results show that mice with experimental BPD have decreased NPC self-renewal and fewer newborn neurons in the SVZ and dentate gyrus, indicating an impairment in postnatal neurogenesis. Furthermore, long-term follow-up revealed significant learning and memory impairments—key cognitive functions linked to postnatal neurogenesis. Together, these findings demonstrate that NPCs are perturbed in experimental BPD, leading to long-term adverse neurodevelopment. These new insights into possible mechanisms of BPD-associated brain damage may help to identify treatments to improve the outcome of preterm infants.

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T-2021

FUNCTIONAL PROPERTIES OF STEM CELL DERIVED NEURONS: CORRELATIONS WITH IN VIVO NEURODEVELOPMENT

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Epileptic encephalopathies are severe disorders characterized by pharmacoresistant seizures, intellectual disability and developmental delay in the affected children. An increasing number of genes has been related to these disorders necessitating the functional verification of detected variants and corresponding pathophysiological explanation. Use of human induced pluripotent stem cells (iPSC) -derived neurons enables disease modeling respecting the unique human biology and enabling studies of neuronal processes in the early stages of development. However, little is known about how the mechanisms of neuronal in vitro maturation

based on different differentiation protocols relate to the particular phases of neuronal development *in vivo*. We investigated here iPSC-derived neurons generated using two differentiation protocols, transgenic overexpression of NGN2 and embryoid body-based dual SMAD inhibition. The single cell electrophysiology was used as readout of the maturity of differentiated neurons. The NGN2 based protocol provided homogenous populations of firing neuronal cells in less than three weeks of differentiation. In contrast, embryoid body procedure required about three months to produce reliably firing populations of neurons, often including cells at different stages of maturity. A more precise look into passive membrane characteristics including capacitance and input resistance, active membrane characteristics such as sodium and potassium currents, or single-cell firing and spontaneous network activity revealed significant differences between the two protocols. The NGN2-derived neurons presented with activity resembling early phases of neuronal development and were lacking synaptic activity, while more mature neuronal and spontaneous oscillatory activity, resembling the activity of cortical neurons of human foetus/newborn were found for the embryoid body-derived neurons. We suggest that the observed differences in neuronal activity and connectivity should be taken into consideration when designing epileptic encephalopathy human in vitro models in order to match timing of disease manifestation to the relevant phase of neurodevelopment.

T-2023

MHC MATCHING IMPROVES ENGRAFTMENT OF IPSC-DERIVED NEURONS IN NON-HUMAN PRIMATES

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The banking of human leukocyte antigen (HLA)-homozygous induced pluripotent stem cells (iPSCs) is considered a future clinical strategy for HLA-matched cell transplantation to reduce immunological graft rejection. On the other hand, brain is less immune-responsive tissue. The object of this study is to confirm the efficacy of major histocompatibility complex (MHC)-matching in allogenic neural cell transplantation in the brain of non-human primate. Two iPSC lines established from MHC homozygous cynomolgus macaque (HT1 and TH4) were used as donors. Dopamine neurons were differentiated from MHC-homo iPSCs and were transplanted to MHC-heterozygous macaques (MHC-matched transplantation) and control monkeys (MHC-mismatched transplantation). Positron emission tomography (PET) imaging, (S)-11C-KTP-Me and 11C-PK11195, revealed neuroinflammation associated with an immune response against MHC-mismatched grafted cells. Immunohistological analyses reveal that MHC-matching reduces the immune response by suppressing the accumulation of microglia (Iba-1+) and lymphocytes (CD45+) into the grafts. Consequently, MHC-matching increases the survival of grafted dopamine neurons (tyrosine hydroxylase: TH+). The effect of an immunosuppressant, Tacrolimus, is also confirmed in the same experimental setting. We show the efficacy of major histocompatibility complex (MHC)-matched allogeneic neural cell grafting in the brain, which is considered a less immune-responsive tissue. Our results demonstrate the rationale for MHC matching in neural cell grafting to the brain and its feasibility in a clinical setting. We will discuss the strategy of a coming Japanese clinical trial of stem cell therapy in Parkinson's disease from the point of immunological rejection.

Funding Source: Japan Agency for Medical Research and Development (AMED)

T-2025

SINGLE-CELL RNA SEQUENCING REVEALS DIFFERENTIATION TRAJECTORIES DIRECTING HUMAN NEURAL CREST FORMATION AND DIFFERENTIATION

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Neural crest (NC) is a transient population of multipotent progenitor cells in vertebrates, contributing to the formation of various organ systems. NC induction occurs at the neural plate border and involves the orchestration of multiple events. After NC cells emerge from the closing neural tube, they delaminate from the neural tube through an epithelium-mesenchymal transition and then migrate extensively to their target sites, depending on the axial level of their origin. NC cells are of ectodermal origin but exhibit a remarkably broad differentiation potential. The different phases of NC development are mediated by unique sets of specifiers and that confer

to the cells the ability to migrate and differentiate. A stepwise differentiation protocol has been established for generation of NC from human pluripotent stem cells (hPSC). However, how the in vitro differentiation relates to embryonic development is still unclear. Here, we applied single-cell RNA sequencing to establish the differentiation trajectories directing human NC formation and differentiation in vitro. Due to asynchrony in differentiation, five distinct cell populations were identified from hPSC-derived NC cells. They represent cells at the neural plate border, specified NC and the post-migratory NC, resembling the key intermediate states during embryonic development. Intriguingly, after NC specification, NC differentiation diverges into three transitional states in which the NC intermediates possess distinctive differentiation plasticity towards the neurogenic, skeletogenic and myogenic lineages and with different proliferation capacity. In summary, NC formation and its stereotypical sequence of intermediate states can be nicely recapitulated in vitro using hPSC.

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NEURAL DISEASE AND DEGENERATION

T-2027

RAPID GENERATION OF MATURE CORTICAL AND SPINAL ASTROCYTES FROM HUMAN IPSCS

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Growing evidence implicates glia, particularly astrocytes, in neurological and psychiatric diseases. Astrocytes perform a variety of essential functions including glutamate regulation, axonal guidance, trophic support, inflammatory response, wound healing, formation of the blood-brain barrier, and neuronal synapse formation. Human cortical astrocytes are larger, structurally more complex and diverse, and respond differently to extracellular glutamate compared to their rodent counterparts. Given the unique biology of human astrocytes, it is critical that improved human-specific cell-based systems be established to enable the study of human astrocytes in health and disease. Because of the limited availability of primary human astrocytes, human iPSCs are currently used as a source of astrocytes. However, existing methods for astrocyte generation are slow (up to 6 months) or require additional selection to reduce heterogeneity. To rapidly generate mature astrocytes for disease modeling, we have developed a novel protocol that uses inducible expression of astrocyte differentiation master transcription factors NFIA and SOX9 and an optimized astrocyte differentiation medium. Human cortical or spinal astrocytes can be generated from normal or disease iPSCs in only one month. They express key astrocyte markers GFAP and S100β at >90% and exhibit mature process-bearing morphologies. These astrocytes can promote neuron synapse formation and functional activity in MEA and calcium imaging applications, and elicit a strong and rapid pro-inflammatory response. This protocol represents an important tool for modeling neurological diseases using a human iPSC-based astrocyte-neuron coculture platform, allowing the role of diseased astrocytes in neuronal degeneration to be investigated.

T-2029

GLOBAL TRENDS OF CLINICAL TRIALS FOR STROKE BY CELL THERAPY

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Treatment of stroke is an important thesis of regenerative medicine in the cerebrovascular field. So far, any cell therapy products for stroke are not approved nor on the market. In this study, we examined the clinical research trends related to cell therapy products in the stroke field based on data obtained from the ClinicalTrial.gov website. Although this website does not provide comprehensive results of clinical trials, it offers information on prospective clinical trials, including work in progress, and thus allows chronological analysis of the data. We selected 58 studies for cell therapy related to the field of stroke treatment from ClinicalTrial.gov. China with Taiwan had 17 studies, ranking the top of the countries, followed by the US, India, France, and Spain. Classifying with the cell source used, the studies used bone marrow (BM)-based cells occupied a half of the whole. Investigating this in chronological order, the use of BM as the cell source had been initially predominant, but thereafter several kind of cells were tried, and especially the use of umbilical cord-derived cells increased from around 2012. Classifying with the origin (autologous or allogeneic) of cell source used, the studies used autologous cells occupied 53% of the whole, but any chronological bias was not found. Regarding the clinical development status, we found 22 studies at Phase I, 17 at Phase I / II, 14 at Phase II, 2 at Phase II / III, and 1 at Phase III. Of the target diseases, 71% was against ischemia and 3% was against hemorrhage. Of ischemic stroke, 47% was for acute phase and 41% was chronic phase. Regarding the target diseases, 71% of whole was against ischemia and 3% was against hemorrhage. Of ischemic stroke, 47% was for acute phase and 41% was for chronic phase. Considering this in chronological order, from 2005 to 2009 acute ischemic stroke was the dominant target disease, whereas studies on chronic ischemia has become dominant from 2010

to 2014. However, acute ischemia has returned to be the dominant again after 2015. Thus, we found a trend that the target of clinical studies shifted from those for chronic ischemic stroke to acute one. Currently, the above two Phase II/III studies are being implemented in this trend. The use of ClinicalTrials.gov as the sole data source can yield a perspective view of the global clinical translational trends.

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T-2031

COMPARISON OF THERAPEUTIC EFFICACY OF HUMAN NEURAL CORTICAL PROGENITORS TO HUMAN UMBILICAL CORD STEM CELLS IN A RAT MODEL OF NEONATAL ISCHEMIC BRAIN DAMAGE.

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Stem cell therapies have been postulated as a viable therapeutic tool in neuro-protection along with both neural repair and regeneration. While work with human umbilical cord blood cells (hUCBCs) can be described as safe, there is little evidence of substantial and consistent gain of function. Currently there is no preclinical work examining the differences in therapeutic efficacy between hUCBCs and pluripotent cells that have been differentiated to a neural lineage. It was the purpose of this study to elucidate these differences and determine whether any evidence exists for the therapeutic use of either or both cell sources following neonatal ischemic stroke. Long Evan Rats (N=192) underwent induction of ischemic stroke at P1 or were held as naïve controls over two experiments. Lesioned animals were separated by treatment at P3 where multiple doses and methods of administration for hUCBCs were included. Motor and cognitive behaviour was assessed through rotarod, staircase, digigait and touchscreen methods. Holistically the data indicated that grafts of neural progenitors but not hUCBCs improved behavioural outcomes compared to ischemia alone. Systematic histological analysis was also performed. This analysis revealed a comparable level of atrophy among stroke induced groups at the target site. However, a reduction in atrophy relative to lesion alone groups distal to the target site was observed in the group which received cortically differentiated pluripotent cells. This suggests a neuroprotective effect, one that was not present in the group which received cord blood cells. Moreover, cortical progenitor cells were found to significantly protect white matter while hUCBC administration was found to show exacerbated white matter damage relative to controls. This analysis failed to find any surviving cord blood cells in the brain at either 7 days or 12 weeks of age, while cortically differentiated pluripotent grafts were present and displayed appropriate markers at both time points. Ultimately this study provides compelling evidence to support the use of neural progenitor cells as a therapeutic option following neonatal ischemic stroke, over the use of HuCBCs. Nonetheless, more research is required with this study raising poignant questions for future investigation.

T-2033

HUMAN NEURAL STEM CELLS FOR THE TREATMENT OF SPINAL CORD INJURY

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Previous studies have demonstrated functional recovery of rats with spinal cord contusions after transplantation of rat fetal neural stem cells adjacent to the site of injury. The purpose of this study was to assess the efficacy of fetal derived hNSC injected either locally or distally to the site of injury in the rat model of acute and chronic spinal cord injury (SCI). The human fetal cortical cells (14 weeks of gestation) were propagated using a proprietary patented scale up technology in the low concentration of fetal bovine serum (0.1%) and low O2 (5%). They were tested for the expression of specific markers, in vivo migration capabilities, and ability to restore locomotor functions in rats using BBB Rating Score following either local (at the site of injury) or distal (intrathecal) injection. The cells expressed positive markers such as Nestin, Sox-1, and Sox-2 and differentiated in vitro into mature neuronal and glial cells. Furthermore, hNSCs demonstrated the ability to migrate in vivo following the injection into chicken embryonic brain. In the acute model of SCI, a statistically significant functional improvement measured using the BBB Score was observed in subjects that received hNSCs injected either locally or distally to the site of injury compared to operated control rats which received isotonic solution alone (p=0.001 and 0.004 respectively). Rather unexpectedly, in the chronic model, the distant hNSC injection led to dramatically better recovery compared to control or locally injected rats. Taken together, this study suggests that fetal derived

hNSCs grown under low serum low O2 conditions can be efficient in the treatment of both acute and chronic SCI using clinically safe intrathecal injection procedure distal to the site of injury.

T-2035

HEALING EFFECTS OF INTRAVENOUS INJECTION OF HO-1 OVEREXPRESSED ADIPOSE DERIVED MESENCHYMAL STROMAL CELLS FOR ACUTE CANINE SPINAL CORD INJURY

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Mesenchymal stromal cells (MSCs) administered through intravenous (IV) route can migrate to the injured spinal cord segment. This route could be available easily for anti-oxidant and - inflammatory effects on acute spinal cord injury (SCI). Frozen thawed MSCs could be used instantly for emergency treatment. Due to the strong anti-oxidant and -inflammatory effects of heme oxygenase 1 (HO1) we produced canine HO-1 overexpressed MSCs (HO1 MSCs). Freshly cultured HO-1 MSCs and frozen thawed HO1 MSCs (FT-HO1 MSCs) were prepared and administered IV and compared their effects with simple GFP-MSCs. The study was performed on 12 SCI dogs divided into three groups. The pre-cultured fresh HO1 MSCs and GFP MSCs were administered IV, at the rate of 1 × 107 cells resuspended in a Hartmann's solution. In third group we injected 1.5 × 107 frozen thawed HO1 MSCs (FT-HO1 MSCs) IV. The dogs were kept for 4 weeks. At each week the dogs were evaluated for the improvement in their hind limbs locomotion by canine basso beattie bresnahan score (cBBB score). At the end of experimental period the spinal cords were harvested and used in the western blotting to determine the expression of inflammatory, anti-inflammatory cytokines and neural markers. Hematoxylin and eosin staining was used to evaluate the degree of fibrosis. The fresh HO-1 MSCs group showed significant improvement of cBBB score in a short time of 4 weeks compared to fresh GFP MSCs. FT-HO1 MSCs group showed higher cBBB score than GFP MSCs group however it was not statistically significant. The fresh HO1 MSCs showed significantly higher expression of NF-M and reduced astrogliosis compared to GFP MSCs. there was a reduced expression of pro-inflammatory cytokines (IL6, TNF alpha) and a higher expression of anti-inflammatory cytokines (IL-10, HO-1) by fresh HO1 MSCs compared to GFP MSCs and FT-HO1 MSCs. Immunocytochemistry results showed that HO1 MSCs did similar migration towards the injured segment as did by GFP MSCs. All the groups showed positive expression for green fluorescent proteins which indicated the successful migration of transplanted cells and their survival until 4 weeks. Owing to the stronger anti-inflammatory capability of HO1 MSCs they promote healing of the spinal cord more than the GFP MSCs.

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T-2037

PATIENT-SPECIFIC IPSC-DERIVED DOPAMINERGIC NEURONS EVOKE MINIMAL AUTOLOGOUS T-CELL RESPONSES IN VITRO

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One of the primary goals of cell replacement therapy is to use human pluripotent stem cell (hPSC)-derived populations to replace diseased cells. A significant hurdle in achieving this objective is overcoming rejection of transplanted material. Parkinson's disease (PD) is a neurodegenerative disorder that affects the motor system through the loss of dopamine (DA)-producing neurons in the substantia nigra. To address the potential utility of cell replacement therapy for the treatment of PD, we evaluated patient-specific immune responses to autologous and allogeneic iPSC-derived DA neurons. Induced pluripotent stem cells (iPSCs) were derived from PD patient cells and differentiated into DA neurons. The neurons innervated nude rat striatum after transplantation, and continued to express tyrosine hydroxylase and MHC I markers. It has been reported that some mouse PSC derivatives are rejected after autologous transplantation, so to determine whether

autologous human DA neurons could evoke an immune response, we performed mixed lymphocyte reactions (MLR) using patient-specific fibroblasts, iPSCs, and DA neurons in allogeneic and autologous settings. Autologous DA neurons elicited minimal T-cell response in comparison to allogeneic DA neurons. We also found less pro-inflammatory cytokine production in autologous MLR than in allogeneic assays. This is significant because our goal is to achieve DA neuron replacement without a requirement for adjuvant immunosuppressive therapy. Our results support the feasibility of using autologous DA neurons for PD therapy.

Funding Source: California Institute for Regenerative Medicine; Summit for Stem Cells

T-2039

THE NEUROPROTECTIVE AND NEUROREGENERATIVE POTENTIAL OF HUMAN CRANIAL BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Bone marrow mesenchymal stem cells (BMSCs) may have the capacity to differentiate into neural lineages and are clinically attractive because autologous transplantation can be performed. Although most recent studies have used BMSCs from iliac bone, the standard source for BMSCs, a few papers have tested similar stem cells such as tooth-derived stem cells and demonstrated their unique cellular neuroregenerative characteristics. We established human BMSCs from cranial bone waste (cBMSCs) obtained following routine neurosurgical procedures. Here, we investigated the neuroregenerative ability of cBMSCs in vitro and in vivo. Bone marrow samples were obtained from the volunteers' fronto-temporal cranial bone waste from a neurosurgical procedure following informed consent according to the hospital's guidelines. Flow cytometry analysis showed that cBMSCs expressed a set of mesenchymal stem cell markers. RT-PCR, western blotting, and immunocytochemical staining showed that neural differentiation of cBMSCs resulted in remarkably stronger expression of a marker for neural cells compared to human iliac BMSCs. To investigate neuronal differentiation, we studied neurite outgrowth in a neuroblastoma × glioma hybrid cell line (mouse neuroblastoma × rat glioma hybrid, NG108-15) cultured in medium supplemented with cBMSC supernatant for 24 hours. Significant increases in the total length of neurites were induced compared with NG108-15 cells in basic medium. In vivo, adult brain injury model mice were transplanted with cBMSCs directly into the contralateral frontal lobe of the injured brain 24 hours after injury. Compared to control mice that received only PBS, mice transplanted with cBMSCs exhibited significant improvement in the beam-walking test. cBMSCs may have greater trophic effects on reestablishment and survival of brain neural tissues because cavity formation was reduced in the lesion area, and Bdnf expression and apoptosis-inhibiting factor expression were higher in the lesion. cBMSCs appear to have a greater tendency than iliac bone-derived cells to differentiate into neuron-like cells and facilitate neuroregenerative recovery after brain injury in vitro and in vivo. cBMSCs may be a novel, favorable source of autologous adult stem cells to treat neurological disorders.

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T-2041

INTRACEREBROVENTRICULAR ADMINISTRATION OF HUMAN MESENCHYMAL STEM CELLS INDUCES TRANSIENT INFLAMMATION IN MOUSE BRAIN.

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In both preclinical and clinical settings, stem cells have attracted attention as an optimal source to treat a wide range of diseases. Out of the various routes to deliver stem cells to the brain, through the intracerebroventricular (ICV) route, stem cells can be directly delivered to the brain and widespread

distribution is possible. In a previous study, inflammatory responses were observed from a canine model following ICV delivery of human mesenchymal stem cells (MSCs). We performed an additional study using wild-type mice to observe whether the same signs of inflammation are observed following ICV delivery of human MSCs. Mice were sacrificed (total of n=27) at 3 different time points following the administration of MSCs: 3, 9, and 24 hours. At each time point, the following groups (n=3 per group) were sacrificed: (1) Sham (2) Vehicle (Minimal Essential Medium; MEMα1x) (3) MSC. Expression of the inflammatory cytokines, TNFα and IL-1β, were analyzed by using ELISA. Interestingly, we discovered that for the MSC group sacrificed at post 9 hours, both TNFα and IL-1β levels were higher compared to that of the other groups. Histological analysis shows that there is interaction between the brain and the injected stem cells. Nevertheless, administration of MSCs via ICV route for in AD is one of the ways to maximize the potential of neurotrophic paracrine factor of MSCs to infiltrate into the parenchyma without BBB restriction. These results demonstrate that as transient inflammatory reaction occurs due to migration into the parenchyma.

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T-2043

TESTING CELL-SPECIFIC EFFECTS OF APOE4-MEDIATED DEVELOPMENT OF ALZHEIMER'S DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Alzheimer's disease (AD) is progressive neurodegenerative disease which affects the part of the brain controlling memory, thoughts, and behavior. Pathological characteristics of AD include massive neuronal cell death, intracellular neurofibrillary tangles (hyper-phosphorylated tau or pTau), and the formation of senile plaques (Ab plaques). The neuron loss starts deep in the brain and spreads throughout the brain, from hippocampus to cerebral cortex. Although the genetic cause of late-onset AD is unknown, apolipoprotein E (APOE) 4 gene allele has been identified as the most significant risk gene. ApoE protein is one of the main components in high-density lipoprotein (HDL) complexes in human cerebrospinal fluid. HDL is the major phospholipid and cholesterol supply for mature neurons. ApoE has been shown to potentially modulate the production and clearance of Aβ, and also been implied in promoting tauopathy. However, the apoE4-associated progress in early stage of AD is poorly understood. In this study, we hypothesized that apoE4 may differentially promote pathological processes in distinct neuron types in cortical layers and hippocampal formation. To test this hypothesis, we examined the apoE-mediated pathological phenotypes in human cortical and hippocampal neurons. First, both types of neurons are derived from human induced pluripotent stem cells (hiPSCs) using two differentiation protocols. Cortical progenitor and mature neurons are characterized by immunofluorescent staining with neural progenitor markers (Otx2, Pax6, FoxG1, Nestin) and mature neuron markers (SATB2, CTIP2). Hippocampal neurons are characterized by the Prox1 marker. Second, to establish a well-controlled system with minimal interference from other cells, hiPSC-derived neurons are purified by fluorescence-activated cell sorting (FACS) with central nervous system (CNS) neuron surface markers CD184, CD44, and CD24. The purified neurons are treated with apoE4, and the pathological phenotypes (i.e. Ab production and phosphorylated tau) in treated neurons are measured using various biochemical assays, including mRNA expression profiling and enzyme-linked immunosorbent assay (ELISA). Our preliminary study showed that apoE4-treated cortical neurons have increased tau phosphorylation level.

T-2045

PATHOPHYSIOLOGICAL ANALYSIS OF SPINAL-BULBAR MUSCULAR ATROPHY USING DISEASE SPECIFIC IPSCS

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Spinal-bulbar muscular atrophy (SBMA) is an adult onset, slowly progressive lower motor neuron disease caused by a CAG repeat expansion in androgen receptor (AR) gene. Previous analyses of SBMA model mice have revealed that mutant AR with expanded polyglutamine tract form aggregation in testosterone-dependent manner and cause motor neuron degeneration. However, the phenotypes of model mice are different from those of patients in several aspects, and the clinical trial of anti-androgen treatment by LH-RH analogue, leuprorelin acetate, has shown its effectiveness only in patients of early-disease, suggesting the importance of early pathology of SBMA. Moreover, recent analysis has shown the involvements of skeletal muscles in neurodegeneration in SBMA. Here, we generated induced pluripotent stem cells (iPSCs) from SBMA patients to establish more accurate disease models, and investigated the pathogenesis of SBMA from the aspect of pre-aggregation early pathology, disease

accelerating signals, and neuromuscular interactions. We established iPSCs from 4 SBMA patients and 3 age- and sex-matched controls and differentiated them into motor neurons (MNs) followed by 4 week-maturation with dihydrotestosterone (DHT), a ligand for AR. As for differentiation ratio into MNs and neuronal cell death, significant difference was not observed between SBMA patients' and control MNs. Mutant AR aggregations were not detected in SBMA-MNs by immunocytochemistry or western blot analysis, while early pathology of SBMA, such as increased expression of CALCA (CGRP-1) and activation of c-Jun signal, were observed, suggesting the recapitulation of early pathology by SBMA-MNs before mutant AR aggregation. Moreover, these early pathologies were enhanced by ER stress inducers, tunicamycin or thapsigargin, suggesting that ER stress could be a disease accelerating factor, which could be a novel therapeutic target for SBMA. Finally, we co-cultured control-MNs with skeletal muscles expressing mutant AR, and found significant decrease in the number of neuromuscular junctions and significant increase in neuronal cell death, suggesting non-cell autonomous neurodegeneration by skeletal muscles. These iPSC-derived disease models provide powerful tools for the analysis of pathogenesis of SBMA and for drug screening.

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CANCERS

T-2049

PI3K ACTIVATION IN NEURAL STEM CELLS DRIVES TUMORIGENESIS WHICH CAN BE AMELIORATED BY TARGETING THE CAMP RESPONSE ELEMENT BINDING (CREB) PROTEIN

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Hyperactivation of the PI3K signaling is common in cancers but the precise role of the pathway in glioma biology remains to be determined. Some understanding of PI3K signaling mechanisms in brain cancer comes from studies on neural stem/progenitor cells, where signals transmitted via the PI3K pathway cooperate with other intracellular pathways and downstream transcription factors to regulate critical cell functions. To investigate the role for the PI3K pathway in glioma initiation and development, we generated a mouse model targeting the inducible expression of a $PIK3CA^{H1047A}$ oncogenic mutant and deletion of the PI3K negative regulator, PTEN, to NSPCs. Expression of a $Pik3ca^{H1047A}$ was sufficient to generate tumors with oligodendroglial features but simultaneous loss of PTEN was required for the development of invasive, high-grade glioma. $Pik3ca^{H1047A}$-PTEN mutant NSPCs exhibited enhanced neurosphere formation which correlated with increased WNT signaling, while loss of CREB in Pik3ca-Pten tumors led to longer symptom-free survival in mice. Taken together, our findings present a novel mouse model for glioma demonstrating that the PI3K pathway is important for initiation of tumorigenesis and that disruption of downstream CREB signaling attenuates tumor expansion.

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T-2051

LEUKAEMIC ALTERATION OF IKZF1 PRIMES STEMNESS AND MALIGNANCY PROGRAMS IN HUMAN LYMPHOCYTES

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Somatic cells acquire stem cell-like properties in cancerous transformation. Understanding the mechanism underlying the stemness and malignancy of committed cells is important for cancer pathogenesis as well as cell reprogramming. Here we detected identical stem cell program that was commonly activated in the leukaemic lymphoblasts of several subgroups of acute lymphoblastic leukaemia in patients with IKZF1 alterations. Our results of functional assay in vivo demonstrated that the expression of IK6 (the most frequent IKZF1 alteration) could confer the stem cell-like properties in committed lymphocytes. Interestingly, analysing the programs in the whole transcriptome activated by IK6 expression revealed that IKZF1 alteration, as a single event, could prime programs of stemness, impaired differentiation and malignancy in

the lymphocytes. Further analysis revealed that these activated programs were associated with the activation of distinct pathways. Stemness and differentiation were found to be coupled with the TGF-beta and WNT pathways, respectively, whereas malignancy was found to be coupled with the PI3K pathway. Our findings therefore posit a mechanism of cancerous reprograming in an oncogenesis model, which could have great implications in investigating cancerous transformation and in inducing dedifferentiation or transdifferentiating of somatic cells to avoid malignant formation.

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T-2053

CONNECTING THE PHYSIOLOGICAL DEREGULATION IN HEMATOPOIETIC MALIGNANCIES TO APOBEC3 UPREGULATION AND MUTATIONAL LOAD

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The prevalence of upregulated apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC3) transcripts as well as a distinct mutational signature has been found and presented extensively in numerous cancer types. Previously this family of cytidine deaminases has been studied in innate immunity, as they are active in the conversion of cytosine to uracil to restrict retroviral replication. Recent studies employing whole exome sequencing of multiple cancer types have shown an increase in transcript level as well as a known APOBEC3 mutation signature. However, the expression and role of these enzymes in cancer initiation and progression as well as the mechanisms by which the APOBEC3 enzymes elicit a response in the cancer microenvironment remains unknown, especially in hematopoietic malignancies. Here, we elucidate the APOBEC3 mutation phenotype in the hematopoietic malignancy Myelofibrosis (MF), as well as the naïve cell populations of CD34+ cord blood and normal aged samples. Through RNA sequencing we have found that there is a cell type and context specific nature of these enzymes, notably the upregulation of APOBEC3G (A3G) in the MF stem and progenitor cell population as compared to normal aged counterparts. There is also significant differential expression of APOBEC3C (A3C), APOBEC3D (A3D), and APOBEC3F (A3F) in MF disease states compared to normal. By cloning the APOBEC3 enzymes into lentiviral expression vectors, we can now study the physiological effects of this deregulation in relation to the known changes in expression seen in many cancers, focusing on the upregulation of A3G through lentiviral overexpression. With this overexpression, we can connect the prevalence and proposed activity of the enzymes to the physiological deregulation present in hematopoietic malignancies.

T-2055

SELECTIVE ELIMINATION OF CULTURE-ADAPTED HUMAN EMBRYONIC STEM CELLS WITH BH3 MIMETICS

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The selective survival advantage of culture-adapted human embryonic stem cells (hESCs) is a serious safety concern for their clinical application. Using two sets of hESCs, we observed that a subpopulation of hESCs at late passage numbers was highly resistant to various cell death stimuli, such as genotoxic stimuli and single-cell dissociation. Comparison of the apoptosis-associated gene expression profiles of YM155-sensitive (YM155S) and YM155-resistant (YM155R) hESCs demonstrated that BCL2L1 was highly expressed in the latter cells. By matching the gene signature of YM155R hESCs with the CTRP dataset, BH3 mimetics were predicted to selectively ablate these cells. Indeed, short-course treatment with a sub-optimal dose of BH3 mimetics, induced the spontaneous death of YM155R, but not YM155S, hESCs by disrupting the mitochondrial membrane potential. YM155S hESCs remained pluripotent following BH3 mimetics treatment. Therefore, the use of BH3 mimetics is a promising strategy to specifically eliminate hESCs with a selective survival advantage.

T-2057

REPROGRAMMING OF CANCER STEM CELLS USING EXOSOMES FROM HUMAN ADIPOSE-DERIVED STEM CELLS

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Cancer stem cells (CSCs) represent a small fraction of cancer, share many properties of normal stem cells such as self-renewal capacity, drug and toxin resistance, active DNA repair capacity. Because of their stem-like properties, CSCs have been pointed as

a main cause of cancer drug resistance, relapse, and metastasis. Stem cells secrete extracellular vesicles containing various proteins, lipids and genetic materials, which could act as critical signals to recipient cells. In this study, we hypothesized that exosomes secreted from stem cells during differentiation might contain specific biochemical molecules which could reprogram CSCs into non-tumorigenic cancer cells. We isolated exosomes from conditioned media during adipogenic or osteogenic differentiation of human adipose-derived stem cells (hASCs) through sequential steps of pre-centrifugation, dead-end pre-filtration, and tangential flow filtration (TFF). The isolated exosomes were characterized by transmission electron microscopy (TEM), nanoparticle analysis system (NTA), western blot. CSCs were isolated from osteosarcoma (MG63) cell line using magnetic activated cell sorting (MACS). The potency of isolated CD133+ CSCs was evaluated by in vitro differentiation tests. In the presence of osteogenic exosomes, MG63 CD133+ CSCs were differentiated into less-aggressive cancer cells in vitro. Adipogenic exosomes were intratumorally injected into a breast cancer mouse model using MDA-MB-231 breast cancer cells. Real-time polymerase chain reaction (qPCR) arrays showed that drug resistance related genes were down-regulated such as ABC binding cassette transporters (ABC) genes, cyclin-dependent kinases (CDKs) gene, cytochromes P450 (CYPs) genes, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) genes. Overall results suggest that exosomes secreted from stem cells during differentiation could act as a biochemical cue for control of CSC fate toward non-tumorigenic.

T-2059

EFFECT OF 7-KETOCHOLESTEROL ON ABC TRANSPORTS IN HUMAN MESENCHYMAL STEM CELL FROM PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is clinically and molecularly heterogeneous clonal myeloid disorders in which hematopoietic precursors are arrested in an early stage of development. During the last decades, mesenchymal stem (MSC) have been identified as one of the main cellular components of the BM microenvironment holding an indispensable role for normal hematopoiesis. The ATP-binding cassette (ABC) transporter contains membrane proteins that translocate a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and sterols, and drugs. Oxysterols are the products of cholesterol oxidation, formed by both enzymatic and non-enzymatic mechanisms. Several lines of evidence link ABC transporter functions with cholesterol and oxysterol metabolism. Here we describe the effect of 7-ketocholesterol (7-KC) of mesenchymal stem cell from patients with acute myeloid leukemia in the expression of ABC transporters. MSC from bone marrow (BM) of patients with acute myeloid leukemia were obtained from LIM31 pattern inventory. 7-ketocholesterol at 25 μM was added, followed by incubation for 24 h. Total RNA was extracted with Trizol (Invitrogen). cDNA was prepared using high capacity cDNA reverse transcription kit (Applied Biosystems) after treatment with DNase (RQ1 RNase-Free DNase - Promega). Human ABC transporters TaqMan Array 96-well FAST plate was used to performed real time PCR at 7500 fast Real Time PCR (Applied Biosystems). MSC were isolated from BM of patients with acute myeloid leukemia and characterized by flow cytometry, in vitro mesenchymal differentiation assays, Oct-4 and Nanog genes expression by RT-PCR. The MSC did not express the ABCA4, ABCA7, ABCA12, ABCB1, ABCB4, ABCB5, ABCB11, ABCC6, ABCC8, ABCB11, ABCB12, ABCD2, ABCG1, ABCG5, ABCG8 and CFTR genes. The treatment with 25 μM of 7-KC did not change the expression of ABCA2, ABCA3, ABCB6, ABCB8, ABCB10, ABCC1, ABCC5, ABCD1, ABCD3, ABCF1, ABCF2, TAP1 and TAP2 genes. The presence of 7-KC led to an increase of ABCA1 and ABCD4 expression. ABCA5, ABCA6, ABCA8, ABCB7, ABCB9, ABCC2, ABCC3, ABCC4, ABCC6, ABCC9, ABCE1, ABCG2 and ABCG4 has its expression decreased by treatment with 7-KC. The oxystrerol 7-KC influences the expression of several proteins of ABC transporters.

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T-2061

IMPACT OF HIF PATHWAY ACTIVATION ON CANCER STEM CELLS IN ANAPLASTIC THYROID CANCER CELL LINES AND MECHANISMS OF CHEMOTHERAPEUTIC DRUG RESISTANCE

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Anaplastic thyroid cancer (ATC) is the rarest but deadliest form of thyroid cancer. ATC is rapidly growing, highly metastatic and resistant to chemotherapy/radiotherapy. It can co-exist with differentiated thyroid cancers and

shares some genetic abnormalities e.g. BRAF mutations. BRAF mutations are known to independently activate HIF pathways, which are activated during hypoxic stress and in turn activate the cell's transcriptional machinery enabling cell survival. We activated the HIF pathway in ATC cell line SW1736 using cobalt chloride (II) (CoCl2) treatment and observed a significant rise in the cancer stem cell (CSC) containing side population (SP) fraction. Thyroid CSC have been reported previously and form a very small fraction of the tumour cells. They remain largely resistant to many chemotherapeutic drugs due to a variety of mechanisms, including the presence of drug-effluxing ABC transporters. They survive and regenerate ATC. In our studies, we tested SW1736 cells for a panel of ABC transporters and found the ABCG2 was upregulated following CoCl2 treatment. Another critical regulator of the CSC phenotype is Epithelial-Mesenchymal Transition (EMT) which is characterised by cells losing their epithelial phenotype and acquiring mesenchymal features. During this transformation the cells undergo a number of changes enabling them to invade the surrounding tissues. In addition E-cadherin (epithelial marker), becomes downregulated and upregulation of TWIST, SNAIL and Slug (transcription factors) and mesenchymal markers, N-cadherin and Vimentin occurs. Following the induction of hypoxia, EMT pathway in SW1736 was found to be activated. Treatment with doxorubicin (a current in use chemotherapeutic) resulted in 2-3 fold increase in the SP fraction in the CoCl2 treated SW1736 compared to the cells treated with doxorubicin alone. MTS cell viability assays shown increase in the doxorubicin-resistant cell fraction following CoCl2 induced hypoxia, and upregulation of some of the ABC-transporters was detected, although the change was not significant. Hypothesise: HIF pathway contributes to drug resistance by enriching for CSC, mechanisms involved include (1) upregulation of ABC transporters and (2) induction of EMT. Eliminating CSC by targeting these pathways would improve outcomes for ATC.

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T-2063

CRACKING THE CODE OF EPITHELIAL MESENCHYMAL PLASTICITY IN HUMAN EPITHELIAL CARCINOMA

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Intratumour heterogeneity along the epithelial-mesenchymal axis is evident in most epithelial carcinoma. The morphological transition, functionally reflects enriched differentiation signatures with enhanced radiation response in the epithelial compartment, while the mesenchymal cells manifest plastic and stem-like signatures with capacities to resist/evade radiotherapy. In this study we have used human oral squamous carcinoma (OSC) cells and spheroids to explore the molecular mechanisms of epithelial-mesenchymal plasticity and its link to therapy response. Using single cell tracking, immune phenotyping and transcriptomics approaches, we have identified a range of epithelial and mesenchymal states with differential downstream outcomes at the levels of cell cycle progression patterns and radiotherapy response. Further fractionation of the cells along the epithelial-mesenchymal (E-M) spectrum, based on expression of E-Cadherin and N-Cadherin, has identified interesting dynamic response patterns in the subpopulations in response to radiotherapy, with gene signatures reflecting differential capacities in DNA repair machinery and stem cell phenotypes. Using single cell transcriptomic approaches, we have further elucidated on distinct signatures of the epithelial and mesenchymal compartments highlighting candidate pathways to drive tumour epithelialisation. This study, has taken a novel approach to dissect epithelial and mesenchymal heterogeneity in human epithelial carcinoma models, describing novel regulatory pathways with potential use in targeted therapy and tumour epithelialization.

CHROMATIN AND EPIGENETICS

T-2065

TINC - A METHOD TO DISSECT TRANSCRIPTIONAL COMPLEXES AT SINGLE LOCUS RESOLUTION

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Being able to determine the molecular composition of protein complexes that assemble at specific regulatory elements to activate or repress gene transcription is essential for a better understanding of how gene expression is controlled in normal and diseased states. We have developed a transcription activator-like effectors (TALE) based method termed TINC (TALE-mediated Isolation of Native Chromatin), which enables the isolation of a specific chromatin region from mammalian cells and consequent identification of associated proteins by mass spectrometry. For proof of concept, we targeted the Nanog proximal promoter in mouse embryonic stem cells and were able to identify transcription factors known to bind to this locus and most importantly novel proteins that play an essential role in maintaining pluripotency and reprogramming. As TINC does not require any genetic modification of the target sequence, a target-specific antibody nor high copy numbers of the target sequence, we strongly believe that this method is applicable to any scientific field and has immense potential to change the concept of how we study gene regulation.

T-2067

FINE-TUNING OF CHROMATIN REMODELING COMPLEX CHD1 REGULATES UNDIFFERENTIATED STATUS OF MESENCHYMAL STROMAL CELLS

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Accumulating studies point to the epigenetic plasticity as a key characteristics for the primitive state in various stem cells. However, the molecular control for the hierarchical maintenance of epigenetic plasticity in stem cells remain poorly understood. In this study, we found that the changes in expression of chromatin remodeling complex, chromatin-helicase DNA binding protein 1 (chd1) exert a regulatory influence on the undifferentiated state of mesenchymal stromal cells (MSCs). We found that the expression levels of chd1 in murine and human MSCs gradually decrease with increasing culture passages of MSCs, being associated with loss of their clonogenic potential. Similarly, knock-down (KD) of chd1 by shRNA-chd1 caused a significant decrease of clonogenic potential, proliferative activity and multi-lineage differentiation of MSCs into osteogenic or adipogenic tissues. Moreover, the KD of chd1 in MSCs caused transcriptional inhibition of pluripotency-related genes, oct-4, sox-2 and nestin via increase of H3K9-trimethylaiton in the upstream promoter region of these genes. Consistent to the finding, KD of chd1 in MSCs led to more condensed chromatin as demonstrated by decrease in chromatin accessibility to oct-4 promoter. In contrast, retroviral overexpression of chd1 gene in human MSCs did not cause a significant changes in the frequency of clonogenic cells, but led to down-regulation of oct-4, sox-2 or nestin genes. Together, these findings indicate that the fine-tuning in expression of chromatin remodeling complex play an important role for balancing the epigenetic plasticity in MSCs to maintain the primitive state of MSCs.

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T-2069

KDM1A REGULATES CELL PROLIFERATION VIA COMPETITIVELY RECRUITING BY DIFFERENT TRANSCRIPTION FACTORS (TFS)

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Lysine (K)-specific demethylase 1A (KDM1A), also known as lysine-specific histone demethylase 1A (LSD1), is the first discovered histone demethylase and has been shown to play vital roles in a wide range of biological processes through demethylating H3K4me1 and H3K4me2. However, how does KDM1A distinguish those methylated genomic sites remains obscure. Using bioinformatic analysis, we predicted that two transcription factors (TFs), TF1 and TF2, may recruit and interact with KDM1A, thus help KDM1A to demethylate mono- or di-methylation, respectively. Our experimental results showed that TF1 and TF2 do not physically interact with each other. Enrichment analyses of ChIP-seq (chromatin immunoprecipitation [ChIP] followed by high-throughput sequencing) data showed that TF1 and TF2 binding sites were enriched at distinct genomic regions, but they all share the same binding motif with KDM1A. Functional examination revealed that loss of TF1 impedes HEK293T cell growth, whereas knocking-out TF2 promotes HEK293T cell proliferation, as they repress different cell division related genes. Significantly, switching from H3K4me1 and H3K4me2 to H3K4me3 upregulates the expression of corresponding genes in TF1-KO and TF2-KO HEK293T cells due to

the dissociation of KDM1A from the binding sites. Collectively, we propose that TF1 competes with TF2 to recruit KDM1A to demethylate H3K4me1 or H3K4me2, thus regulating cell proliferation.

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T-2071

CANONICAL PRC2 FUNCTION IS ESSENTIAL FOR MAMMARY GLAND DEVELOPMENT AND AFFECTS CHROMATIN COMPACTION IN MAMMARY ORGANOIDS

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Distinct transcriptional states are maintained through organization of chromatin, resulting from the sum of numerous repressive and active histone modifications, into tightly packaged heterochromatin versus more accessible euchromatin. PRC2 is the main mammalian complex responsible for Histone 3 lysine 27 tri-methylation (H3K27me3), and is integral to chromatin organization. Using in vitro and in vivo studies, we show that deletion of Suz12, a core component of all PRC2 complexes, results in loss of H3K27me2/me3, completely blocks normal mammary gland development and profoundly curtails progenitor activity in 3D organoid cultures. Through the application of mammary organoids to bypass the severe phenotype associated with Suz12 loss in vivo, we have explored gene expression and chromatin structure in wildtype and Suz12-deleted basal-derived organoids. Analysis of organoids led to the identification of lineage-specific changes in gene expression and chromatin structure, inferring cell-type specific PRC2-mediated gene silencing of the chromatin state. These data indicate that canonical PRC2 function is essential for development of the mammary gland through the repression of alternate transcription programs and maintenance of chromatin states.

ORGANOIDS

T-2073

USING INDUCED PLURIPOTENT STEM CELLS TO MODEL HUMAN KIDNEY DISEASE

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The generation of induced pluripotent stem (iPS) cells from patients with hereditary diseases and the differentiation of these cells into mini organs (organoids) provides a new way to study and find treatments for illnesses in vitro. The lysosomal storage disease nephropathic cystinosis results from mutations in the CTNS gene, encoding a cystine transporter, and initially causes kidney proximal tubule dysfunction followed by kidney failure. Patients receive the drug-based therapy Cysteamine from diagnosis, however, despite long-term treatment with this drug, patients still progress to kidney failure with the need for transplant inevitable. There is an urgent need for alternative treatments as there is increasing evidence that secondary complications are associated with loss of CTNS that are unrelated to the accumulation of cystine. Here, we describe the characterization of iPS cells from a patient with nephropathic cystinosis as well as a CRISPR/Cas9-induced line and the development of a simple protocol for generating human kidney organoids. As expected, cystine (and cysteine) levels are elevated and basal autophagy flux is reduced in CTNS-iPS cells and CTNS-kidney organoids. Some CTNS-iPS cells displayed large degradative vacuoles with multivesicular inclusions. RNA-Seq analysis of CTNS-iPS cells identified new biomarkers for cystinosis, including the DDIT3 gene (aka CHOP), which encodes a C/EBP-homologous protein which functions as a cellular stress sensor. We found that Cysteamine treatment of CTNS-iPS cells and organoids lowered cystine and cysteine levels, reduced the number of large degradative vacuoles and restored the expression of DDIT3 and other biomarkers to normal levels. However, the basal autophagy flux defect was not corrected. Similar results were found, with the addition of correction of autophagy flux, using the FDA-approved drug Everolimus, which inhibits the mTOR pathway and has recently been implicated in cystinosis. Together, our

results indicate that the iPS cell/organoid platform can be used to model aspects of cystinosis and suggest that mTOR-inhibiting drugs may have therapeutic value in the treatment of this disease.

Funding Source: Cystinosis Ireland; Cystinosis Research Foundation; Auckland Medical Research Foundation

T-2075

FUNCTIONAL APPLICATIONS OF HUMAN INTESTINAL ORGANOIDS ESTABLISHED AND MAINTAINED IN INTESTICULT™ ORGANOID GROWTH MEDIUM (HUMAN)

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We recently developed IntestiCult™ Organoid Growth Medium (Human) (OGM), a novel medium for the establishment and expansion of human intestinal organoids. In the current study, we provide a more detailed phenotypic and functional characterization of organoids maintained in this medium. To establish cultures, intestinal crypts were isolated from patient biopsies obtained from all anatomical regions of the intestinal tract by incubation with gentle agitation in Gentle Cell Dissociation Reagent™. The isolated crypts were embedded within a Corning® Matrigel® dome and flooded with IntestiCult™ OGM. Cultures could be maintained for ≥ 6 months when passaged approximately every 10 days at a 1:4 split ratio. Organoids were analyzed at various passages for expression of markers specific for enterocytes (villin), intestinal stem (LGR5, AXIN2), Paneth (lysozyme), enteroendocrine (CHGA) and goblet (MUC2) cells. Results demonstrate that all of these cell types were present within the organoids, although a selective enrichment of stem cells was observed during passaging. Differentiation of enterocytes and goblet cells could be enhanced by culturing for 5 days in IntestiCult™ OGM Component A, as indicated by increased expression of villin (18.6-fold; 3 7.1, n = 2) and MUC2 (1.4-fold; 3 0.2, n = 2). To determine whether the organoids maintained expression of cystic fibrosis transmembrane conductance regulator (CFTR), 10 day-old colonic organoids were treated with 5 µM forskolin and organoid size was monitored for 2 hours. Results indicated that a functional CFTR protein was, indeed expressed in the cultured organoids, as forskolin-treated organoids exhibited a pronounced increase in size (34.3 3 4%; n = 1, 100 organoids) compared to controls. To further assess the differentiation potential of organoids maintained in IntestiCult™ OGM, organoids were seeded directly on Matrigel® coated coverslips and cultured in this medium for 10 days in the presence of 10 µM Y27632. Subsequent immunostaining to detect villin and keratin 20, as well as ion transport measurements, revealed that seeded cells were able to form a functional, polarized monolayer. Collectively, these results demonstrate that long-term expansion of intestinal stem cells that retain their differentiation potential can be promoted by culture in IntestiCult™ OGM.

T-2077

A 3D MODEL OF HUMAN EMBRYONIC DEVELOPMENT INDICATES DISPENSABLE NATURE OF DYSTROGLYCAN IN BASEMENT MEMBRANE ASSEMBLY

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α-Dystroglycan (α-DG) is a cell surface receptor of extracellular matrix proteins, particularly those constituting the basement membrane, a specialized extracellular matrix structure linked to the plasma membrane of certain cell types and tissues. α-DG receives a unique post-translational glycan modification through which it binds to its matrix ligands. Defective glycosylation of α-DG causes muscular dystrophy often associated with embryonic brain and eye malformations, a syndromic spectrum referred to as α-dystroglycanopathy. Neuropathological findings in α-dystroglycanopathy include cell detachment from the basement membrane surface, cell migration through basement membrane ruptures, and gross malformation of tissue architecture. Here, we sought to determine the role of α-DG as an extracellular matrix receptor in human embryonic basement membranes. We have adapted a protocol to generate embryoid bodies from human induced pluripotent stem cells that mimic the peri-implantation stage of embryonic development. After five days of culture, these embryoid bodies form a spherical structure reminiscent of the inner cell mass, consisting of an inner core of OCT4+ epithelial cells and an outer layer of SOX17+ endoderm separated by a laminin-rich basement membrane. Because mouse models of α-dystroglycanopathy develop ruptures in

the embryonic Reichert's basement membrane and pial basement membrane, we investigated embryoid bodies derived from human patients with α-dystroglycanopathy. Notably, we found that patient embryoid bodies assembled morphologically normal basement membranes, despite complete absence of glycosylated α-dystroglycan. Compared to most embryonic tissues, which undergo rapid expansion during development, embryoid bodies derived by our method show relatively little change in size over time. Our data demonstrate that α-dystroglycan matrix receptor function is dispensable for basement membrane assembly under static conditions, suggesting a function in remodeling, maintaining, or strengthening basement membranes under mechanical stress.

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T-2079

MAGNETIC THREE-DIMENSIONAL BIOPRINTING FOR DEVELOPING FUNCTIONAL SALIVARY "MINI-GLANDS" FROM HUMAN ORAL STEM CELLS

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Radiotherapy, the preferred therapy for head and neck cancers, can irreversibly damage the saliva-secreting cells from salivary glands (SG) in about 40-60% of patients. This damage results in dry mouth, which increases the susceptibility to oral infections, decreasing the quality of life of patients. Current options for dry mouth therapies are heavily dependent on the limited number of residual SG secretory cells. Hence, cell-based approaches to replace and/or regenerate the damaged SG epithelia are paramount, together with the use of mesenchymal-derived stem cells, like human dental pulp stem cells (hDPSC). Thus, our aim was to generate saliva-secreting epithelial cells arranged in 3D organoids using a novel culture system, the magnetic 3D bio-printing (M3DB) from hDPSC. These stem cells were expanded as 3D spheroids followed by an epithelial differentiation step using two systems: M3DB and force aggregation (a conventional 3D system). Cellular ATP was assessed in the 3D spheroids to study viability and cell survival. These spheroids were evaluated for functional secretion, trans-epithelial resistance and characterized genotypically and phenotypically by qPCR and whole-mount immunofluorescence, respectively. 3D spheroids using M3DB system exhibited similar proliferation to conventional 3DFA after 3 days in vitro. The differentiated 3D organoids showed increase expression of α-amylase at gene and protein levels compared to undifferentiated spheroids. Furthermore, these differentiated 3D spheroids express diverse SG cellular markers including acinar epithelial secretory (AQP5, Amy1, Chrm3), ductal epithelial (KRT14, KRT5), pan-neuronal (TUBB3). This was dependant on Fibroblast Growth Factor-10 (FGF-10), a key signaling cue in SG development. Upon parasympathetic and sympathetic neuro-stimulation, salivary α-amylase was increased as well as calcium influx, and trans-epithelial electrical resistance, when compared to conditions when FGF-10 was absent. Overall, this tissue construct indicates the presence of functionally innervated SG-like secretory epithelia organoid, comparable to a "mini-SG". This project offers a promising 3D bio-printing therapeutic solution to alleviate dry mouth in irradiated cancer patients.

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T-2081

A HIGH THROUGHPUT, HOMOLOGOUS AND LOW COST HUMAN 3D BRAIN ORGANOIDS CULTURING AND IMAGING SYSTEM

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Based on the RONA method, we can derive large amount of high quality human neural stem cells and progenitor cells from human pluripotent stem cells including patient iPSCs. We have now developed a low cost culturing system to produce thousands of brain organoids with homologous size, structure and cell component, with markers of six cortical layers and PV, SST, nNOS interneurons. These organoids can reach a dimension of 3-5 mm and cultures up to years. This system can also make the matrigel wrapping and imaging of these organoids more high throughput. This novel system will provide great aid to the high throughout 3D organoids screening with hiPSC models.

T-2083

MODELLING GLIOBLASTOMA MULTIFORME BRAIN TUMOURS IN HUMAN PLURIPOTENT STEM CELL-DERIVED CEREBRAL ORGANOIDS

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The brain tumour glioblastoma multiforme (GBM) is the most common malignant central nervous system tumour and is known for its extreme aggressiveness leading to a median patient survival of 15 months. GBM is highly resistant to therapy, with tumour cell and microenvironmental heterogeneity proposed as major contributing factors. Standard in vitro cell culture is limited in its ability to recapitulate this complex niche-tumour cell interaction, while genetic mouse-modelling and xenograft studies of GBM lack species-specificity. Collectively these deficiencies in pre-clinical models could contribute to the poor treatment outcomes in the clinic. Alternatively, human pluripotent stem cell (hPSC)-derived cerebral organoids (COs) may provide a new platform for studying GBM that can overcome some of the current limitations in GBM models. These self-organized 3D-structures cultured in vitro recapitulate the structure and organization of a human brain, and have been shown to reliably model a number of neurodevelopmental disorders. Our lab has optimized the original Lancaster, 2014 CO protocol to limit intra-organoid variability and produce COs with high efficiency. These COs display consistent 3D-architectures and cellular physiology mimicking that of the developing human cortex and its unique microenvironment. I hypothesize that studying GBM in different microenvironments captured through hPSC models of brain development will provide great insight into the molecular and cellular behaviour of the disease. In particular, that the developmental organization and cellular landscape of hPSC-derived cerebral organoids creates a cortical microenvironment that when combined with GBM better reflects the cellular and molecular behaviours of a GBM tumour-tissue niche in humans in vivo. Here I discuss my work on establishing human GBM allografts, human COs engrafted with human GBM cell lines and patient-derived glioblastoma stem cell lines where GBM cell marking, viability, and tumour progression will be explored. Allograft modelling of the GBM microenvironment will allow us to investigate oncogenic properties of GBM and identify new druggable targets that can be translated to the clinic.

T-2085

PREDICTING COLON CANCER PATIENT RESPONSE TO CHEMOTHERAPY USING TUMOUR DERIVED ORGANOIDS

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Colorectal cancer is a leading cause of cancer mortality worldwide. Clinicians face many challenges in the treatment of this disease and approaches that can predict an individual's response to therapy is vital for future treatment strategies. There has been considerable effort in analysing the genetic and epigenetic markers of colorectal carcinogenesis. However, these substantial resources cannot experimentally validate therapeutic targets. This requires a living, preclinical model to understand the evolution of cancer and to discover susceptibilities. Cancer organoids are rapidly becoming a valuable pre-clinical model that mimics biological features of the original tumour from which they are derived. Cancer cells, isolated from resected colon cancer tissues and cultured in a cocktail of growth factors, develop into 3D structures called organoids. Organoid technology holds great promise to trial therapies before they reach the patient. We have established patient-derived primary colon cancer and metastatic organoid cultures. These organoids recapitulate the morphology and cellular composition of the primary tumour. We have examined their response to chemotherapeutics including 5FU, Oxaliplatin, and other selected reagents. Interestingly, our initial results show that the patient-derived cancer organoids display different sensitivities to drugs. Some organoid lines are resistant to 5-FU (IC50= 35.3 and 42.9 μM) while others are sensitive (IC50= 8.3 and 11.1 μM). These preliminary data will be correlated to patient response to therapy recorded in the Cabrini Monash University Department of Surgery Colorectal Neoplasia database. Our pilot data also suggests that cancer organoids derived from metastatic lesions show similar responses to their associated primary tumour-derived organoids when treated with 5FU and Oxaliplatin. This study will provide insight into the clinical relevance of using organoid technology to predict response to therapy and whether organoids generated from primary tumours can predict drug sensitivity of secondary metastatic tumours.

TISSUE ENGINEERING

T-2087

THE INTERACTION OF MESENCHYMAL STEM CELLS WITH POLY(LACTIC-CO-GLYCOLIC ACID) - GALANTAMINE MICROPARTICLES OBTAINED BY ELECTROSPRAYING

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Bone marrow-derived mesenchymal stem cells (MSCs) are under investigation as a source of neurons to replace damaged or lost cells in various neurological disorders or injuries. Galantamine is a reversible, competitive acetylcholinesterase inhibitor that also acts as a potentiating ligand for nicotinic acetylcholine receptors, exhibiting neuroprotective effects. However, the need of repeated dosing and cholinergic side effects of galantamine are the major hurdles in the optimum usage of this drug. In this study, a novel carrier system was developed, using poly(lactic-co-glycolic acid) (PLGA) microparticles with encapsulated galantamine. They were tested for their cytocompatibility with rat MSCs. Microparticles containing galantamine were electrosprayed from PLGA polymeric solutions with three different concentrations (4%, 6% and 8%). The morphology of these nanoparticles was evaluated by scanning electron microscopy (SEM) and the zeta potential of the particle suspension was measured by the Zetasizer. Bone marrow-derived MSCs were isolated and prepared from 12-week-old Wistar rats and characterized by flow cytometry and differentiation assays. The MSCs were cultivated with the particles and their viability was analyzed by the WST8 assay. The 4% PLGA particles showed the most uniform morphology, as seen by SEM and a zeta potential of -32.731.44, indicating good stability. Thus, the 4% PLGA particles were chosen as the galantamine carrier. Microparticles with an average size of 434.733 49.67 nm and 393.223 121.01 nm were observed for the PLGA only and galantamine-loaded PLGA particles, respectively. Flow cytometry analysis experiments showed that the rat MSCs were negative for CD45 and positive for CD90 and that 69% of the cell population incorporated the PLGA particles after an incubation period of 24 hours. The WST8 assay showed that the MSCs cultivated in the presence of the PLGA-galantamine particles presented a higher viability (2.5430.43) than the control cells (1.1130.11). Effectiveness of the electrospray method as a technique for preparing galantamine-loaded nanoparticles was confirmed and the particles showed good biological activity when co-cultivated with the MSCs. This shows that the combination of stem cells with nanoparticles has a great potential for neural tissue engineering.

Funding Source: MCTIC, CNPq and Stem Cell Research Institute

T-2089

EXOSOMAL MICRORNA-21-5P MEDIATES MESENCHYMAL STEM CELL PARACRINE EFFECTS ON HUMAN CARDIAC TISSUE CONTRACTILITY

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The promising clinical benefits of delivering human mesenchymal stem cells (hMSCs) for treating heart disease warrant a better understanding of underlying mechanisms of action. hMSC exosomes increase myocardial contractility; however, the exosomal cargo responsible for these effects remain unresolved. This study aims to identify lead cardioactive hMSC exosomal microRNAs to provide a mechanistic basis for optimizing future stem cell-based cardiotherapies. Integrating systems biology and human engineered cardiac tissue (hECT) technologies, partial least squares regression analysis of exosomal microRNA profiling data predicted microRNA-21-5p (miR-21-5p) levels positively correlate with contractile force and calcium handling gene expression responses in hECTs treated with conditioned media from multiple cell types. This motivated testing the human-specific role of miR-21-5p in hMSC-exo-mediated increases of cardiac tissue contractility. Treating hECTs with miR-21-5p alone was sufficient to recapitulate effects observed with hMSC-exo on hECT developed force and expression of associated calcium handling genes (e.g., SERCA2a and L-type calcium channel). Delivering exosomes from hMSCs depleted of miR-21-5p significantly diminished pro-contractile and associated calcium handling gene expression effects on hECTs. Western blots supported miR-21-5p effects on calcium handling gene expression at the protein level, corresponding to significantly increased calcium transient amplitude and decreased decay time constant

in comparison to miR-scramble control. Mechanistically, co-treating with miR-21-5p and LY294002, a PI3K inhibitor, suppressed these effects. In conclusion, miR-21-5p plays a key role in hMSC-exo-mediated effects on human cardiac tissue contractility and calcium handling, likely via PI3K signaling. These findings may open new avenues of research to harness the role of miR-21-5p in optimizing future stem cell-based cardiotherapies.

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T-2093

DELIVERY OF MECHANOSENSITIVE MIR-100-5P AND MIR-143-3P FROM HYDROGELS CAN DRIVE OSTEOGENESIS OF MESENCHYMAL STEM CELLS

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When combined with biomaterials, mesenchymal stem cells (MSCs) hold significant promise in bone-tissue engineering. However, the efficient differentiation of MSCs must improve if this is ever to become a reality. Understanding events in mechanotransduction and impacts upon MSC fate provides information that can be applied to improve the differentiation of MSCs in tissue-engineered constructs, for example by informing the design of biomaterials with the appropriate properties to direct MSC fate. In this study, we have taken an alternative approach and used this knowledge to manipulate signalling pathways and overcome potential limitations in the mechanotransductive cues available. We have previously shown that miR-100-5p and 143-3p are upregulated in human MSCs (hMSCs) in response to stiff substrates and that mimics of these miRNAs promote osteogenesis via their effects on mTOR signalling. We confirmed expression changes of mTOR pathway components at both mRNA and protein level in hMSCs treated with miRNA inhibitors or mimics and direct interaction with miR-100-5p via a 3'UTR reporter of mTOR. We could also mimic the effects of miR-100-5p and miR-143-3p through the use of the mTOR inhibitor, Rapamycin. Here, we demonstrate the utility of this information, by developing a system to deliver miR-100 and miR-143 directly from a hydrogel to MSCs encapsulated within. We determined the release kinetics of miRNA:delivery agent complexes, showing high release within the first 48 h post gelling. hMSCs within the hydrogels demonstrated high viability and transfection efficiency up to 72 h post-encapsulation. Finally, we investigated osteogenesis and showed that in situ transfection of MSCs via gelatin-PEG hydrogels loaded with miR-100-5p and 143-3p increased osteogenesis. Overall our study provides insights into miRNA and mTOR-mediated regulation of osteogenesis and proof-of-concept that this knowledge can be used to improve the differentiation of MSCs for tissue-engineering.

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T-2095

HUMAN INDUCED PLURIPOTENT STEM CELLS BASED 3D COMPOSITES FOR CARDIAC TISSUE ENGINEERING

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Cells are the functional center of every tissue, operating in synergy with their surrounding extracellular matrix (ECM). Cardiac tissue, in particular, has compartmentalized and hierarchically complex cell-ECM composition. Cardiac tissue engineering (CTE) requires, therefore, both a robust cell source—effectively differentiating into all cardiac cell types—and the use of cardiac ECM mimicking biomaterials—enabling the "correct" cell-ECM dialogue. Induced pluripotent stem cells (iPSCs) are an ideal cell-choice for CTE as they can be effectively differentiated to all cardiac lineages, including cardiomyocytes (CM). How undifferentiated iPSCs and iPSC derived CM (iPSC-CMs) interact with 3D ECM mimicking biomaterials, and the role of these interactions in possible cardiac regeneration, however, remain largely uncharted. Two major characteristics of biomaterial scaffolds govern iPSC attachment, proliferation and differentiation: ultrastructural architecture, and biochemical composition. In this study, composite 3D scaffolds were fabricated, which enable modular control of both characteristics. Architecturally mimicking ECM structures were generated by wet electrospinning of PLGA; and cardiac-specific biochemical cues were added by modifying the synthetic scaffold with decellularized cardiac ECM hydrogels. The resulting composites display cardiac-ECM-like architecture and chemistry as characterized by SEM, FTIR and LC MS-MS. Primary Human iPSCs seeded on these composites, exhibited favorable interaction profiles, assessed by proliferation and qRT-PCR assays, compared to non-modified controls. Human iPSC-CMs seeded on these composites further improved beating functionality, compared to controls. Taken together, our results suggest that human iPSCs and iPSC-CMs recognize the composite scaffold as a supportive and conducive microenvironment improving cell survival and functionality. Our platform could potentially aid in the further understanding of the iPSC-ECM dynamic interaction profile—a knowledge required for CTE, cell therapy and regenerative medicine.

T-2097

OSTEOCHONDRAL REGENERATION BY HUMAN MESENCHYMAL STEM CELLS EXPANDED WITH FGF2-BINDING HEPARAN SULFATE

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Articular cartilage damage can lead to chronic pain and joint disability. Recent advances in therapies that use human mesenchymal stem cells (hMSCs) offer great hope to suffering patients. However, their scarcity in bone marrow necessitates *ex vivo* culture expansion for therapeutic use and fibroblast growth factor 2 (FGF2) is a common culture supplement to accelerate such expansion. We show that heparan sulfate (HS) glycosaminoglycans isolated based on FGF2 affinity (termed HS8), bind and potentiate endogenously produced FGF2, so removing the exogenous growth factor burden. Human MSCs from 11 healthy bone marrow donors were cultured for two weeks 3 HS8 supplementation (termed $hMSC^{HS8}$ and $hMSC^{control}$, respectively) resulting in a 2~3-fold increase in the population doublings of $hMSC^{HS8}$ compared with that of $hMSC^{control}$ without a reduction in their telomere length, colony-forming efficiency, MSC marker expression or tri-lineage potential. Notably, the level STRO-1 was markedly increased in $hMSC^{HS8}$ by up to 64% compared to $hMSC^{control}$. Next, the therapeutic efficacy of $hMSC^{HS8}$ for osteochondral repair was assessed in both small and large animals. Defects were created in the femoral trochlear groove of thirty-six NIH nude rats and forty-five immuno-compromised micropigs and left untreated or treated either with fibrin or fibrin loaded with $hMSC^{HS8}$ or $hMSC^{control}$. Rat osteochondral defects treated with $hMSC^{HS8}$ resulted in ~60% of defects achieving normal or nearly normal ICRS I scores and >70% of defects with high ICRS II and O'Driscoll scores. In comparison, $hMSC^{control}$ treatment had less (~50%) defects with high ICRS II and O'Driscoll scores and untreated or fibrin-treated defects had no (0%) defects with high scores. When trialed in pig osteochondral defects, $hMSC^{HS8}$ significantly increased ICRS I, ICRS II and O'Driscoll scores, had reduced osteochondral lesions as assessed by Magnetic Resonance Imaging (MRI) and enhanced biomechanical properties compared with control treatments. This study details the use of a novel glycosaminoglycan as a culture supplement for the expansion of naive hMSCs with high therapeutic value and advocates for the further development of this strategy for clinical purposes.

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T-2099

CKD TREATMENT STRATEGY: SELF-ASSEMBLING BETA-PEPTIDE HYDROGELS FOR THERAPEUTIC DELIVERY OF MESENCHYMAL STROMAL CELLS

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Kidney fibrosis is a hallmark of chronic kidney disease (CKD). Currently no therapies exist which halt the progression of fibrosis and restore renal function. Mesenchymal stromal cells (MSCs) modulate inflammation underling fibrosis and potentially reverse existing fibrosis, however their reparative effect is transient when delivered systemically. Local delivery and longer retention of MSCs holds potential to improve their efficacy in reversing established fibrosis. We aim to develop an injectable, non-degrading hydrogel to promote long term MSC retention following in vivo transplantation. N-acetylated β-tripeptides self-assemble into 14-helical ropes which form hydrogels at sufficient concentrations. We introduce functionality into the peptide monomer by the incorporation of a specially designed β-amino acid with an orthoganol protecting group. Using this strategy, we can optimise the biological signals within the gel to improve MSC anti-inflammatory efficacy in the kidney. β-tripeptides functionalised with cell adhesive epitopes, RGD and SIKVAV, and fluorophores, are synthesised using solid-phase peptide synthesis. Human bone-marrow derived MSCs with a GFP+ luciferase reporter will be encapsulated and delivered in hydrogel to the kidney subcapsular space of mice with fibrotic kidney damage. Hydrogels and cells will be tracked for up to 60 days using in vivo fluorescence and bioluminescence imaging, respectively, and kidneys will be periodically harvested for assessment of fibrosis and inflammation. Development of an injectable hydrogel to deliver MSCs directly to the kidney could provide a novel treatment option for CKD patients who require dialysis or transplant, greatly improving their quality of life and easing the mounting pressure on our healthcare systems.

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T-2101

BIO-ENGINEERING A TISSUE FLAP USING AN ARTERIAL/VENOUS PEDICLE AND A HUMAN iPSC ENDOTHELIAL CELL DERIVED PRE-VASCULARIZED SCAFFOLD

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For wound healing applications we have developed **pre-vascularized scaffolds** using human induced Pluripotent Stem Cell derived endothelial cells (**hiPSC ECs**) seeded into a porous polyurethane scaffold. When implanted subcutaneously hiPSC EC capillaries survive and unite (**inosculate**) with the host vasculature. In "difficult to heal wounds" (over bone and tendon, deep burn wounds and chronic wounds), inosculation of host and donor capillaries will not occur and a tissue flap [capillary bed connected to a large artery and vein (a pedicle)] is required for wound coverage. Anastomosis of the flap pedicle to a host site pedicle will provide immediate blood flow in the flap. This study describes mice and rat studies where a tissue flap comprising an artery/vein connected to a hiPSC EC capillary network is assembled. In vitro: Scaffolds [6mm diam. X 1.0mm thick (PolyNovo, Melb.)] were seeded with 4 x10^5 hiPSC EC in 40 ml of fibrin. In vivo flap assembly: A vascular pedicle [epigastric vessels in SCID mice, femoral arterio-venous loop (AVL) in nude rats] was isolated and surrounded by a plastic chamber for one (rats) or 3 weeks (mice). During this period a capillary network sprouted from the pedicles. After the incubation period an iPSC EC seeded scaffold was positioned over the pedicle, and left for a further week when FITC dextran tail vein infusion was undertaken to demonstrate circulation between host and human vessels, and chamber tissues harvested for immunohistochemical/morphometric assessment. Human blood vessels survived *in vivo* (anti-human $CD31^+$) and demonstrated FITC intra-luminally indicating inosculation with host blood vessels. Scaffolds connected to mouse pedicles (N=4) had a human percent vascular volume (hPVV) of 1.18+/-0.54 % (Mean +/-SEM), and a human vascular density of 40.63+/-22.36 vessels/mm^2, whilst scaffolds connected to rat pedicles (N=3) had a higher hPVV (2.51%+/-0.08, Mean +/-SEM) and density (71.62+/-2.82 vessels/mm^2). [Note: human abdominal skin (N=4) hPVV is 0.66+/-0.17% and vessel density is 17.04+/-2.94]. Ongoing experiments will transplant rat scaffold flaps to a secondary site, to model clinical applications. As iPSC ECs can be patient derived, we have established 'proof of principle' that bio-engineered personalized tissue flaps are clinically feasible.

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T-2103

BIOPRINTED MOUSE SKELETAL MUSCLE FIBRES IN A GELATIN METHACRYLATE HYDROGEL INK

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Skeletal muscle defects remain a challenge for patients and surgeons. Volumetric loss of muscle can lead to scarring, denervation, loss of function and loss of limb. Restoring mobility and independence is a major reconstructive challenge, for which tissue engineering may offer a solution. Bioprinting is an emerging technology for skeletal muscle tissue engineering. It is advantageous for rapid fabrication of three-dimensional (3D) structures, with careful design of architecture to promote tissue maturation. The geometry of a scaffold is critical for creating an optimal environment that mimics the extracellular matrix, particularly for tissues that require cellular alignment such as skeletal muscle. This work presents a temperature controlled printing technique with post-print crosslinking, that enables the delivery of muscle precursor cells (primary mouse myoblasts) in a gelatin methacrylate (GelMA) bioink. The GelMA hydrogel can be covalently cross-linked by photo-initiated polymerisation. It is biocompatible with myoblasts, and we have developed a protocol that achieves a viability of over 99% of cells when encapsulated in GelMA by day 14 of differentiation. Molecular markers of muscle maturation, including MyoG and MYH8, support the observed morphological changes characteristic of myotube formation. Extrusion

printing of the cell-laden GelMA has produced aligned hydrogel fibres with diameters of 100-200um. Cell migration and differentiation were characterised at different time-points using 3D confocal imaging. By day 4 of differentiation, the majority of myoblasts had migrated to the surface of the fibre and begun to fuse into myotubes. By day 7 onwards, long multinucleated muscle fibres had formed, spiralling along the length of the GelMA fibre. In summary, myoblasts can be printed with high viability in a GelMA bioink, and can successfully migrate and differentiate around the fibre scaffold. This study represents the preliminary step for bioprinting functional 3D muscle constructs.

T-2105

ENHANCED PROGENITOR DELIVERY TO DYSTROPHIC SKELETAL MUSCLE VIA TROJAN HYDROGEL FIBRES

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Duchenne Muscular Dystrophy is characterised by the progressive loss of muscle tissue over time due to mutations in the dystrophin gene. There are a number of exciting gene therapy and cell replacement strategies aimed at alleviating this disease, with some approaches at the clinical trial stage, however there are some remaining challenges in the field. The majority of cell replacement strategies involve direct injection of a stem or progenitor cell bolus directly into the muscle to facilitate remodelling of diseased tissue. This approach generally results in uptake of donor cells, however previous studies have indicated that many cells are lost during the injection process and that there is some loss of donor derived muscle fibres over time. In our recent work, we have used additive fabrication technologies to create cell laden hydrogel fibres for in vivo delivery of muscle progenitors with a focus on improving the delivery and retention of donor cells in dystrophic skeletal muscle. Cell laden hydrogel fibres supported cell viability and proliferation of encapsulated progenitor cells for at least 6 weeks and facilitated cell migration from the fibres into surrounding areas in vitro. Importantly, flow cytometry confirmed the retention of myogenic markers in migrating cells for at least 12 weeks. We used this encapsulation method to deliver myogenic progenitors to the gastrocnemius muscles of *mdx* mice (murine model of Duchenne Muscular Dystrophy). We found low numbers of donor cells encapsulated in single fibres were capable of remodelling up to 50% of recipient muscles, with high numbers of donor fibres persisting for at least 12 weeks *in vivo*. In addition, significantly higher levels of donor cell remodelling were seen in animals implanted with cell laden fibres vs. animals injected with cell bolus alone. Encapsulation of progenitor cells in clinically compliant hydrogels prior to implantation may be an important step in improving the clinical delivery of progenitor cells to skeletal muscle. This approach may also be applied to the delivery of progenitor cells to other tissues to facilitate uptake and prolonged delivery of cells to tissue requiring cell remodelling due to disease or for tissue engineering approaches.

T-2107

DUAL RECORDINGS OF CONTRACTILE FORCE AND CALCIUM TRANSIENTS IN HUMAN ENGINEERED HEART TISSUES USING GENETICALLY ENCODED CALCIUM INDICATORS

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Defects in excitation contraction coupling are a hallmark of cardiac dysfunction and their investigation in human iPS-derived cardiomyocytes (hiPSC-CM) in engineered heart tissue (EHT) format may give important insight into disease pathogenesis. Our study aimed at establishing an in vitro system for measurement of contractile force and calcium transients (CaT) in EHTs using genetically encoded calcium indicators (GECI). Three-dimensional, force-generating fibrin-based EHTs were prepared from hiPSC-CM and were lentivirally transduced to express GECIs (GCaMP5G or GCaMP6f). We measured contractile force (video-optical recording) and CaT (fluorescent light intensity) sequentially with 10 sec delay under stable frequency and screened 8 indicator compounds affecting cardiac contraction and relaxation: myofilament Ca2+ sensitizer (EMD-57033), myosin activator (omecamtiv), L-type calcium channel agonist/antagonist (Bay K-8644, nifedipine), beta-adrenergic agonist (isoprenaline), ryanodine receptor antagonist (ryanodine), SR Ca2+-ATPase inhibitor (thapsigargin), Na+/K+ ATPase inhibitor (digoxin). Both GECIs showed a strong signal to noise ratio. GCaMP6f showed faster on- and off-kinetics than GCaMP5G, better reflecting physiological CaT kinetics. EMD-57033 (10 µM) increased

force (+170%) without a change in CaT. Omecamtiv (1 μM) increased force (+25%) and contraction time (+17%). Bay K 8644 (300 nM) increased force (+55%), relaxation time (+64%) and calcium decay time (+55%). Nifedipine (100 nM) reduced force (-79%), contraction time (30%), CaT (76%) and calcium rise time (26%). Isoprenaline (10 nM) showed increased force (+32%), decreased relaxation time (17%), increased CaT (+13%) and decreased calcium decay time (-21%). Ryanodine (10 μM) and thapsigargin decreased force (37%, -28%) and CaT (-49%, 33%) respectively. Digoxin (0.3 μM) increased force (+36%) and CaT (+46%), reduced contraction time (-14), relaxation time (-18%), calcium rise (-18%) and calcium decay time (-23%). Force/calcium loops also revealed compound-specific changes illustrating the mechanism of action. Dual force and Ca2+ measurements in hiPSC-EHTs provide important information on drug actions on cardiac excitation contraction coupling under stable experimental conditions.

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ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

T-2109

VULNERABILITIES AND THE USE OF AUTOLOGOUS STEM CELLS FOR MEDICAL CONDITIONS IN AUSTRALIA

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Australia has a booming market of unproven autologous stem cell-based interventions (SCBI) for a wide range of medical conditions. Multiple SCBIs are provided in private practices outside of formal clinical trials. Some defend the provision of unproven SCBIs on grounds of patient choice. In this presentation, I interrogate this argument for patient choice and explore patients' vulnerabilities in clinical practice with autologous SCBIs. While all patients are inherently vulnerable, the regulatory framework for autologous stem cells in Australia exacerbates the problems associated with inherent vulnerabilities and generates situational and pathogenic vulnerabilities. I argue that a just state ought to implement regulatory measures that mitigate vulnerabilities and foster patients' autonomy.

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CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

T-2113

SELECTIVE CELL DEATH INDUCTION OF PLURIPOTENT STEM CELLS WITH LUTEOLIN FOR TERATOMA INHIBITION

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Because of tumorigenic potential of the residual undifferentiated human pluripotent stem cells (hPSCs), complete elimination of these cells with minimal or no damages to the differentiated cells would be an important prerequisite for safe stem cell-based therapy. Previously, we have shown that quercetin (QC) but not kaempferol (KP), induces the selective cell death of hPSCs and inhibits teratoma formation while functionality of the differentiated cells remains intact. Considering structural similarity between QC and KP except one hydroxyl group of hydroxylflavonol backbone, we hypothesized that structure of flavonoid would affect the cytotoxicity of hPSCs. Through screening of total 11 flavonoids with different number of hydroxyl groups, we identified luteolin but not apigenin, was more potent than QC for inducing selective cell death of hPSCs compared to the smooth muscle cells through p53 stabilization, although both luteolin and apigenin induced strong cell death of a various cancer cell lines. These data suggest that two hydroxyl groups of hydroxylflavone structure are important for cell death induction of hPSCs.

T-2115

INHIBITION OF LYSOPHOSPHATIDIC ACID RECEPTOR 2 EXPEDITES ODONTOBLASTIC DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS

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Dental stem cells are divided into five different types, including dental pulp stem cells (DPSCs), human exfoliated deciduous teeth, periodontal ligament stem cells (PDLSCs), apical papilla stem cells, and dental follicle progenitor cells, depending on their origins. Our research focus is on the relation of dental stem cells and lysophosphatidic acid (LPA) signaling. Previous studies have shown the relation of LPAs with the proliferation and differentiation of mesenchymal stem cells. However, the effect of LPA signaling on dental stem cells has not been reported. In this study, we investigated the effect of LPA receptor inhibitors on the proliferation and differentiation of dental stem cells. Among LPA receptor inhibitors, LPAR2 inhibitor, Beck 35, promoted the proliferation of DPSCs but inhibited the proliferation of PDLSCs, which showed the opposite effects on two different dental stem cells. In osteogenic differentiation, RT-PCR analysis showed a gradual decrease of LPAR2 expression in DPSCs. When DPSCs were treated with different concentration of LPAR2 inhibitor during osteogenic differentiation, increasing dose of LPAR2 inhibitor promoted osteogenic differentiation, which reduced the differentiation period from three weeks to one week according to Alizarin Red S staining. The proliferation of DPSCs, however, during osteogenic differentiation decreased by LPAR2 inhibitor dose-dependently. When differentiating DPSCs were subjected to real-time PCR analysis, Beck35-treated cells showed the increased expression of odontoblast differentiation markers, such as DSPP, DMP-1, osteogenic differentiation markers, such as RUNX2, Osteopontin and the accelerated decrease of LPAR2 expression in comparison with control cells. When LPAR2 inhibitor was applied to molar pulp in mice after cavitation, LPAR2 inhibitors increased calcification of pulp cells. These results suggest that the inhibition of LPAR2 signaling positively regulates the proliferation and osteogenic differentiation of DPSCs, which may contribute to the development of the regenerative medicine in dentistry.

T-2117

TRANSIENT IN VIVO MODULATION OF PTEN AMELIORATES FUNCTIONAL MOTOR RECOVERY FOLLOWING SPINAL CORD INJURY IN MICE

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Spinal cord injury (SCI) is a debilitating condition which progresses to paraplegia due to the secondary cascade of events that follows a primary assault. Clinical, cell and rehabilitation therapy has had minimal impact on regaining lost function. Recently, knockdown of PTEN seems promising but safety concerns with toxicity and immunogenicity employing shRNAs and viral vectors questions translatability. Our primary aim was to establish an inducible, virus-free, non-toxic, safe and localized modulation construct for PTEN in SCI mice model targeting functional recovery. For this, we developed a Doxycycline (Dox) inducible miR-E based knockdown of PTEN. After contusion injury, endotoxin-free DNA constructs were electroporated in vivo caudally and rostrally to the lesion epicenter. For PTEN knockdown, Dox was administered intraperitoneally and supplemented in drinking water; Dox negative served as controls. Manual expression of bladder thrice daily and weekly behavioral assessment using BMS scale were performed along with in vivo GFP tracking and CT once a fortnight. Trans-cranial stimulation and motor evoked potentials were recorded before termination of the experiment. Our results show that Dox+ mice exhibit a marked increase in BMS scores (day 35 onwards) and significant amplitudes and latency in evoked potential from hind-limb. There was a complete absence of evoked potentials in controls. Histopathological and immunohistochemical analysis showed reduced lesion size and a significant reduction in hypertrophy, and reactive astrogliosis in Dox-treated, compared to control. Our findings collectively suggest that an in vivo electroporation mediated miR-E based modulation of PTEN could be a safer potential therapeutic strategy for intervention in SCI, which may have translational potential.

T-2119

CHARACTERIZATION OF NOVEL HUMAN ENDOMETRIAL STROMAL CELL PRODUCT

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Infertility due to conditions such as Asherman's Syndrome (endometrial adhesions/ fibrosis) or thin, unresponsive endometrium represent a significant clinical problem. Little is understood regarding the mechanisms or role of the stroma in the pathogenesis of these clinical indications. The aim of this study was to isolate and characterize endometrial stromal cells for the development of a translational, clinical grade

product for cell therapy suitable for the treatment of Asherman's Syndrome or unresponsive endometrial disorders. Endometrial biopsies (n=6) were taken from healthy, fertile women during the proliferative phase of the menstrual cycle (day 7-9) and stromal cells were isolated by enzymatic digestion. Cells were expanded under xeno-free conditions for multiple passages to assess both colony forming potential (CFU-F) and proliferative capacity. Expanded cells were assessed for cell surface expression of the mesenchymal stromal cell (MSC) markers CD90, CD105, CD73, CD29, human leukocyte antigen (HLA) I, HLA II and for the haematopoietic and endothelial markers CD34 and CD45. Multipotency was determined by differentiation down adipocyte and osteoblast lineages. Expanded cells (passage 3) were evaluated for chromosomal stability by karyotyping and tumorigenic potential using the soft agar colony forming assay. Cells were co-cultured with allogeneic lymphocytes (+/- activation with anti-CD2/CD3/CD28 microbeads) to assess immunoreactivity and immunomodulation respectively. Stromal cells were reliable isolated and expanded in vitro under xeno-free, clinical protocols. Cells were uniformly positive for MSC markers and demonstrated no expression of HLA II or haematopoietic or endothelial markers. Expanded cells demonstrated an MSC like phenotype, with CFU-F formation and multipotency down both adipocyte and osteoblast lineages. Importantly expanded cells demonstrated no in vitro tumorigenic potential or alloreactivity. Our continuing work focuses on further evaluating the potential for these cells in the development of a local cell therapy application in the treatment of endometrium-led infertility.

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GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-2123

IMPLANTATION-COMPETENT BLASTOCYST-LIKE STRUCTURES FROM MOUSE PLURIPOTENT STEM CELLS

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Soon after fertilization, the few totipotent cells of mammalian embryos diverge to form a structure called the blastocyst(BC). Although numerous types of cells including germ cells and extended pluripotency stem cells have been generated from pluripotent stem cells(PSCs) in-vitro, generating functional BCs only from PSCs has not yet been reported. Here we describe induced self-organizing 3D BC-like structures(iBCs) generated from mouse PSC culture in-vitro. Resembling natural BCs, iBCs have a blastocoel-like cavity, and were formed with outer cells that are positive for an extraembryonic lineage markers and with inner cells that are positive for embryonic pluripotency markers. iBCs transplanted to pseudopregnant mice uteruses implanted, induced decidualization, and exhibited growth and development before resorption, demonstrating that iBCs are implantation competent. iBC production required the transcription factor Prdm14 and concomitantly activates the MERVL totipotency related cleavage-stage reporter. Thus, our system may contribute to understanding molecular mechanisms underpinning totipotency, embryogenesis, and implantation.

T-2125

POST-TRANSCRIPTIONAL REGULATORS OF MOUSE GERMLINE STEM CELL FUNCTION

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The maintenance of adult tissue integrity and function is dependent upon the proliferation and differentiation of tissue-specific adult stem cells. The balance between self-renewal and differentiation of adult stem cells is regulated through extrinsic stimuli that includes growth factors produced in a supportive niche, as well as cell intrinsic factors such as transcription factors and post-transcriptional gene regulatory mechanisms. Mammalian spermatogenesis is a complex process that is maintained by a population of adult stem cells known as undifferentiated spermatogonia. These cells enable the lifelong production of spermatozoa through tightly co-ordinated transcriptional and post-transcriptional mechanisms. Recently, the RNA helicase DDX5 has been identified as a candidate marker of spermatogonia; however, its role in spermatogenesis remains unknown. DDX5 is known in other systems to play essential role in splicing, mRNA export, maintenance of transcript stability, rRNA biogenesis and microRNA processing. Using a murine knockout model, we demonstrate that DDX5 is required for the maintenance of male fertility. In addition, we used an in vitro germline stem cell culture system to elucidate the molecular mechanisms underlying DDX5 function. We show through a combination of approaches that DDX5 can associate with other RNA binding proteins to post-transcriptionally regulate key genes involved in germ cell function. We conclude that DDX5 is essential for post-transcriptional regulation in spermatogonia and its loss results in male infertility.

T-2127

SAFEGUARDING SPERMATOGONIA STEM CELL GENOMIC INTEGRITY BY NON-CANONICAL FUNCTIONS OF EPIGENOMIC MODULATORS

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Male germ cell development and spermatogenesis is a good model for studying stem cell based hometostasis and differentiation. Spermatogonial stem cells (SSCs) are also the foundation of long-term male fertility. The genetic and epigenetic integrity of these cells determine the health of the next generation. The physiologically or pathologically derepressed endogenous retrotransposons during male germ cell development pose significant threat to the genetic and epigenetic integrity of SSCs. We study how epigenetic modulators, piRNA pathway components and germinal stem cell surviving factors collaborate to ensure retrotransposons are re-repressed during prospermatogoina and spermatogonia development, after the physiological epigenome wide derepression during primordial germ cell development. DNA methyltransferase 3-like (DNMT3L) is an epigenetic modulator without enzymatic activity, but is one of the key components for facilitating de novo DNA methylation on retrotransposon in prospermatogonia. In the postnatal spermatogonia stem cell (SSCs) enriched THY1^{+} population from 8 dpp pups, DNMT3L expression can be observed in a subset of (~30%) quiescent cells with perinuclear localized stem/progenitor cell marker PLZF, potentially contributing to the over proliferation and differentiation defects observed in the SSCs enriched THY1^{+} cells from *Dnmt3l* KO mice. The subcellular localization of DNMT3L in postnatal SSCs is surprisingly in both the nucleus and cytoplasm. We also observed the physical interactions of PLZF-DNMT3L, PLZF-MILI, PLZF-MAEL in the SSCs enriched THY1^{+} population. On top of the epigenomic and piRNA pathway modulation activity for PLZF and MAEL, respectively, PLZF and MAEL have both been implicated for retrotransposon transcripts binding and translational inhibition. In addition, we also observed aberrant piRNA compositions and origins in THY1^{+} cells, as well as significant down regulation of piRNA pathway components, MILI, TDRKH, VASA, and the depletion of TDRDKH-MILI interaction in 8 dpp testes of Dnmt3l KO mice. We therefor deduced a crosstalk among DNMT3L-PLZF-piRNA pathway network for safeguarding the epigenomic integrity of spermatogonia stem cells in both epigenomic and post-transcriptional levels, for germline maintenance and healthy offspring.

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TECHNOLOGIES FOR STEM CELL RESEARCH

T-2129

A NON-INVASIVE ROUTINE TEST TO CAPTURE RECURRENT GENETIC ABNORMALITIES IN HUMAN PLURIPOTENT STEM CELL SUPERNATANTS

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Human pluripotent stem cells (PSC) are used for basic research and regenerative medicine. However, their genetic stability during passaging is critical to avoid result misinterpretation, and unexpected complications in clinical therapies. Thus, PSC genetic integrity should be regularly checked. Among the genetic abnormalities found in PSC, recurrent abnormalities are a major concern because their recurrence might reflect a selection pressure that favors cell proliferation and survival, or blocks PSC differentiation capacities. Here, we report the development of a rapid, cost-effective and highly sensitive test for the detection of recurrent abnormalities in PSCs. To this purpose, we gathered data from 110 published studies (n=942 PSC samples). We identified 757 recurrent genetic abnormalities (i.e., genome sequence with abnormalities found in at least five different publications), and 243 hyper-recurrent abnormalities (i.e., found in at least 20 different publications). Strikingly, a limited set of 24 PCR targets, designed based on the 24 most common altered sequences, covered more than 90% of all recurrent and over 99% of all hyper-recurrent genetic abnormalities. A major hurdle in the routine PSC genome integrity screening is the requirement of a significant amount of cultured cells for these tests, and the inconstant success of karyotyping, the gold standard test. We thus developed a PARalleled and Multiplexed droplet digital PCR (ddPCR) approach (called PARM ddPCR) to measure recurrent copy number variations (CNV) in PSC. We show that the CNVs detected by PARM ddPCR in DNA extracted from culture supernatant was highly correlated with that in DNA from adherent PSC. Hence, PARM ddPCR of culture supernatant, using the 24 most common recurrently altered sequences in PSC, is a non-invasive technology to routinely assay PSC genetic stability by analysis of the majority of recurrent genomic abnormalities. This new test can change how quality control is implemented for PSC use in basic research and regenerative medicine.

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T-2131

NEWLY DEVELOPED XENO-FREE MEDIUM FOR HUMAN MESENCHYMAL STEM CELLS SHOW ROBUST CELL-EXPANSION CAPACITY

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Human mesenchymal stem cells (hMSCs) are an attractive candidate for cell therapy due to their multipotential differentiation activities into each cell type or immunomodulatory properties. For therapeutic applications, hMSCs are needed to be expanded to appropriate cell number because primary hMSCs obtained from bone marrow, adipose or cord blood are usually limited and thereby the required cell number can't be obtained. Although many medium for culturing hMSCs are currently proposed, further improvements in their cell-expansion capacity or the maintenance of multipotential differentiation activities have been needed. Furthermore, medium for clinical use should be at least xeno-free formulation because of the potential risks of FBS, such as virus or prion contamination. Thus, we have newly developed xeno-free medium for hMSCs, having especially robust cell-expansion capacity. Both bone-marrow and adipose derived hMSCs expansions using new medium were several-fold higher than those using conventional other medium (commercially available). Moreover, new medium could be used even in the extracellular matrix coating-free condition. After several passages, we confirmed the expression of cell surface markers identified as hMSCs such as CD73, CD90 and CD105 by flow cytometry and colony-forming unit-fibroblast (CFU-F) capacity of the expanded cells. The cells expanded by new medium were shown to be almost 100% of CD73, CD90 and CD105 positive population, but a little decrease of CD105 expression was observed in the cells by other medium. Since CFU-F capacity was almost equal among tested medium, the obtained colony-forming cells in new medium were several-fold larger than those in other medium. We have also confirmed their multipotential differentiation activities into adipocytes, chondrocytes and osteocytes. Overall, it is considered that our newly developed xeno-free medium have desired properties to expand hMSCs for therapeutic application.

T-2133

PROTEOMIC COMPARISON OF XENO FREE AND TRADITIONAL MEDIA FOR HUMAN ADIPOSE STEM CELLS

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Human adipose stem cells are yet to have their phenotype fully characterised. However, they are being utilised as treatments for a plethora of disorders, despite the minimal evidence of efficacy. A lack of standardisation of procedures of the maintenance of clinical standards is also apparent in the expansion of human adipose stem cells for clinical use. Traditional media for growing human adipose stem cells is supplemented with fetal bovine serum, which provides essential nutrients, growth and attachment factors, hormones and a variety of macromolecules. However, its animal origin can present safety issues and the potential risk of inducing an immune response or infection within patients. An alternative to traditional fetal bovine serum containing media is xeno-free media, which does not face any of the aforementioned issues. This project compares human adipose stem cells isolated and cultured in DMEM with 10% fetal bovine serum to StemMACS Xeno-free alternative. The comparison is achieved through characterisation of the expressed proteome through data independent acquisition mass spectrometry with a Q Exactive™ Plus Orbitrap Mass Spectrometer, and evaluation of 27 cytokines with a Multiplex Immunoassay. The resulting data provides an analysis of whether a xeno-free alternative for culturing human adipose stem cells alters the phenotype and will contribute towards standardised procedures for maintenance of human adipose stem cells. This research is vital as human adipose stem cell therapies are still readily available on the market despite the lack of standardisation of protocols as apparent through the minimal investigation into xeno-free alternatives.

T-2135

HIGH-CONTENT ASSAY FOR MORPHOLOGICAL CHARACTERIZATION OF 3D NEURONAL NETWORKS IN AN ORGAN-ON-A-CHIP MICROFLUIDIC PLATFORM

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The increasing prevalence of neurological disorders could possibly be correlated to the large number of untested hazardous compounds in the environment. Therefore, a pressing need is to develop efficient screening tools to identify chemicals that could potentially causes neurological disorder and affect neurological development. There is an increasing interest in using three-dimensional (3D) cultures for assay development applicable to study neurodegenerative diseases and to perform neurotoxicity screens. The OrganoPlate, a microfluidic-based organ-on-a-chip platform allows development of long-term 3D tissue cultures from different cell types. We developed methods for monitoring the effect of compounds on the formation and integrity of 3D networks of spontaneously active iPSC-derived human neurons. The 3D neuronal cultures were treated for five days across a 6-point concentration range with a set of 22 neurotoxic compounds including methyl mercury, lead, rotenone, DDT, and other chemicals with known neurotoxicity effect. The complexity of neuronal networks, cell viability, and mitochondria integrity were assessed in multi-parametric phenotypic assay using automated confocal imaging and high content analysis. Neurite outgrowth and cell integrity were evaluated using a combination of neuronal markers, viability dyes, calcium and mitochondria potential probes. Phenotypic readouts allowed quantitative characterization of the extent and complexity of the neural networks in 3D. The 3D analysis readouts included counting the number of neurites, processes, branching points, characterization of viability and mitochondria integrity. The method is applicable for compound screening and prediction of neurotoxicity. Furthermore, a library of representative neurotoxic compounds was screened and compound effects were compared between 3D and two-dimensional (2D) model systems. Significant concordance in the phenotypes between 3D and 2D models was observed after compound exposure, but essential differences were observed in the concentration-responses in a time-dependent manner. The results indicate that 3D neuronal cultures constitutes a functionally distinct biological model system from traditional flat 2D cultures, and therefore could be applied for predictive toxicity screenings.

T-2137

HIGHLY REPRODUCIBLE INTEGRATED SYSTEM FOR HUMAN MIDBRAIN DA NEURON PRODUCTION FROM FIBROBLAST VIA EPISOMAL IPS CELL

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Human iPS cell lines suffer from significant heterogeneity in their differentiation capabilities. A practical solution to this problem is an implicit but fundamental premise for fully utilizing this technology for the medical industry. With the advent of the TET1-introduced human iPS cell reprogramming, we took advantage of obtaining in-house vector-free human iPS cell clones using episomal vectors from both male and female fibroblasts. We have also set a biologically-relevant standard threshold for the amounts of residual vectors after their establishment. Challenging the clones through a default differentiation protocol revealed that only about a quarter of the obtained clones had adequate differentiation capabilities toward neuroectodermal cell specification. A systematic survey of the gene expression profiles before and after cell differentiation uncovered the newly proposed “Pluripotency Indicators” which prospectively diagnose the differentiation capabilities. In other words, these Pluripotency Indicators represent true “pluripotency-relatedness” unlike other conventional pluripotency-genes such as POU5F1, SOX2, and NANOG with no such value. Moreover, clones with good differentiation capabilities had significantly better performance in their differentiation efficiencies into NURR1-positive midbrain dopaminergic (DA) neurons to an unprecedented level attained using any kind of human pluripotent stem cells. Importantly, these clones displayed far more elaborated dendritic arborization, often hypoplastic in cultures derived from conventional clones, an overt problem common in most drug screening platforms. The proof-of-principle differentiation success exemplified here encouraged us to question the true requirement for the human pluripotency. We will discuss some key features which may have segregated the mouse versus human pluripotency, with special concerns about their difference in the early cell lineage specification. We feel confident that this developed system can be traced in most research facilities and hence, merit to be considered as a de facto standard for human iPS cell production.

T-2139

THE INVERTED QUASI-SPHERICAL THREE-DIMENSIONAL CELL CULTURE SYSTEMS FOR EFFICIENT EX VIVO GENE-CELL THERAPY

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In the field of gene and cell therapy, the gene delivery and expression efficiency are the important factors to determine efficacy of therapeutic effects by gene-cell based recovering of malfunctioned tissues. Herein, the inverted quasi-spherical three-dimensional (iQS-3D) ex vivo cell culture substrates were developed to increase the delivery efficiency of viral and non-viral gene delivery vehicles into targeted cells. The iQS-3D substrates were fabricated with stable polydopamine (pDA) hydrophilic layers and superhydrophobic nano-roughness titaniumdioxide (TiO2) layers. In this study, by controlling the exposed area of hydrophilic layers with sub-micron dot pattern, the contact angle of aqueous hanging droplet were regulated and the stable iQS-3D droplets conditions were optimized. The iQS-3D substrates provided the dramatically increased spherical curvature of hanging droplets and the unique bioactive microenvironments with suitable suspending cell culture conditions without hypoxia of culture medium. The iQS-3D substrates showed high potential of platform technique for ex vivo gene delivery with using adeno-associated viral vectors (AAV) and human neural stem cells (hNSCs). The iQS-3D culture condition induced the enhanced AAV transduction efficiency, the regulation of the size of neurospheres, and improved secretion rate of interleukin-10.

T-2141

GENERATION OF KIDNEY MICRO-ORGANOIDS FROM HUMAN IPSCs VIA SUSPENSION CULTURE FOR HUMAN KIDNEY CELL SCALE UP

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The directed differentiation of human pluripotent stem cells (hPSCs) to distinct cellular endpoints has enabled the generation of organoid models of a variety of human tissues, including the kidney. While kidney organoids has provided a future for disease modelling, drug screening and regenerative medicine, the approach is expensive and faces limitations to long term culture. As a result, novel cost effective techniques are needed to enable scale up of kidney cells types in vitro and facilitate higher throughput screening approaches. Kidney micro-organoids were generated using a swirling suspension culture. Briefly, hPSCs were differentiated to intermediate mesoderm using CHIR and FGF9 in 2D monolayer for 7 days. Derived intermediate mesoderm cells were dissociated using EDTA and allowed to form cell aggregates via low speed swirling using a media containing a cocktail of growth factors. Within 24hr, cell aggregates formed an outer laminin basement membrane. These organoids further matured for 12 to 18 days to yield complex kidney micro-organoids in suspension culture, enabling the simultaneous generation of organoids in a single culture flask. Mature kidney micro-organoids revealed the presence of intact patterning nephrons, including podocytes (NPHS), proximal (LTL) and distal (ECAD) nephron and collecting duct (ECAD+GATA3) segments. 3D reconstruction revealed contiguous nephrons with clear tubular lumens. Each micro-organoid contains only 6-10 nephrons. As a result, imaging through an entire organoid is feasible. 10x Chromium single cell RNA sequencing analysis identified the presence of 9 distinct cell types, both confirming the presence of anticipated renal cell types and revealing a reduced level of surrounding stroma compared to our previously described protocol where kidney organoids are cultured on trans-well filters . With this novel method, a starting population of 4.5 million cells (D7+0) was expanded to 90-100 million kidney cells (20 fold) within 10 days, providing a platform for the economical production of kidney cells for various biological applications. In conclusion, this is a cost effective method for the generation of large numbers of human kidney cells. As a result, this technique will improve the feasibility of drug screening and regenerative cell therapy.

T-2143

AUTOMATED DEEP LEARNING-BASED SYSTEM FOR THE IDENTIFICATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS

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Deep learning technology has been rapidly developing. Deep neural networks can extract data patterns automatically, to solve complex issues. Convolutional neural network (CNN) has important applications in image classification, as using it, the labeled images can be accurately recognized and classified. Induced pluripotent stem cells (iPSCs) can differentiate into different cellular types, but the potential of iPSCs varies between the cells and external conditions, making it difficult to identify differentiated cells and their types without applying immunostaining or lineage tracing. Therefore, an automated system for the identification of the differentiated iPSCs may lead to the wider use of these cells. The aim of this study is to identify iPSC-derived endothelial cells (ECs) using CNN and the morphological information of the phase contrast images, and to develop simple and reliable automated recognition system. We induced the differentiation of iPSCs to ECs, and phase contrast images were used as the input datasets. After obtaining phase contrast images, ECs were immunostained with anti-CD31 antibody and these images were binarized, creating the answers. CNN was trained to identify the ECs on the input images by referring to the binarized answers, and had two predictions: the target blocks were either stained or unstained for CD31. Interestingly, F1 score and accuracy, indicating CNN performance, showed positive correlation with the input image size, indicating surround environment of the target blocks is important for the identification of the target cells. Moreover, deeper neural network improved F1 score and accuracy. Error analysis showed that the area with the heterogenous appearance, such as a border of a high cell density area, may generate incorrect answers. Reconfiguration of answer to avoid errors increased network performance. Finally, K-fold validation confirmed that CNN can identify iPSC-derived ECs with high accuracy and generalization (F1 score: 0.73, Accuracy: 0.93). CNN was able to identify iPSC-derived ECs in just 275 msec per block. We demonstrated here that CNN can effectively identify iPSC-derived ECs, and can be applied as the automated identification system for the detection of the iPSC-derived target cells.

T-2145

DEVELOPING STANDARDS AND VALIDATION METHODS FOR XENO-FREE STEM CELL RESEARCH

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Research with human induced pluripotent stem cells (iPSCs) can afford both basic science and clinical insights into human physiology and disease within a human-based framework. However, the microenvironments of many iPSC-based studies often contain extensive xenogenic materials. Beyond the ethical concerns, these components of animal-origin introduce several other issues, including: (i) they are highly variable (ii) they are foreign to the original system (iii) in therapeutic settings, they pose the risk of transferring pathogens and/or unwanted biological material to the model systems. Together, these issues unnecessarily reduce the predictive and therapeutic value of iPSC research. We have previously reported an ongoing effort to help the field transition to xeno-free studies by developing an online toolkit and database to increase transparency of methods and materials as well as support broader access and refinement of xeno-free protocols. Here, we describe our process for creating practical standards and clear validation steps in order to help researchers improve the human-relevance of their studies, increase efficiency, lower costs, address ethics, and increase transparency via well-defined protocols. By analyzing the methodology of peer-reviewed articles and comparing protocols obtained through direct requests from laboratories, we determine and assess the potential challenges in reproducibility. Our preliminary validation framework consists of a crowdsourcing approach to engage laboratories and material suppliers thereby providing detailed materials and methods in order to determine the minimal necessary standards to generate and maintain effective xeno-free model systems. Using a mix of expert reviews, standards and/or validation guidelines from established fields, we report our preliminary methodology, versioning and templating of protocols that are so essential for developing robust examples of xeno-free research models. The development of these resources and an open research network are necessary for the expansion of more relevant human research approaches and increasing translatability of basic research in the age of personalized medicine.

T-2147

ASCORBIC ACID 2-GLUCOSIDE PROMOTES THE PRIMITIVE STATE IN EMBRYONIC AND ADULT STEM CELLS THROUGH TET- AND CREB1-DEPENDENT MECHANISMS

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The naïve or primitive states of stem cells (SCs) residing in specific niches are unstable and difficult to preserve in vitro. Vitamin C (VitC), in addition to suppressing oxygen radicals, exerts pleiotropic effects on primitive SC functions. However, this compound is labile and readily oxidized, resulting in cellular toxicity and preventing its reliable application in this context. We found that a VitC derivative, ascorbic acid 2-glucoside (AA2G), which lacks cellular toxicity, stably maintains the primitive state of embryonic (ESCs), inducible pluripotent (iPSCs), and mesenchymal SCs (MSCs). AA2G supplementation recapitulated the well-known effects of VitC, including promotion of TET-dependent DNA demethylation in murine ESCs and suppression of p53 during generation of iPSCs. Particularly, activation of the cAMP-responsive element-binding protein-1 (CREB1) pathway contributed to the potency of AA2G in multiple SC types. Importantly, supplementation of MSCs with AA2G improved therapeutic outcomes in an asthma mouse model by promoting the self-renewal, anti-inflammatory, and engraftment properties of these cells. Therefore, AA2G supplementation should be broadly useful in providing an environment that supports the naïve pluripotent or primitive state of several types of SCs, which would have a major impact on their developmental potency and the efficacy of therapeutic applications using these cells.

T-2149

MAKING CHIMERAS WITH NON-RODENT PSCS BY OVEREXPRESSING BCL2

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When the cells in more advanced development stages transplanted into pre-implantation embryos, their progenies fail to contribute to morphogenesis. However, we previously found that if cell survival is transiently promoted by expressing the anti-apoptotic gene *BCL2*, epiblast stem cells (EpiSCs) and even lineage-committed progenitors can form chimera with pre-implantation stage embryos. It has been reported that conventional non-rodent pluripotent stem cells (PSCs), including humans, carries similar characteristics to rodent EpiSCs, so that they are corresponding to post implantation epiblast. This mismatched developmental stages between the grafted PSCs and pre-implantation embryos might be the reason to make chimeras from PSCs within non-rodent animals. Here we report that overexpression of BCL2 promotes chimera formation within various non-rodent PSCs, such as chimpanzee, macaque monkey, marmoset, and rabbit, with mouse embryos. However, we also find that the progeny of transplanted cells did not proliferate unlikely to BCL2-overexpressing EpiSCs, despite the high possibility of chimeric embryos. This low proliferation of the engrafts might be caused by interspecies setting.

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T-2151

STRATEGIES TO ENHANCE AND MODULATE HEMATOPOIETIC STEM CELL CONTRIBUTION TO BRAIN MYELOID CELL/MICROGLIA TURNOVER

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Our recent data indicate that the delivery of hematopoietic and progenitor stem cells (HSCs) in the brain lateral ventricles (intracerebral ventricular - ICV - delivery) of conditioned recipient results in a rapid, robust and central nervous system (CNS)-restricted engraftment of transplant-derived myeloid cells sharing functional features of microglia. This repopulation is driven by early homing after transplant and long term engraftment of HSCs in the brain that generate a myeloid local progeny (Capotondo et al, 2017). The ICV delivery route per se has therapeutic relevance because it allows for delivery of therapeutic molecules to the brain and modulation of microglia function for the treatment of disease affecting the CNS. Moreover, it also offers the opportunity of enhancing and fastening brain engraftment and therapeutic benefit of a standard HSPC transplantation strategy in the context of a

combined approach. In order to develop this strategy toward the clinics we tested different combinatorial HSC based transplant protocols modulating the contribution of intravenous (IV) versus ICV injected cells to the CNS and to medullar hemapoiesis in terms of population employed at each site and timing of cell delivery. In the context of this study, we could characterize and fate map the CNS-progeny of the transplanted cells and obtain relevant insights on the mechanism of HSC engraftment and differentiation in the CNS. Indeed, we observed that the route of cell delivery determines i) a different CNS microglial and medullar hematopoietic reconstituting potential of the transplanted HSCs, ii) a specific pattern of distribution of the transplanted progeny in the brain and iii) a differential maturation stage of IV versus ICV-derived cells, as shown by morphological and transcriptional studies. Importantly, we documented the unique role of the ICV delivery route for a not only fostered myeloid engraftment of the transplanted HSCs, but also their more efficacious differentiation into morphologically- and functionally-defined microglia cells distributed throughout the CNS. Overall, our results demonstrate the biological relevance of HSC transplantation within the CNS. Its therapeutic potential was also shown in neurodegenerative disease animal models in the context of gene therapy and allogeneic transplantation

T-2153

LARGE SCALE EXPANSION AND ABERRANT PROLIFERATIVE BEHAVIOR IN DENTAL PULP STEM CELLS UNDER XENO-FREE CULTURE CONDITION

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Mesenchymal stem cells (MSC) are cultured using culture medium supplemented with fetal bovine serum (FBS) and stored in dimethyl sulfoxide (DMSO)-containing medium. However, FBS and DMSO hinder clinical application of MSCs due to immunoreactions or cytotoxicity. The purpose of this study was to establish a xenogeneic serum-free culture medium (XFM) for clinical handling of dental pulp stem cells (DPSCs). DPSCs were isolated from eight wisdom teeth using XFM or FBS-containing medium (SCM). Cells at passage 3 or 4 were subjected to morphological, proliferation, karyotype, marker expression, cryopreservation, and cytotoxic susceptibility analyses in vitro and transplantation in vivo. The adhesive area of XFM cells was significantly smaller than that of SCM cells. XFM cells exhibited significantly greater growth than SCM cells and maintained a normal karyotype. XFM cells showed typical MSC characteristics in vitro and in vivo. However, XFM cells were susceptible to extrinsic cytotoxic stimuli. Moreover, upon reaching over-confluence, XFM cells formed a multilayered structure showing cell death/division and the cell number decreased. Furthermore, over-confluent XFM cells could not be subcultured. DMSO-free cryopreserved XFM cells exhibited similar results as those obtained using XFM cells in the aforementioned experiments. Dental pulp cells cultured under xenogeneic serum-free culture conditions have a high proliferative potential and retain MSC characteristics; these can also be effectively cryopreserved. However, excessive XFM cultivation until over-confluence results in aberrant proliferative behavior in DPSCs. These results suggest that XFM cultivation and DMSO-free cryopreservation are useful for the expansion of DPSCs and clinical application to cell-based therapies.

T-2155

SIS-SEQ: A MOLECULAR "TIME MACHINE" LINKS GENE EXPRESSION WITH STEM CELL FATE

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While single cell RNA sequencing (scRNA-seq) has revolutionised the understanding of cellular heterogeneity, due to its destructive nature, it precludes testing of the same cell for its putative unique biological property. In the absence of a time machine, we reasoned that a small pre-expansion step of single cells might allow daughters to act as biological surrogates of the mother. While it only applies to cells for which division is possible and for biological features that are intrinsic and therefore heritable upon division, cells from these clones can be assessed in parallel for multiple molecular and cellular aspects. In this way, we determine the earliest gene expression correlates of dendritic cell subtype development. More generally, this principle could be applied to identify the gene expression correlates of rare stem cells, cancer cells or other systems where molecular characterisation of a subset of cells with a unique biological property is desired.

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T-2157

DEVELOPMENT OF STEALTH RNA VECTOR: AN IDEAL TOOL FOR CELL REPROGRAMMING

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Since the discovery of induced pluripotent stem cells (iPSCs), it is widely accepted that the somatic cell fate can be redirected with forced expression of multiple reprogramming factors. As cell reprogramming is a relatively slow process, recombinant retro- and lenti-virus vectors have been used as the standard tools for achieve this goal. However, for clinical application of reprogrammed cells, random integration of exogenous genes into host chromosomes should be avoided. We have been focusing on RNA-based technology for achieving stable gene expression without chromosomal integration. One of the outcomes of this project, SeVdp (defective and persistent Sendai virus) -iPS vectors installed OCT4/SOX2/KLF4/c-MYC on a single genome RNA, have been widely appreciated as a tool for iPSC generation. Lessons from SeVdp vector project lead us to develop "Stealth RNA Vector (SRV)", a new generation gene delivery/expression platform for basic as well as for clinical applications. SRV was reconstructed from structure-optimized synthetic RNA for escaping from the defense system of host cell against invading pathogen. SRV can install one to ten cDNAs (total size up to 14 kbp) on a single vector, and can express them stably in the cytoplasm at physiological level. In addition, SRV can be erased from the cells quickly and completely by interfering vector-derived RNA-dependent RNA polymerase, either with endogenous miRNA or with specific siRNA. SRV installed with six reprogramming factors can reprogram various human tissue cells (dermal fibroblasts, cord blood cells and peripheral blood monocytes) into iPSCs under feeder- and xeno-free condition. The reprogramming occurs with extremely high efficiency (5 ~ 50%) and footprint-free iPSCs can be generated within a short period (~ four weeks). These characteristics are ideal for generating clinical-grade human iPSCs. We will present our recent progress of SRV technology and its application.

T-2159

GENE EDITING TOOLS FOR HIGH THROUGHPUT CELL ENGINEERING AND COMBINATORIAL GENE INTERACTION STUDIES

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Systematic mapping of underlying cellular pathways and functional interactions between genes is fundamental to advancing our understanding of biological systems. Decoding these gene interactions is also vital to functional genomics and drug discovery research. Without high throughput (HTP) gene editing tools and screening platforms the time and monetary investment towards these efforts will remain a large bottleneck for both pharmaceutical companies and research driven organizations. Next generation gene editing technologies like the CRISPR/Cas9 system allows for more efficient generation of disease relevant cell models and genome wide loss of function screening thereby enabling drug discovery research. My work at Thermo Fisher Scientific is focused on two main goals 1) developing ready-to-use CRISPR/Cas9 ribonucleoprotein (RNP) formulations to enable HTP cell engineering workflows including single and multiplexed gene editing and 2) Testing lentiviral based tools for large gene deletions and synthetic lethality screening applications. This work will demonstrate optimal conditions for combinatorial gene knockout using recently developed Invitrogen CRISPR/Cas9 RNP and LentiArray™ based platforms using both coding and noncoding gene examples across variety of cell types including iPSCs. Using Genomic Cleavage Detection assays and Next Generation Sequencing the efficiency of the ready-to-use CRISPR/Cas9 RNP has been assessed. Editing efficiency ranges based on both cell type and gene target. At this point, preliminary results show effective gene editing comparable to previous products (up to 80% gene knockout). Work is currently being done to determine long-term stability of the reagents. The lentiviral plasmid construct has been assembled, and future work will be done to determine its effectiveness. The tools developed through this work will increase the efficiency in disease modeling and drug discovery research by increasing throughput, thereby accelerating and enabling drug discovery research.

T-2161

3D BIOPRINTING PERSONALIZED HUMAN NEURAL TISSUES FOR DRUG SCREENING APPLICATIONS

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Neurological drugs entering clinical trials fail over 90% of the time due to lack of efficacy or unforeseen toxicity. Cultured neural cells and animal disease models currently serve as the main pre-clinical tools for the development of therapeutics against neurodegenerative disorders such as Parkinson's and Alzheimer's. 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 the healthcare burden of neurodegenerative diseases. Developing novel 3D multi-cellular neural tissue models that recapitulate the features of neurodegenerative diseases can serve as a convenient drug screening tool with increased physiological relevance. Human induced pluripotent stem cells (hiPSCs) can be used as the cellular component of engineered neural tissues as they can be expanded and differentiated into neurons. Crucially, hiPSCs enable patient-specific disease modeling as hiPSCs derived from patients suffering from neurological diseases can generate neural tissues that recreate the specific disease phenotype of the patient. However, current methods for generating physiological neural tissue from human pluripotent stem cells are low throughput, inconsistent, and labor intensive. We are using the novel Lab-On-a-Printer (LOP)™ bioprinting technology (Aspect Biosystems) to optimise the production of functional 3D neural tissues containing hiPSCs, and to validate that these tissues mimic the properties of real human brain tissue. Here we show proof of concept data for a novel printable bioink formulation that supports the differentiation of hiPSC-derived neural progenitors into mature 3D neural tissue. We also show that our 3D tissue models can be used as tools for assessing drug toxicity using a set of known toxins. These 3D bioprinted tissues exhibit novel properties compared to traditional 2D hiPSC-derived neural cultures.

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PLURIPOTENCY

T-2163

ADEQUATE CONCENTRATION OF B CELL LEUKEMIA/LYMPHOMA 3 (BCL3) IS REQUIRED FOR PLURIPOTENCY AND SELF-RENEWAL OF MOUSE EMBRYONIC STEM CELLS VIA DOWNREGULATION OF NANOG TRANSCRIPTION

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B cell leukemia/lymphoma 3 (Bcl3) plays a pivotal role in immune homeostasis, cellular proliferation, and cell survival, as a co-activator or co-repressor of transcription of the NF-κB family. Recently, it was reported that Bcl3 positively regulates pluripotency genes, including Oct4, in mouse embryonic stem cells (mESCs). However, the role of Bcl3 in the maintenance of pluripotency and self-renewal activity is not fully established. Here, we report the dynamic regulation of the proliferation, pluripotency, and self-renewal of mESCs by Bcl3 via an influence on Nanog transcriptional activity. Bcl3 expression is predominantly observed in immature mESCs, but significantly decreased during cell differentiation by LIF depletion and in mESC-derived EBs. Importantly, the knockdown of Bcl3 resulted in the loss of self-renewal ability and decreased cell proliferation. Similarly, the ectopic expression of Bcl3 also resulted in a significant reduction of proliferation, and the self-renewal of mESCs was demonstrated by alkaline phosphatase staining and clonogenic single cell-derived colony assay. We further examined that Bcl3-mediated regulation of Nanog transcriptional activity in mESCs, which indicated that Bcl3 acts as a transcriptional repressor of Nanog expression in mESCs. In conclusion, we demonstrated that a sufficient concentration of Bcl3 in mESCs plays a critical role in the maintenance of pluripotency and the self-renewal of mESCs via the regulation of Nanog transcriptional activity.

T-2165

MOLECULAR MECHANISM OF TELOMERE ELONGATION IN EARLY EMBRYOS AND ITS ROLE IN SOMATIC CELL REPROGRAMMING

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Activation of alternative lengthening of telomeres (ALT) mechanism can significantly benefit the generation and quality of induced pluripotent stem cells (iPSCs). However, the overexpression of Zscan4, which plays critical roles in ALT mechanism, can lead to genome instability and increased risk of tumorigenesis. How to control Zscan4 expression precisely remains elusive due to the limited information of ALT mechanism. Based on

the expression pattern of Zscan4, we proposed that early embryos and embryonic stem cells might share similar mechanism in regulating Zscan4 expression. We used proteomic information of different stage embryos to identify candidate genes associated with Zscan4 regulation. We found that Dcaf11 significantly activated Zscan4 expression in embryonic stem cells. Besides, Dcaf11 plays important roles in the ALT process and iPSCs induction. Our study will improve our understanding of ALT mechanism and provide valuable information for further developing safe and efficient strategy for telomere elongation in iPSCs induction.

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T-2167

SURVIVAL MOTOR NEURON PROTEIN AFFECTS PLURIPOTENCY IN HUMAN EMBRYONIC STEM CELLS

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Survival motor neuron (SMN) is a multifunctional protein that plays essential roles in the assembly of small nuclear ribonucleoproteins (snRNPs), the components of the RNA spliceosome. Despite of its well-known mechanisms in RNA processing, SMN also affects maturation and maintenance of neuronal cells, which the deficiency causes inherited spinal muscular atrophy (SMA). Previously SMN has been shown to regulate stem cell pluripotency, division, proliferation and differentiation in Drosophila and mice; on the other hand, induced pluripotent stem cells (iPSCs) have been generated from SMA-like mice and human SMA patients as well. The objective of this study is to understand the role of SMN in human stem cell pluripotency. Results showed that SMN is abundantly enriched in human embryonic stem cells (hESCs). When three independent short-hairpin RNA (shRNA) were applied to knockdown SMN1 respectively, the expression level of SMN1 RNA and other ESC pluripotent markers, including OCT4 and SOX2, were dramatically decreased. Western blotting data confirmed that SOX2 was down-regulated after SMN depletion, while OCT4 displayed diverse expression pattern between clones. Interestingly, slightly increasing of NANOG was discovered in both transcriptional and translational levels in shSMN1 hESCs. These findings indicate the SMN may regulate human cell stemness through key pluripotency genes such as SOX2. The detailed mechanisms are worth further discovery.

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T-2169

CELL-MATRIX ADHESION COULD REGULATE GENES EXPRESSION AND MORPHOLOGY OF PLURIPOTENT STEM CELLS VIA SERUM RESPONSE FACTOR BASED SIGNALING

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It has been reported that microenvironment could affect the reprogramming efficiency of induced pluripotent stem cells through changing cell morphology as well as the cytoskeleton. However, the interactions among cell morphology, pluripotency as well as micro environment have not been well investigated. In this study, we developed a single hPSC culture platform by using a low-matrix adhesion substrate that we have previously reported. From the conventional hPSC culture, we isolated and cultured two different morphological type of single-cell derived clones (flat and domed). Interestingly, the domed clones demonstrated higher proliferation ratio and up-regulated pluripotent genes expression than the other type of clones. These morphological and genetical differences were systematically investigated and a serum response factor (SRF)-based regulatory double-loop were proposed as the signalling pathway that mediate these differences. In conclusion, we have provided a mechanistic view on the interactions among stem cell morphology, pluripotency as well as substrate adhesion, suggesting that the physical environment may play a critical role in the processes of creating and maintaining hPSC.

T-2171

ENDOGENOUS ACTIVATION OF NANOG USING CRISPR TRANSCRIPTIONAL ACTIVATOR REPROGRAMS PLURIPOTENCY NETWORK AND ENHANCES VIABILITY OF HUMAN PLURIPOTENT STEM CELLS

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Conventional cultured human pluripotent stem cells (hPSCs) are in a primed state. Previous research showed that ectopic expression of pluripotency factors and/or small molecule treatment could convert hPSCs to a naive state. However, high level ectopic overexpression may suppress the level of endogenous counterpart genes and cause artifacts. To better understand how the pluripotency network operates, we constructed a CRISPR-ON system that can efficiently upregulate endogenous genes in hPSCs. A doxycycline (Dox) inducible dCas9-VP64-p65-Rta (dCas9-VPR) transcriptional activator and a reverse Tet transactivator (rtTA) expression cassette were knocked into the two alleles of the AAVS1 locus to engineer an iVPR hESC line. Subsequently, we generated iNANOG hPSCs with plasmid containing multiplexed gRNA targeting the NANOG promoter. Upon Dox addition and removal, NANOG expression can be controlled from its endogenous locus at desired time window. Activation of endogenous NANOG promoted naïve pluripotency gene expression, enhanced cell survival and clonogenicity, and enabled hESCs to integrate with the inner cell mass (ICM) of mouse blastocysts in vitro. Moreover, transcriptome profiling showed that iNANOG and NANOG cDNA overexpression cells up and downregulated distinct gene networks, implying that they may have different chromatin organization. Our results, for the first time, clearly demonstrated that gene activation from endogenous locus may represent a better means to reprogram cell states. Moreover, iVPR cells provide a convenient platform to control gene transcription as well as for high-throughput gene activation screen in hPSCs.

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T-2173

A RHO SMALL GTPASE REGULATOR ABR SECURES MITOTIC FIDELITY IN HUMAN EMBRYONIC STEM CELLS

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Pluripotent stem cells can undergo repeated self-renewal while retaining genetic integrity, but they occasionally acquire aneuploidy during long-term culture, which is a practical obstacle for medical applications of human pluripotent stem cells. In this study, we explored the biological roles of ABR, a regulator of RHO-family small GTPases, and found that it has pivotal roles during mitotic processes in human embryonic stem cells (hESCs). Although ABR has been shown to be involved in dissociation-induced hESC apoptosis, it does not appear to have direct effects on cell survival unless cell-cell contact is impaired. Instead, we found that it is important for faithful hESC division. Mechanistically, ABR depletion compromised centrosome dynamics and predisposed the cell to chromosome misalignment and missegregation, which raised the frequency of aneuploidy. These results provide insights into the mechanisms that support the genetic integrity of self-renewing hESCs.

T-2175

SINGLE-CELL RNA-SEQUENCING OF HUMAN NAIVE, PRIMED AND EXTENDED PLURIPOTENT STEM CELLS

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During early human development, from blastocyst formation to a few days after implantation, the embryo contains pluripotent epiblast cells. Thus, pluripotency can be divided into at least two states corresponding to the preimplantation and postimplantation epiblast. Conventional human primed embryonic stem cells (ESCs) are considered to represent the postimplantation epiblast, whereas naive ESCs represent the preimplantation epiblast. Recently, a new stem cell type was reported, called extended pluripotent stem cells (EPSCs), with extraembryonic potential. Interestingly, these cells were suggested to share some molecular features with even earlier embryos, from zygote to morula. Whether the EPSCs represent a certain pluripotency state in vivo, however, is still unclear. Here, we used an isogenic cell line in primed and naive ESC and EPSC condition for single-cell RNA-sequencing with 10X genomics technology and obtained the molecular signatures for over 1000 cells in each condition. We found that the different stem cell types had distinct transcriptional features and clustered separately. Preliminary analysis also revealed few subpopulations within each stem cell type, with more intense expression of their corresponding signature markers. Comparing the molecular signatures to available single cell human preimplantation embryo data, we confirmed that naive ESCs had the closest correlation with the epiblast cells of embryonic day 5-7 blastocysts. The global gene expression of EPSCs was distinct from preimplantation embryos, however, few of the EPSC specific genes, such as RARRES2, were expressed specifically in embryos up to morula stage. By analyzing the protein expression of known naive and primed specific cell-surface markers by flow cytometry and immunostaining, we found that EPSCs expressed the naïve markers CD75 and CD77 at low levels, but not CD7 or CD130. In addition, they expressed the primed markers CD24, CD57 and CD90. Our data indicate that although EPSCs share few markers with early preimplantation embryos, they may represent a possible intermediate pluripotency state,

between naive and primed ESCs. However, molecular signatures of postimplantation epiblast cells are needed to confirm whether or not EPSCs represent a specific in vivo cell type.

PLURIPOTENT STEM CELL DIFFERENTIATION

T-3001

THE CELL CYCLE REGULATOR CDK1 CONTROLS HUMAN PLURIPOTENT STEM CELL FATE COMMITMENT BY REGULATING PROTEIN SYNTHESIS OF KEY TRANSCRIPTION FACTORS.

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Control of cell proliferation is critical for embryonic development as interplays between cell cycle and differentiation are essential for cell fate decisions. However, the mechanisms coordinating cell cycle progression and the first stages of lineage commitment remain to be fully uncovered. Here, we investigated these mechanisms using a new culture system allowing human pluripotent cells (hPSCs) to differentiate into definitive endoderm (DE) after synchronisation of their cell cycle. We found that hPSCs divide two times before generating DE cells. A first cell division correlated with the acquisition of mesendoderm (ME) identity, while the second, shorter, cell cycle was associated with DE specification. We also observed that each cell division was associated with a change in capacity of differentiation with ME being competent for mesoderm generation derivatives but not competent to express pluripotency markers. These results suggest that cell cycle progression is coupled to definitive change in cellular identity associated with differentiation. However, blocking cell division during differentiation did not inhibit ME formation. Furthermore, chromatin remodelling, RNA expression, and protein synthesis of key ME genes, such as EOMES and T, were independent of physical cell division as well. Together, these data reveal that cell cycle progression is not necessary for hPSCs to initiate epigenetic and transcriptomic changes associated with differentiation. We then perform analyses to determine the importance of cell cycle regulator in ME specification. Inhibition of CDK1 function either through the use of chemical inhibitors or an inducible knock-down system completely impeded ME formation. This included a lack of EOMES and T protein production. Thus, cell cycle regulators seem to have an essential role in changing cellular identity during cell cycle upon differentiation. Together, these results show a tight association between the cell cycle machinery and the acquisition of a new cell identity. Furthermore, they reveal a novel mechanism of control that coordinates cell cycle progression, protein synthesis, and cell fate commitment. Ultimately, these observations are likely to be relevant for adult stem cells and for organ homeostasis and regeneration.

T-3003

MEIS1 REGULATES HEMOGENIC ENDOTHELIAL GENERATION, MEGAKARYOPOIESIS AND THROMBOPOIESIS IN HUMAN PLURIPOTENT STEM CELLS BY TARGETING TAL1 AND FLI1

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Human pluripotent stem cells (hPSCs) provide an unlimited source for generating various kinds of functional blood cells. However, efficient strategies for generating large-scale functional blood cells from hPSCs are still lacking, and the mechanism underlying human hematopoiesis remains largely unknown. In this study, we identified Myeloid Ectopic Viral Integration Site 1 homolog (MEIS1) as a crucial regulator of hPSC early hematopoietic differentiation. MEIS1 is vital for specification of APLNR+ mesoderm progenitors to functional hemogenic endothelial progenitors (HEPs), thereby controlling formation of hematopoietic progenitor cells (HPCs). TAL1 mediates the function of MEIS1 in HEP specification. In addition, MEIS1 is vital for megakaryopoiesis and thrombopoiesis from hPSCs. Mechanistically, FLI1 acts as a downstream gene necessary for the function of MEIS1 during megakaryopoiesis. Thus, MEIS1 controls human hematopoiesis in a stage-specific manner and can be potentially manipulated for large-scale generation of HPCs or platelets from hPSCs for therapeutic applications in regenerative medicine.

T-3005

EFFECT OF NITRATE TRANSPORTER SIALIN ON THE BIOLOGICAL BEHAVIORS OF HUMAN PERIODONTAL LIGAMENT STEM CELLS

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To study the effect and possible mechanism of nitrate transporter sialin on the biological behaviors of human periodontal ligament stem cells (HPDLSC). SLC17A5 shRNA or control plasmid was transfected to HPDLSC to interfere the expression of sialin. CCK8 assay was used to analyze the proliferation rate of SLC17A5 knockdown cells compared to the control cells. In addition, the osteogenic differentiation potential was compared using ALP (Alkaline phosphatase) staining and AR-S staining (Alizarin red-staining) between SLC17A5 knockdown

cells and control cells. To explore the mechanism how sialin affected HPDLSCs, the cGMP expression and NO3- level were compared in the two groups. The knockdown of SLC17A5 greatly inhibited the proliferation rate and the osteogenic differentiation potential of HPDLSC ($p<0.05$). However, cGMP and NO3- levels were not found to be significantly different between SLC17A5 knockdown cells and control cells. The knockdown of SLC17A5 has been shown to decrease the proliferation and osteogenic differentiation potential of mesenchymal stem cells, which is not due to the nitrate transportation function of sialin.

T-3007

PRODUCTION OF HLA HOMOZYGOUS SCNT-HESC DERIVED RPE CELLS FOR THERAPEUTIC APPROACHES TO MARCULAR DEGENERATION

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Retinal pigment epithelium (RPE) is important for retinal development and function, and has a critical role of retinal degenerative diseases such as Age-related macular degeneration (AMD) and retinitis pigmentosa (RP). The defects in RPE function can affect the integrity and viability of photoreceptors, so RPE is the source and the target of many retinal degenerative diseases. Because of their proliferation and differentiation abilities, hES derived RPE cells represent a potentially unlimited resource for cell replacement therapy for AMD. The human leukocyte antigen (HLA) system is important for transplantation. Matching donor and recipient tissue or cells for HLA antigens reduces the chance of a cytotoxic T cell response in the recipient, and thus greatly increase the survial rate of transplanted cells. Therefore, HLA homozygous stem cell derived RPE cells have the potential for cell-based thrapy in a significant number of individuals, provided the HLA haplotype is prevalent. In this study, we produced RPE cells derived from HLA homozygous somatic cell nuclear transfer (SCNT)-hESC, CHA-hES NT6, and analyzed the characteristics and functional efficacy of the RPE cells compared to human embryonic stem cell (hESC) derived RPE cells. The HLA homozygous RPE cells show high similarity compared to hES derived RPE cells in typical RPE characteristics; pigmented polygonal shape, expression of RPE-related markers, epithelial polarization and phagocytosis activity. From these results, we produced the successful derivation of functional RPE cells, which can be HLA matched with large segments of the population.

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T-3009

GENERATION OF TRICHOGENIC DERMAL PAPILLA PRECURSOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR HUMAN HAIR FOLLICLE REGENERATION

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During development, dermal papilla precursor cells (DPPCs) initiate embryonic hair follicle (HF) formation with epidermal placode cells. Obtaining DPPCs with trichogenic ability is critical for human HF regeneration because dermal papilla cells (DPCs) rapidly lose their trichogenic ability in culture. Here, we generated trichogenic DPPCs from human induced pluripotent stem cells (iPSCs) via neural crest stem cells (NCSCs), based on the developmental evidence at the hair placode stage. SDC1+CD133− cells showed signature DP gene expression, spontaneous sphere formation and represented intermediate population in the differentiation way from parental NCSCs to DPCs. hiPSC-derived DPPCs generated HF equivalents in vitro and reconstituted de novo human HFs in vivo combined with hiPSC-derived epithelial stem cells. Remarkably, trichogenic ability of DPPC was proven only in the specific time window, providing insights into the loss of trichogenicity in cultured DPC. Thus, this study provides an in vitro model for studying DPC development and biology.

T-3011

IDENTIFICATION OF A KEY REGULATOR FOR ACTIVATING BMP SIGNALLING AND PROMOTING MESODERMAL DIFFERENTIATION IN INDUCED PLURIPOTENT STEM CELLS

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Recently, patient's own induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) are expected to be cell sources for regenerative therapy. In stem cell differentiation into cardiomyocytes, activation of BMP and Activin signallings in early phase and suppression of Wnt signalling in late phase are important. Although small molecule chemical compounds and recombinant proteins are currently used for differentiation, however, it is better that all recombinant proteins are replaced by chemical compounds, which cost cheaper and improve the efficiency of iPSC-CM differentiation. Several chemical compounds inhibiting Wnt signalling have been reported in previous researches. Regarding BMP signalling, a few activator have been also reported, however, there are many BMP signalling modifiers. Therefore, we tried to screen candidate factors for the development of novel chemical compounds activating BMP signalling in the future. To develop the screening system, we used luciferase assay including BMP-responsive element (BRE) and confirmed that our screening system promptly responds to BMP-4 stimulation. Then, we focused on the molecular target, which has a strong impact on BMP signalling. We screened the possible BMP signal regulators which were reported in previous papers by RNA interference in HEK293T cells. Among many candidate regulators, we found that knockdown of Tripartite motif 33 (TRIM33), E3 type ubiquitin ligase, significantly and reproducibly enhanced BRE-luciferase assay and induced the expression of Id-1 gene as innate reporter gene of BMP signalling. Next, we investigate whether the suppression of TRIM33 promotes the differentiation of iPSCs into mesodermal cells. We confirmed that TRIM33 knockdown by RNA interference promoted the expression of mesodermal marker Brachyury T and epithelial-mesenchymal transition marker Slug2 in 2-dimensional cardiac differentiation. We successfully detected the factor targeted by compounds for activating BMP signalling. TRIM33 inhibition enhanced the expression of Id-1 gene and promoted mesodermal differentiation. In the future, chemical compounds targeting TRIM33 may be useful for regenerative medicine as BMP signalling activators.

T-3013

IDENTIFICATION AND CHARACTERIZATION OF MARKER GENES FOR PREDICTION OF DIFFERENTIATION PROPENSITY OF HUMAN INDUCED PLURIPOTENT STEM CELL LINES

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Human pluripotent stem cells (hPSCs) have the ability to differentiate into a variety of cells and to self-renew in vitro. Because of these two characteristics, hPSCs are expected to provide new regenerative medicine/cell therapy. In recent years, many clinical-grade human embryonic stem cell (hESC) lines and human induced pluripotent stem cell (hiPSC) lines have been established for use as raw materials for cell-based therapeutic products (CTPs). Essentially, hESC and hiPSC lines exhibit variation in their individual differentiation propensities for generating specific cell lineages. Therefore, selection of hPSC lines capable of efficiently differentiating into desired cells is quite important for the practical application of CTPs. Here, we tried to identify the marker genes for predicting propensity of differentiation into the three germ layers among hiPSC lines. In the first effort to identify marker genes, of which correlate with the differentiation propensity, we examined comprehensive mRNA expression in 10 undifferentiated hiPSC lines. Next, to investigate the differentiation propensity of the hiPSC lines, we differentiated hiPSC lines into embryoid bodies, and their gene expression profiles with germ layer marker genes were obtained. With principal component analysis, we quantified differentiation propensity of each hiPSC line for three germ layers. Finally, the correlations between the rank of the gene expression at the undifferentiated state and the rank of differentiation propensity for three germ layers was determined using Spearman's rank correlation. Furthermore, we found relevant genes to predict differentiation propensity among our identified marker candidates and also functionally characterized

the marker genes. Our research will allow us to present a novel factor for defining the molecular mechanism of differentiation propensity of hiPSCs and to select the suitable cell lines for future translation applications.

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T-3015

IDENTIFICATION OF LATROPHILIN-2, A NOVEL RECEPTOR THAT SPECIFIES CARDIAC PROGENITORS FROM PLURIPOTENT STEM CELLS AND IS ESSENTIAL FOR MOUSE EMBRYONIC HEART DEVELOPMENT

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The identification of a lineage-specific marker plays a pivotal role in understanding developmental process and is utilized to isolate a certain cell type with high purity for the therapeutic purpose. When mouse pluripotent stem cells (PSCs) were stimulated with BMP4, Activin A, and bFGF, they differentiated into cardiac cells. To screen cell-surface expressing molecules on cardiac progenitor cells (CPCs) compared to undifferentiated PSCs, we isolated Flk1+/PDGFRα+ cells at differentiation day 4 and performed microarray analysis. Among candidates, we identified a new G protein-coupled receptor, latrophilin-2 (LPHN2). Here, we report a new cardiac-specific cell surface marker, latrophilin 2 (LPHN2), expressed specifically by CPCs and cardiomyocytes (CMCs) during mouse and human PSCs differentiation in vitro. In sorting experiments under cardiac differentiation condition, LPHN2(+) cells derived from PSCs strongly expressed cardiac-related genes (Mesp1, Nkx2.5, αMHC, and cTnT) and exclusively gave rise to beating cardiomyocytes, as compared with LPHN2(-) cells. To verify the in vivo functional significance of LPHN2 during development, we generated Lphn2-KO mice. While Lphn2 heterozygous mice (Lphn2+/-) were alive and fertile, homozygous Lphn2 (Lphn2-/-) mice showed embryonic lethality. Next, we performed RNA-Seq analysis to explore gene expression differences associated with defects in heart development from wild-type and Lphn2-homo KO embryos at E9.5. Interestingly, most genes associated with heart development were downregulated in Lphn2-homo KO embryos. For the purpose of cardiac regeneration, we transplanted PSC-derived LPHN2(+) cells into the infarcted heart. LPHN2(+) cells differentiated into cardiomyocytes, and systolic function of the left ventricle was improved and infarct size was reduced. In summary, LPHN2 is a functionally significant and cell-surface expressing marker for cardiac progenitor and cardiomyocytes. Our findings provide a valuable tool for isolating cardiac lineage cells from PSCs and shed light on heart development and regeneration.

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T-3017

GENERATION OF INSULIN-EXPRESSING CELLS IN MOUSE SMALL INTESTINE BY PDX1, MAFA AND BETA2/NEUROD

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To develop surrogate insulin-producing cells for diabetes therapy, adult stem cells have been identified in various tissues and studied for their conversion into beta cells. Pancreatic progenitor cells are derived from the endodermal epithelium and formed in a similar manner as gut progenitor cells. Here, I generated insulin-producing cells from the intestinal epithelial cells that induced many of the specific pancreatic transcription factors using adenoviral vectors carrying three genes: Pdx1, MafA, and BETA2/NeuroD (PMB). By direct injection into the intestine through the cranial mesenteric artery, adenoviruses (Ad) were successfully delivered to the whole intestine. After virus injection, I could confirm that the small intestine of the mouse was appropriately infected with the Ad-Pdx1 and triple Ad-PMB. Four weeks after the injection, insulin mRNA was expressed in the small intestine, and the insulin gene expression was induced in Ad-Pdx1 as well as Ad-PMB compared to control Ad-GFP. In addition, the conversion of intestinal cells into insulin-expressing cells was detected in parts of the crypts and villi located in the small intestine. In Conclusions, These data indicated that Pdx1, MafA, and BETA2/NeuroD facilitate the differentiation of mouse intestinal cells into insulin-expressing cells. In conclusion, the small intestine is an accessible and abundant source of surrogate insulin-producing cells.

T-3019

BRAINPHYS™ NEURONAL MEDIUM SUPPORTS THE MATURATION AND ELECTRICAL ACTIVITY OF HUMAN PLURIPOTENT STEM CELL (HPSC)-DERIVED NEURONS IN LONG-TERM CULTURES

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Neuronal cultures derived from human pluripotent stem cells (hPSCs) are powerful in vitro models for studying neurological development and disease. To support maturation and synaptic function of neurons in long-term culture, we developed BrainPhys™ Neuronal Medium (BrainPhys™) based on a previously published formulation (Bardy et al., PNAS 2015). Here we describe the effect of BrainPhys™ on the electrophysiology of hPSC-derived neurons during long-term culture. Neural progenitor cells derived from hPSCs (H9 and XCL-1) were differentiated in BrainPhys™ with NeuroCult™ SM1 Neuronal Supplement and other growth factors (BP/SM1). Cells were cultured on microelectrode array (MEA) plates containing recording electrodes embedded within each well. We performed half-medium changes every 3 - 4 days, and measured the electrical activity of neurons twice per week. Our data demonstrate that in the presence of BP/SM1, H9- and XCL-1-derived neurons gradually became electrically active over a 10- and 18-week maturation period, respectively. For H9-neurons (n = 1; 128 electrodes), the mean firing rate (MFR) progressively increased from 0.003 Hz on day 29 to 0.8 Hz by day 71. Similarly, the MFR for XCL-1-neurons (n = 1; 128 electrodes) also increased from 0.1 Hz on day 30 to 1.6 Hz by day 125. Network bursts, a measure of synaptic connections within a neuronal population, were first detected on day 61 and day 30 for H9- and XCL-1-neurons, respectively. In a 10-minute recording, the number of network bursts increased from 0 on day 30 to 42 by day 71 for H9-neurons; whereas the number of network bursts increased from 2 on day 30 to 19 by day 71 for XCL-1-neurons. This progressive increase in network bursts indicates synchronous firing was enhanced as hPSC-neurons matured in BP/SM1. Furthermore, we measured the electrical activity of H9-neurons on a single-cell level using patch clamp analysis. The resting membrane potential recorded from 5 individual neurons cultured in BP/SM1 on day 63 was -32.1 3 0.4 mV (mean 3 SEM), which indicates the neurons are not fully mature at this stage. However, they do display normal synaptic activities since they generated AMPA receptor currents. Collectively, these results demonstrate that BrainPhys™ promotes the physiological maturation and synaptic activity of hPSC-derived neurons in long-term culture.

T-3021

THE VENTX HOMEOBOX GENE IN HUMAN PRIMORDIAL GERM CELL (PGC) AND HAEMATOPOIETIC DEVELOPMENT

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The Ventx genes are nonclustered 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). In order to investigate the uneexplored role of VENTX during early human differentiation, a pluripotent stem cell (PSC) reporter line was generated in which GFP was inserted at the start codon of VENTX. Our studies revealed that early expression of VENTX identified a subset of primordial germ cells (hPGCs). hPSCs from a double reporter line (VENTXGFP/w OCT42ACHERRY/w) and VENTX knock-out line (VENTXGFP/- OCT42ACHERRY/w) were differentiated in vitro into hPGCs. Cells were flow sorted on the basis of VENTX and OCT4 reporter expression and EPCAM and CD49f surface markers that allowed the identification of PGCs (GFP+OCT4+EPCAM+CD49f+ and GFP-OCT4+EPCAM+CD49f+). Transcriptional profiling of GFP-positive and GFP-negative OCT4+EPCAM+CD49f+ cells confirmed expression of early PGC-associated genes, including BLIMP1, PRDM14, SOX17 and TFA2PC, in populations from both cell lines. The RNA-seq data demonstrated that VENTX is expressed in a subset of human PGCs, but did not reveal significant transcriptional differences between hPGCs derived from both the VENTX knock-out and the VENTX-heterozygous double-reporter cell lines. These results suggest that VENTX is not required for early human PGC ontogeny. We also addressed the role of VENTX during haematopoietic development. Using an 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 (CD90+

and CD34+) with a higher clonogenic capacity. Gene expression analysis revealed increased expression of HOXA genes in haematopoietic cells following VENTX induction, consistent with conservation of an immature phenotype. We are investigating the consequences of VENTX overexpression, alone and in combination with other oncogenes, on haematopoietic cells derived from hPSCs.

T-3023

ANALYSIS OF MIRNA EXPRESSION DURING ENDODERM LINEAGE SPECIFICATION IN MOUSE EMBRYONIC STEM CELLS

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Pluripotent stem cells hold great promise in treating chronic liver diseases through cell replacement therapy, owing to their ability to self-renew and differentiate into all cell types. However, the current available differentiation protocols have yet to generate fully functional hepatocytes in vitro. Increasing evidence shows that microRNAs (miRNAs) play a crucial role in controlling the embryonic stem cell (ESC) state. Although there are many reports about their role in maintaining ESC pluripotency, the mechanisms by which they promote cell differentiation remain largely unknown. In the present study, we aim to identify miRNAs involved in endoderm lineage specification, which is an early stage of hepatocyte differentiation. Here, we induced mouse embryonic stem cells (mESCs) to definitive endoderm and characterized them with endoderm specific markers, Sox17 and Foxa2. We performed miRNA array analysis on mESCs during the course of endoderm differentiation. By validation through qPCR, we uncovered the miRNAs that are differentially expressed during endoderm differentiation. We further evaluated the functional roles of the selected miRNAs by over-expressing or inhibiting them in mESCs during embryoid body (EB) formation and examined their impact on the induction of the markers of the three germ layers in both mRNA and protein level. We successfully discovered a key miRNA that is associated with the regulation of endoderm lineage specification in mESCs and studied the mechanism by which this miRNA regulates endoderm differentiation through identification of its target via 3'UTR reporter assay. Collectively, our findings revealed a regulatory network between the studied miRNA and its target, showing its importance in mESC lineage commitment.

T-3025

GENOME-WIDE DEFINITION OF REGULATORY REGIONS AND TRANSCRIPTS DURING PLURIPOTENT TO NEURAL STEM CELL TRANSITION

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Human fetal-derived neural stem cells (hfNSCs) are under clinical evaluation for several neurodegenerative diseases. These cells display a favorable safety profile but their clinical application to large cohorts of patients is still hampered by ethical and safety concerns. NSC populations derived from human induced pluripotent stem cells (hiPSCs) represent an attractive alternative since their combination with gene therapy strategies might open new avenues for autologous cell therapy approaches aimed to treat genetic diseases with unmet medical need. In this scenario, obtaining hiPSC-derived NSCs showing a reliable "NSC signature" is mandatory. We recently generated a collection of hiPSCs that we differentiated into NSCs (hiPS-NSC) sharing molecular, phenotypic and functional identity with hfNSCs, which we used as a valuable "gold standard" in a side-by-side comparison when validating the phenotype of the resulting hiPS-NSC. In hiPSCs, hiPS-NSCs and hfNSCs we are performing a genome-wide mapping of regulatory elements, integrating RNA-seq and ChIP-seq data in an attempt to: i) better identify hiPS-NSC identity and safety profile, ii) define the molecular circuitry associated with the transition from pluripotent to neural restricted stem cells. Transcriptomic analysis revealed a distinct "neural signature" of hiPS-NSC with no donor- or clone-related bias underlining both known and novel master regulators characterizing the transition from pluripotent to neural restricted stem cells that we are currently investigating. ChIP-seq analysis led us to identify promoter, enhancer and super-enhancers (SE) usage specific of each cell population. Overall, our study will provide a collection of coding and non-coding RNAs differentially expressed between hiPSCs, hiPS-NSCs and hfNSC as well as a genome-wide description of promoter and enhancer usage characterizing the transition from hiPSCs to hiPS-NSCs. We expect that combining genetic and epigenetic analyses will allow devising a reliable overview of the dynamic changes occurring during iPSC

to neural differentiation, helping to define a consistent "NSC identity and safety signature" that might aid strategies for increasing safety and efficiency of hiPS-NSC populations to be used for cell therapy approaches.

T-3027

IN VITRO AND IN VIVO MIRNA-MEDIATED CONTROL OF SATB2 EXPRESSION DURING CORTICOGENESIS IN 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 Bcl11b, Satb2 may occupy a central position in transcriptional networks significant to Autistic Syndrome Disorders. Throughout corticogenesis, SATB2 protein acts as a transcriptional factor expressed in the upper layer neurons of the cortex and inhibits the transcription of the deep layer gene, Ctip2, establishing the end of the corticogenesis. When the level of SATB2 positive neurons increase, CTIP2 positive neurons decrease and so, it is worth to know by which mechanism SATB2 translation is repressed while deeper neurons are being generated. Based on this aim and on our previous results, we looked for miRNAs candidates that could be involved in targeting Satb2 mRNA during corticogenesis. We have observed that two candidates, miR-409 and miR-380, have predicted binding sites in the Satb2 3'UTR. Using LNA oligonucleotides against the two miRNAs we were able to observe SATB2 protein expression in ES cells corticalized in vitro at DIV (day in vitro) 12, while in control experiment SATB2 it is not expressed until DIV 15.5. Similar experiments were repeated in vivo by in utero intraventricular injections of the same LNA sequences at E12, with posterior analysis at E14. In vivo, high SATB2 protein expression was induced at E14 in the injected cortical hemisphere as compared to control hemisphere. In order to evaluate Satb2 mRNA translational repression from miRNAs at different time-points of corticogenesis, we have developed: 1) a cell line that expresses Satb2 3'UTR tagged with a GFP marker; 2) an RNA immunoprecipitation (RIP) protocol which allows to evaluate the binding of Satb2 mRNA with RISC at different time-points of ES cell in vitro corticalization; 3) a protocol of miR-CATCH technique, that allows to identify the exact miRNAs bound to Satb2 3'UTR. The experiments concerning all these three strategies are currently in progress.

T-3029

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-3031

GENERATION OF SMALL INTESTINAL ENTEROCYTE-LIKE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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The enterocyte-like cells differentiated from human induced pluripotent stem (iPS) cells (hiPS-ELCs) are expected to be utilized in drug development and research. We previously reported that the enterocyte differentiation from human iPS cells could be promoted by using epidermal growth factor, SB431542 and Wnt3a (Sci Rep. 2015 Nov 12;5:16479), but there is room for improvement in terms of differentiation efficiency. In addition, enterocytes are known to have different properties in the small intestine and the colon. Nevertheless, there is almost no report that verified whether the hiPS-ELCs have the properties of the small intestinal enterocyte or colonial enterocyte. In this study, we attempted to improve the enterocyte differentiation efficiency by referring to the developmental process of the small intestine and the method of culturing intestinal organoids. Furthermore, we examined whether hiPS-ELCs have the properties of the small intestinal enterocytes or colonial enterocytes. To investigate whether the enterocyte differentiation was promoted by treatment with several compounds or cytokines, we analyzed the expression levels of enterocyte markers. Next, we evaluated whether the hiPS-ELCs have the properties of the small intestinal enterocytes or colonial enterocytes, the gene expression levels of the small intestinal enterocyte markers and colonial enterocyte markers were analyzed. The villin-positive cells were approximately 95% by treatment with several compounds or cytokines. The gene expression levels of small intestinal enterocyte markers (apolipoprotein A4, fibroblast growth factor 19) were similar between the hiPS-ELCs and the human adult small intestine. Interestingly, the gene expression levels of colonial enterocyte markers (carbonic anhydrase 1, carbonic anhydrase 2) in the hiPS-ELCs were lower than the human adult colon. In addition, the gene expression levels of drug transporters, such as breast cancer resistance protein and peptide transporter 1, were similar between the hiPS-ELCs and the human adult small intestine. The small intestinal enterocyte-like cells were efficiently generated from human iPS cells by using our protocol. The small intestinal enterocyte-like cells would be utilized for evaluation of drug absorption, metabolism and excretion.

T-3033

PREVENTION OF TUMORIGENESIS IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES BY IMMUNOLOGICAL CYTOTOXICITY AGAINST ONCOFETAL ANTIGEN

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Recently, transplantation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) was evaluated to treat patients with severe heart failure. However, to ensure safety of the transplanted hiPSC-CMs, complete removal of contaminating immature cells is essential. Additionally, management of patients in whom iPSC-derived tumors develop after post treatment transplantation of hiPSC-CMs has not been established. To address this issue, we applied anti-cancer immunotherapy with peptide vaccination to prevent hiPSC-derived tumorigenesis for ensuring safety of hiPSC-CM transplantation. To ensure immunological elimination of contaminating undifferentiated-hiPSCs in hiPSC-CMs, we explored carcinoembryonic antigens and identified glypican-3 (GPC3) as a pluripotent state-specific immunogenic antigen. In the course of cardiac differentiation of hiPSCs, expression of GPC3 was decreased and was almost undetectable in terminally differentiated hiPSC-CMs. Undifferentiated hiPSCs were rejected by cytotoxic T cell (CTL) clones that were sensitized by using HLA-class I-restricted GPC3 peptides; however, hiPSC-CMs were not rejected. Furthermore, GPC3-reactive CTLs removed undifferentiated cells from hiPSC derivatives selectively in vitro and inhibited formation of teratoma in vivo. These results indicated that GPC3-reactive CTLs could be applied for preventing hiPSC-derived tumorigenesis caused by contaminating undifferentiated cells in hiPSC-CMs. Our results demonstrated that oncofetal antigen GPC3 worked as a pluripotent state-specific immunogenic antigen in hiPSCs and was an effective target for purification of hiPSC-CMs. Additionally, these results showed future applicability of immunotherapy against GPC3 to manage cases that are unmanageable by surgical resection of hiPSC-derived tumors owing to invasiveness of the operation. Therefore, our results could be useful for ensuring the safety of regenerative therapy involving hiPSC-derivatives.

T-3035

DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO INNER EAR HAIR CELL-LIKE CELLS USING VESTIBULAR CELL-CONDITIONED MEDIUM

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The vestibular system in the inner ear is essential for maintaining spatial orientation and balance. Current options for treating vestibular disorders are limited and include vestibular rehabilitation, medication, and surgery. However, to fully restore vestibular function, the only method presently available is vestibular hair cell (V-HC) regeneration and subsequent nerve innervation. Embryonic stem (ES) cells are a candidate source for cell therapy as treatment for a range of organs because of their potential for self-renewal and pluripotency. Recently, we developed a simple and efficient technique, termed the HIST2 method, to obtain ES-derived HC-like cells within a relatively short period of cultivation, for which only conditioned medium from cultured ST2 stromal cells is used. In the present study, we investigated differentiation of ES cells into inner ear V-HCs using the differentiation-inducing activity of supernatant obtained from cultures of vestibular cells isolated from the inner ear of adult mice. We utilized the mouse ES cell line EB5 and subline Math1-GFP ES cells carrying the GFP gene driven by the Math1 promoter. Mouse vestibular cells (VCs) were isolated from inner ear utricles and cultured, then the resultant conditioned medium (V-CM) was collected. Embryoid bodies (EBs) were prepared for 4 days using a hanging drop method, then cultured for 14 days in V-CM. GFP expression was examined using FACS and fluorescence microscopy. Furthermore, expressions of HC-related markers including those of V-HCs were examined by real time RT-PCR and immunocytochemistry. :The number of GFP-positive cells was increased in EB outgrowths cultured in V-CM, whereas few cells were detected in its absence. Moreover, expressions of HC-related markers including some V-HC-related markers were increased in those cultured in V-CM. The differentiation-inducing activity of V-CM was effective to induce differentiation of ES cells into HC-like cells including V-HCs.

PLURIPOTENT STEM CELL: DISEASE MODELING

T-3037

PERSONALIZED MEDICINE FOR RARE DISEASES: TOWARDS MODELING LIMB-GIRDLE MUSCULAR DYSTROPHIES USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Limb-girdle muscular dystrophies (LGMDs) are a group of clinically and genetically heterogeneous disorders that share the primary symptom of progressive weakness and atrophy of the hip and shoulder muscles. Although these disorders have been classified as rare, our preliminary findings show the autosomal recessive forms to be largely overrepresented in Kuwait due to the high rate of consanguinity. Currently, there is no treatment available for LGMD and it thus represents a major unmet medical need. A key challenge in the field of rare disease research is the scarcity of human cell and tissue samples needed to study the disease. To overcome this challenge, we exploit patient-derived induced pluripotent stem cell (iPSC) technology as an unlimited source of disease-relevant cells. This research aims to use LGMD iPSC-derived skeletal muscle cells to develop phenotypic disease-in-a-dish cellular models suitable for personalized high-throughput screening and drug discovery. Towards this aim, eleven genetically undiagnosed families in Kuwait that presented with weakness in the shoulder and hip region were screened against a panel of 63 genes associated with LGMDs and other neuromuscular disorders. The panel identified LGMD mutations in 64% of the families (7/11: 4 LGMD2A, 2 LGMD2I and 1 LGMD2B). Consanguinity was present in six of the seven positively diagnosed cases and all cases exhibited a recessive inheritance pattern with homozygous pathogenic variants. Notably, 2 of the remaining 4 undiagnosed patients carried unreported genetic variants in known LGMD genes. LGMD iPSC lines were successfully generated from the skin fibroblasts of all consenting LGMD2I (dystroglycanopathy, FKRP mutations) and LGMD2A (calpainopathy, CAPN3 mutations) patients, the most prevalent forms of LGMD found in Kuwait. A highly efficient scalable monolayer differentiation protocol is now being implemented to generate skeletal muscle cells from these LGMD iPSC lines. The generation of LGMD patient-specific skeletal muscle cells will provide a platform to study and model the pathology of the disease-in-a-dish and screen

for therapies in a personalized manner in the future. Additionally, the genetic testing results demonstrate the advantage of using neurological gene panels in the diagnosis of heterogeneous disorders such as LGMDs.

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T-3039

A SHORT INVESTIGATION OF MIXED HAEMATOPOIETIC CHIMERISM: AN NHP MODEL OF HAEMATOPOIETIC STEM CELL TRANSPLANTATION (IUHSCT)

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Intrauterine hematopoietic stem cell transplantation (IUHSCT), a non-myeloablative approach for prenatal treatment of various congenital hematological and immunological disorders, may serve as an alternative to mainstream treatment of congenital haemoglobinopathies. A small cell dose given to the fetus during the period of pre-immunity may effect greater engraftment while minimizing the risk of graft versus host disease (GVHD). We have developed a non-human primate (NHP) model of IUHSCT through fetal injections of maternal bone marrow-derived mononuclear cells (MNCs) and examined donor cell engraftment and feasibility of this approach. Maternal MNCs were isolated and labelled with carboxyfluorescein succinimidyl ester (CFSE) prior to fetal intrauterine injection via umbilical cord fetoscopically or transabdominal intra-cardiac puncture under ultrasound guidance, at 0.46-0.74 gestational age (71-144 days). Fetuses (n=10) received 18.9-170E+6 MNCs (median dosage 49.8x10E+6) and were sacrificed at 24-48 hours post-injection. Targeted organs were harvested for analysis through stereoscopy, flow-assisted cytometry (FACS) and qPCR of MHC polymorphisms. Fetal weights ranged from 36-160g. Stereoscopy results revealed detectable levels of CFSE-positive MNCs in fetal liver, spleen, heart, lung and placenta, in accordance with FACS results. Low levels of maternal MNCs were found in haematopoietic tissues like fetal liver, spleen and heart (2-8%). In contrast, qPCR indicated that maternal MNCs were mostly found in placenta (6.9%) and present in relatively low levels in other organs (<0.01%). It is technically challenging to deliver donor cells via fetoscopy compared to ultrasound-guided intrauterine injection. Although maternal cell chimerism may be low in some organs, there is hope that microchimerism may help to ameliorate or arrest disease pathology until further interventions can be carried out post-natally. Moving forward, we will explore the ability of early-gestation high-dose IUHSCT at 0.4G to achieve stable significant engraftment and generate donor specific tolerance (DST) in our NHP model. The use of lentiviral vector carrying green fluorescent protein (GFP) transgene for HSC transduction could be an alternative to CFSE-labeling and complement qPCR in detecting chimerism.

T-3041

KINESIN DEFICIENCY IN RETT-SYNDROME LIKE PATIENTS: 3D-CELLULAR MODELLING AND IN VITRO FUNCTIONAL STUDIES

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Rett Syndrome (RTT) is a severe disorder of neuronal plasticity, predominantly caused by mutations in the methyl-CpG-binding (MECP2) gene. Between 5 - 40% of RTT patients (classical and atypical patients) remain without a genetic diagnosis. Using next generation based sequence technologies, we are performing variant screening in a cohort of 80 MECP2 negative RTT patients. Candidate disease gene variants are further investigated with three dimensional (3D) protein modelling and functional assays. In one patient we have identified a novel de novo missense variant in the motor domain of KIF1A, which encodes a neuronal specific kinesin molecular motor that is essential for adenosine triphosphate dependent axonal trafficking of key synaptic cargos. Subsequently, different missense KIF1A variants were identified in three further patients with overlapping clinical features. The 3D modelling of the first missense variant predicts it to be located in a critical protein domain, resulting in loss of interaction with Mg2+ and defective ATP hydrolysis. It is also located close to a salt bridge, critical for effective γ-phosphate release to complete ATPase cycling steps. Molecular modelling of the other missense variants predicts inhibition of proper protein folding. Moreover, targeted sequencing of the first batch of samples revealed one atypical RTT patient with the variant in another kinesin gene, which is also involved in dendritic microtubule trafficking, and is predicted to be pathogenic based on in silico analyses. To test for pathogenicity, we are currently performing an in vitro microtubule-gliding assay to assess the change in velocity of synthetic microtubules over a lawn of purified recombinant protein consisting of specific KIF1A patient variants. We are also generating iPSCs from patient fibroblasts along with isogeneic CRISPR/Cas-9 gene corrected controls, which will be differentiated into neurons to examine microtubular kinesin-specific cargo trafficking, using fluorescent tagged cargo for live cell imaging. This work has potentially identified a novel biological process causing RTT, which may be tractable to future targeted therapeutic approaches.

T-3043

INVESTIGATING ALPHA-SYNUCLEIN PROPAGATION IN PARKINSON'S DISEASE USING AN IPSC-BASED ALPHA-SYNUCLEIN REPORTER SYSTEM

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In Parkinson's disease [PD], alpha synuclein [α-syn] propagation has been well-documented and is thought to be pathogenic. Misfolding of α-syn and the spreading of α-syn aggregates between cells could play a central role in PD progression. In this study, we describe the generation of a SNCA-triplication induced pluripotent stem cell [iPSC] reporter line, where we used the CRISPR/Cas9 technology to engineer α-syn-FLAG fusion protein. Using this iPSC line, we show that this differentiates efficiently into FOXA2+LMX1A+ floor plate progenitors, which then differentiates and matures into midbrain dopaminergic neurons. We observed a-syn aggregates in dopaminergic neurons derived from this cell line, and verify that the FLAG reporter accurately labels α-syn localization, aggregation and propagation.

Funding Source: National University of Singapore, Yong Loo Lin School of Medicine; Institute of Molecular and Cell Biology

T-3045

EVALUATING THE EFFECT OF AMYLOID BETA ON SYNAPTIC HEALTH OF FOREBRAIN NEURONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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The lack of appropriate disease models limits the understanding of disease mechanisms and therefore opportunities for developing new treatments. This is especially the case with neurodegenerative diseases including Alzheimer's disease (AD). To address

this critical issue, it is important to develop a cell culture model that highly resembles the human brain environment. Since the loss of synapses is the most common aetiology observed from post-mortem brains of AD patients, synaptic health of neurons derived from both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) was examined in this study. To generate forebrain neurons from pluripotent stem cells (PSCs) we used a combination of small molecules for the first 9 days of differentiation. The small molecules include inhibitors of SMAD signalling (LDN193189 and SB431542; LSB) and a Wnt signalling pathway inhibitor (XAV939) to derive central nervous system lineages, followed by other small molecules (PD0325901, SU5402 and DAPT; PSD) to derive forebrain neurons. Immunocytochemistry confirmed that the protocol produces mature neurons (positive for β-III-tubulin and MAP2) and early astrocytes (positive for S100β and Aquaporin4) by day 30 of differentiation. The neurons also possess characteristic forebrain markers (positive for TBR1 and PSD95). Release of glutamate into the cell culture medium after stimulation of neurons was assayed by LC-MS, indicating that the hPSC-derived forebrain neurons are functional and have the potential to provide a promising disease model for AD. Co-localisation of PSD95 and GluR2 indicated the presence of functional glutamatergic neuronal synapses, and changes in these markers after exposure to amyloid beta (Aβ) are under investigation. Detection of synaptophysin and PSD95 using a high-content plate-based imaging assay is under examination to establish its utility for small molecule drug screening. Overall, these results suggest that current differentiation protocol successfully produces forebrain neurons with functional synapses and that loss of synaptic function is a potential way to screen for compounds that can protect neurons from degeneration.

T-3047

MODELING AND RESCUE OF DEFECTIVE BLOOD-BRAIN BARRIER FUNCTION OF INDUCED BRAIN MICROVASCULAR ENDOTHELIAL CELLS FROM CHILDHOOD CEREBRAL ADRENOLEUKODYSTROPHY PATIENTS

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For X-linked adrenoleukodystrophy (X-ALD) patients with an ABCD1 mutation, 40% will convert to the deadly cerebral form (ccALD) characterized by rapid breakdown of the blood-brain barrier (BBB). Mutation information and molecular markers investigated to date are not predictive of conversion. Prior reports have focused on toxic metabolic byproducts as instigators of cerebral inflammation and subsequent immune cell invasion leading to BBB breakdown. This study focuses on the integrity of the BBB itself by modeling the BBB of ccALD patients and WT controls using directed differentiation of induced pluripotent stem cells (iPSCs) into induced brain microvascular endothelial cells (iBMECs). Immunocytochemistry and PCR confirmed characteristic expression of brain microvascular endothelial cell (BMEC) markers. Barrier properties of iBMECs were measured via trans-endothelial electrical resistance (TEER), sodium fluorescein permeability, and frayed junction analysis. RNA-seq and electron microscopy were used to further characterize disease-specific differences. Oil-Red-O staining was used to quantify differences in lipid accumulation. Addition of a diblock copolymer was optimized to increase TEER and decrease lipid accumulation in ccALD-iBMECs. We found that ccALD-iBMECs have significantly decreased TEER (2592 3 190 ff•cm2) compared to WT-iBMECs (5001 3 297 ff•cm2). They also accumulated lipid droplets to a greater extent than WT-iBMECs. To improve the barrier integrity of the ccALD-iBMECs, custom-designed block copolymers were added during the differentiation process. Upon treatment with the block copolymers, a 60% increase in TEER was observed for the ccALD-iBMECs compared to untreated controls. Treatment with block copolymers also reduced lipid droplet accumulation in the ccALD-iBMECs. We are currently investigating whether the decrease in barrier integrity and accumulation of lipid droplets is specific to ABCD1 using CRISPR-Cas9 to knockout ABCD1 in WT iPSCs and an inducible Tet-on system to express ABCD1 in ccALD iPSCs. Our finding that BBB integrity is decreased in ccALD and can be rescued with block copolymers opens the door for the discovery of BBB-specific molecular markers that can indicate the onset of ccALD and has therapeutic implications for preventing the conversion to ccALD.

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T-3049

ESTABLISHMENT OF AN IPSC-DERIVED SKELETAL MUSCLE CELLS PLASMA MEMBRANE REPAIR MODEL FOR DRUG SCREENING IN LIMB-GIRDLE MUSCULAR DYSTROPHY

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Limb-girdle muscular dystrophy (LGMD) is a diverse group of disorders by several genes mutations and inheritance. Main symptoms include the weakness and atrophy of the limb-girdle muscles. We focus on Limb-girdle muscular dystrophy type 2B (LGMD2B), that deficient dysferlin leads to poor plasma membrane repair. Currently, there were no efficient drugs for this tragic disease. Molecules which enhance the plasma membrane repairing rate are supposed to be beneficial to LGMD2B. Thus, we established a platform which could evaluate the skeletal muscle cell membrane repair ability based on healthy and dysferlin-deficient human iPSC (LGMD2B-iPSC). C2C12 mouse muscle cell line was used to establish the functional assays for high-throughput screening. Skeletal muscle cells derived from human iPSC will be incorporated into a microplate-based assay to monitor plasma membrane healing and repair. This platform will be used to screen new drugs for diseases associated with defective membrane repair ability, like LGMD and other neuromuscular disorders.

T-3051

MODELLING MITOCHONDRIAL DISEASE IN HUMAN EMBRYONIC STEM CELLS

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Mitochondrial diseases affect cellular energy production and are among the most complex inherited genetic disorders. Affecting approximately 1 in 5000 live births, these disorders are both clinically and genetically heterogeneous and currently have no known cures or treatments, despite a number of agents showing therapeutic promise. With more than 280 genetic causes of oxidative phosphorylation (OXPHOS) disorders, proving therapeutic efficacy remains challenging as homogeneous groups of patients are difficult to achieve for clinical trials. This project leverages human embryonic stem cell models that, when differentiated into clinically relevant cell types, could facilitate preclinical treatment studies and investigation of the underlying cellular mechanisms. CRISPR-Cas9 gene editing technology was used to generate knockouts of nuclear-encoded OXPHOS genes in H9 human embryonic stem cells (hESCs) to study tissue-specific effects via differentiation. We focused on generating cell lines for well-defined disease genes such as SURF1, and other genes in which patients often have biallelic Loss-of-Function mutations. This strategy permitted modelling of clinically relevant null mutations for multiple genes using a single isogenic control cell line. The two SURF1-/- cell lines generated using this technique show complex IV deficiency equivalent to patient fibroblasts. This cell line may better mimic complex IV based forms of Leigh syndrome since the SURF1-/- mouse model does not portray the classical human phenotypes. Both SURF1-/- clones form beating cardiomyocytes and maintain decreased complex IV expression post differentiation. Additionally, these cells show abnormal calcium handling and a significant decrease in contraction force in a cardiac organoid system. Label-free quantitative proteomic analysis of mature cardiomyocytes shows downregulation of complex IV associated proteins, while nearly all other mitochondrial proteins trend toward upregulation. Although cardiac involvement in SURF1 patients is atypical, cardiomyopathy is seen in rare cases. Overall, data generated from the SURF1-/- model show promise and we plan to trial disease-specific treatment options and study neural differentiations in the near future.

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T-3053

BUILDING BETTER IPSC MODELS OF HUMAN DISEASE AND DEVELOPMENT

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Since its inception in 1998, RUCDR Infinite Biologics (RUCDR, www.rucdr.org) has provided the world-wide scientific community with the highest quality biomaterials, technical consultation, and logistical support. In 2011 with the rising interest in utilizing iPSC to model human development and disease progression, for drug screening and for toxicology testing RUCDR began offering stem cell services and housing stem cell collections, including those from the National Institute of Neurological Disorders and Stroke and the National Institute of Mental Health. Although early iPSC were generated from skin fibroblasts, new methods involve generating iPSC from different tissue types such as blood. The advantages of blood as a source cell include the relative ease with which blood can be drawn, the small amount of blood that is required to generate iPSC and the existence of extremely well characterized blood samples in repositories around the world. At RUCDR Infinite Biologics, we have shown that we can reprogram a wide range of cell types and that the resulting iPSC from different source cells of a single subject are indistinguishable from each other. Despite the gains made by the use of blood as a source cell for iPSC, the diverse genetic background of the human race has hampered the usefulness of iPSC. Currently, controls often consist of age and sex-matched non-affected subjects or non-affected family members. The use of the CRISPR (clustered regularly-interspaced short palindromic repeats)/Cas 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, creating isogenic lines. Using this strategy, we have generated edited iPSC lines that are footprint free and meet all of the criteria necessary to certify the cell lines as pluripotent.

T-3055

PATHOLOGICAL INNATE IMMUNITY AND NEUROINFLAMMATION IN THE FIBROBLASTS AND INDUCED PLURIPOTENT STEM CELLS DERIVED FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by damage of motor neurons. Recent reports indicate that inflammatory responses occurring within the central nervous system contribute to the pathogenesis of ALS. We aimed to investigate disease-specific gene expression associated with neuroinflammation by conducting transcriptome analysis on fibroblasts from three patients with sporadic ALS and three normal controls. Several pathways were found to be upregulated in patients with ALS, among which the toll-like receptor (TLR) and NOD-like receptor (NLR) signaling pathways are related to the immune response. Genes-toll-interacting protein (TOLLIP), mitogen-activated protein kinase 9 (MAPK9), interleukin-1β (IL-1β), interleukin-8 (IL-8), and chemokine (C-X-C motif) ligand 1 (CXCL1)-related to these two pathways were validated using western blotting. This study validated the genes that are associated with TLR and NLR signaling pathways from different types of patient-derived cells. Not only fibroblasts but also induced pluripotent stem cells (iPSCs) and neural rosettes from the same origins showed similar expression patterns. Furthermore, expression of TOLLIP, a regulator of TLR signaling pathway, decreased with cellular aging as judged by changes in its expression through multiple passages. TOLLIP expression was downregulated in ALS cells under conditions of inflammation induced by lipopolysaccharide. Our data suggest that the TLR and NLR signaling pathways are involved in pathological innate immunity and neuroinflammation associated with ALS and that TOLLIP, MAPK9, IL-1β, IL-8, and CXCL1 play a role in ALS-specific immune responses. Moreover, changes of TOLLIP expression might be associated with progression of ALS.

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T-3057

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 a monogenic diabetes condition caused by an autosomal dominant mutation in the hepatocyte nuclear factor 4A (HNF4A) gene. Although Hnf4α is expressed during embryonic foregut development, the consequences of MODY1 mutations in HNF4A on foregut endoderm development have not been reported. Additionally, rodent models are unable to recapitulate the MODY1 phenotype as heterozygous or conditional knockouts do not present with diabetes. To determine if mutations in HNF4A affect human foregut development which can subsequently impact on liver and pancreas development and function, we generated human induced pluripotent stem cells (hiPSCs) from members of a MODY1 family and differentiated them down the foregut endoderm lineage towards hepato-pancreatic progenitors (HPPs), before bifurcating to hepatic or pancreatic β-like cells. In differentiated cells derived from MODY1-iPSCs, HNF4A protein was found to be predominantly sequestered in the cytoplasm, suggesting a loss of its function as a transcription factor. Transcriptional and RNA-Seq analyses revealed that numerous foregut pancreas- and liver-related genes, including HNF4A, were downregulated in MODY1-HPPs whereas hindgut HOX genes were found to be upregulated. Subsequent differentiation revealed that the decreased HNF4A expression in mutant foregut cells impacted on downstream liver and pancreatic development, resulting in altered morphology of differentiated hepatic cells and decreased expression of several hepatic and pancreatic β cell genes. Molecular studies of wild type and mutant HNF4A confirmed that mutant HNF4A was unable to activate downstream gene targets such as HNF1A, which is also a MODY gene. This HNF4A-HNF1A regulatory defect was demonstrated in both hepatic progenitors and β cells. Collectively, our patient-derived stem cell models revealed that this is a loss-of-function HNF4A mutation that affects the proper development of the foregut and its subsequent derivatives due to impaired transcriptional regulation by HNF4A. Both the developmental and functional defects may account for the progressive deterioration of the β cells and eventual loss of glucose homeostasis in MODY1 patients.

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T-3059

INNATE IMMUNE RESPONSES IN MURINE BLASTOCYST IN VITRO MODEL USING EMBRYONIC AND TROPHOBLAST STEM CELLS

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Mammalian early-stage embryo is protected from infection by zona-pellucida, but a hatched blastocyst has no physical defense. It is not clear how pre-implantation blastocyst protects from the infection of viruses by itself. We tried to study innate immune responses in the in vitro model of mouse blastocyst to culture murine embryonic stem (ES) and trophoblast stem (TS) cells as inner cell mass and trophectoderm of blastocyst, respectively. Each type of the cells were cultured separately on the cell-culture insert system. This model was stimulated by transfection with poly[I:C], which is a synthetic double-stranded RNA and induces an interferon response. The interferon response was induced in only TS cells, whereas the expression of pattern recognition receptors (PRRs) was detected in both ES cells and TS cells. Interestingly, the expression of interferon-stimulated genes were induced in both types of stem cells in our model. In the blastocyst, trophectoderm layer is the outermost layer and first barrier for infection. Our findings support the idea that virus-infected trophectoderm, i.e. the outer layer of the embryo, may have sensing and protective roles producing type I interferon and also give anti-viral signals to inner cell mass.

T-3061

DEVELOPMENT OF AN IPSC-BASED CELLULAR MODEL FOR INHERITED PERIPHERAL NEUROPATHIES TO STUDY AXONAL DEGENERATION USING CMTX6 PATIENT DERIVED MOTOR NEURONS.

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Charcot-Marie-Tooth neuropathy (CMT) is a group of inherited diseases caused by length dependent axonal degeneration of the peripheral motor and sensory neurons. A missense mutation (c.G473A; p.R158H) in the pyruvate dehydrogenase kinase 3 (PDK3) gene causes an X-linked form of CMT (CMTX6). PDK3 negatively regulates the pyruvate dehydrogenase complex (PDC) by reversible phosphorylation. Mitochondrial PDC catalyses the oxidative decarboxylation of pyruvate to acetyl CoA and critically links glycolysis to the energy producing Krebs (TCA) cycle. We have shown the p.R158H mutation causes hyperactivity of PDK3 and hyperphosphorylation of the E1 subunit of PDC and leads to both reduced PDC activity and ATP production in patient fibroblasts. We have generated a line of induced pluripotent stem cells (iPSCs) by re-programming CMTX6 patient fibroblasts carrying the PDK3 p.R158H mutation (iPSCCMTX6). Our data confirms iPSCCMTX6 maintain the PDC hyperphosphorylation phenotype found in CMTX6 fibroblasts. Treatment of iPSCCMTX6 with a pan PDK inhibitor reduces E1 hyperphosphorylation to levels found in control iPSC lines. We also have engineered an isogenic control using the CRISPR/Cas9 system (iPSCisogenic) and demonstrated that genetic correction of the disease causative p.R158H PDK3 mutation reverses the CMTX6 cellular phenotype. Using our 24 day motor neuron differentiating protocol followed by L1CAM magnetic sorting, we have successfully cultured mature motor neurons (MN) derived from these lines. The MNCMTX6 and MNisogenic motor neurons are ideal cells to model axonal degeneration and will provide an excellent neuronal cell system for compound screening approaches to identify effective drugs for the treatment of CMTX6 neuropathy.

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T-3063

STEM CELL-BASED NEURAL MODELLING OF GENETIC CHILDHOOD EPILEPSY

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Epileptic Encephalopathies (EE) are a group of devastating disorders presenting in childhood with numerous features including developmental delay and drug resistant seizures. Children born with EE typically have a poor prognosis and a high risk of sudden death. Variants in the SCN2A gene encoding the voltage-gated sodium channel type II α subunit (Nav1.2) represent a relatively common cause of EE. Biophysical approaches in non-neural cells have demonstrated that the SCN2A variants can give rise to Nav1.2 channels with hyper-excitability ('gain-of-function') or hypo-excitability ('loss-of-function'). We sought to examine the electrophysiological properties of neurons derived from patients with a severe form of EE carrying a heterozygous de novo R1882Q SCN2A variant. Excitatory neurons expressing SCN2A, vGLUT1 and GAD65/67 were generated from patient iPS cells by the forced expression of the neural transcription factor Neurogenin 2 (NGN2). Sequencing and restriction digest experiments confirmed the heterozygous expression of the variant transcript (R1882Q) from patient-derived NGN2 neurons. We compared the electrophysiological properties of patient-derived neurons to control neurons in which the mutation was 'corrected' using CRISPR-Cas9. Single-cell patch clamp analysis demonstrated that R1882Q neurons exhibited increased action potential firing and a lower rheobase (the minimal input current required to evoke an action potential) compared to control neurons. Together these results demonstrate that the R1882Q variant exhibits increased excitability at the neuronal level and indicates that reducing Nav1.2 channel activity may be a useful therapeutic approach to restore normative neural behaviour in these EE patients. Here, we demonstrate that a stem cell neural model can recapitulate the cellular phenotypic consequence of a gain-of-function SCN2A variant and may be a useful approach to test patient-specific pharmoco-therapies.

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T-3065

HUMAN IPSC BASED PHOSPHOLIPASE D1 KNOCK-OUT MODEL OF CONGENITAL CARDIAC DEFECT

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Phospholipase D1 (PLD1) is phosphatidylcholin specific enzyme catalyzing the hydrolysis of phosphatidylcholine to phosphatidic acid and choline. The reaction products are suspected to play a role in cellular signaling pathways and in regulation of subcellular trafficking. Alternative splicing of the transcript leads to multiple variants with both catalytic and regulatory functions. Recently, it has been described that mutations in PLD1 gene are associated with some congenital valvular defects which are among the most common birth defects, with an reported prevalence of 7.2 per 1000 live births. In present study, we created iPS knock out model of PLD1 using CRISPR/Cas9 protocols to evaluate of the role PLD1 in cardiomyocytes differentiation. Urinary cells from healthy individuals were used for generation of transgene-free iPSC with Sendai virus vectors expressing the reprogramming factors (CytoTune(R)). The iPSC were a morphologically similar to embryonic stem cells, expressed the pluripotency markers OCT3/4, LIN28, SSEA4, TRA-1-81, SOX2 and could be differentiated into all three germ layers. CRISPR/Cas9 nuclease system was used to introduce biallelic frameshift mutations disrupting the expression of PLD1 in this control iPSC line. Functional cardiomyocytes (CM) were generated by temporal application of a glycogen synthase kinase 3 inhibitor (CHIR99021) combined with chemical Wnt inhibitor (IWP2) and were positive for typical cardiac marker Troponin T, I and Nkx2.5 without observable signs of any disorganization. Robust spontaneous contraction of CM trough whole cell culture plates were observed in control cells in comparison with small restricted beating clusters in all mutant cells. Generated CMs provide suitable material for further pathology studies.

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REPROGRAMMING

T-3067

MODELLING THE NEURODEVELOPMENTAL DISORDER FRAGILE X SYNDROME BY DIRECT CELLULAR REPROGRAMMING: PROOF-OF-CONCEPT.

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Fragile X Syndrome (FXS) is the leading monogenic cause of intellectual impairment and autism spectrum disorder (ASD). FXS results from epigenetic silencing of the FMR1 gene due to expansion of the CGG repeat in the 5'UTR of FMR1. Study of the early molecular and cellular mechanisms that link FMR1 inactivation to impaired neuronal development and function is limited by the inability to access live FXS affected human neurons. We have established a direct cellular reprogramming strategy using a chemically modified mRNA gene delivery system to enhance the conversion of adult human dermal fibroblasts (HDFs) to induced neural precursor cells (iNPs) from which multiple mature neural lineages can be derived. We propose that use of our direct-to-iNP reprogramming strategy will provide a disease-relevant human model of FXS to study the pathogenesis of FXS with potential to identify therapeutic targets. The current study provides proof-of-concept supporting this strategy. FXS patient-derived HDFs were directly reprogrammed to iNPs by transient ectopic expression of the pro-neural transcription factors, SOX2 and PAX6. Gene analysis showed expression of early neural positioning genes indicative of dorsal and ventral telencephalic progenitors in both control and FXS-derived iNPs. A mixed population of iNP-derived neurons and astrocytes were derived after 30 days in defined differentiation media, exhibiting expression of the astrocyte marker S100B as well as glutamatergic genes TBR2 and VGLUT1 and GABAergic genes MEIS2 and GAD67. FMR1 was not detected in independent FXS lines following differentiation. Initial findings demonstrated an increase in neurite length as well an alteration in the ratio of neurons to astrocytes in FXS iNP-derived neurons compared to unaffected controls. These observations were correlated with the CGG-repeat dynamics and epigenetic profile of independent FXS lines using genotyping and DNA methylation analyses. Overall, these findings provide proof-of-concept for the application of direct-to-iNP reprogramming to undertake further research analyzing the molecular and cellular basis of FXS.

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T-3069

UNDERSTANDING HOW TELOMERASE TRIGGERS IN VIVO REPROGRAMMING OF MOUSE KIDNEY EPITHELIUM

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Telomerase serves two independent and critical roles in normal stem cells and in cancer. As a reverse transcriptase that synthesizes telomere repeats, telomerase prevents the severe adverse consequences of telomere dysfunction. In order to add telomere sequences to chromosomes ends, telomerase requires both TERT, the telomerase protein component, and TERC, the telomerase RNA component. In addition, recent studies demonstrated that TERT possess a non-canonical activity that foster cancer cell proliferation, and adult stem cells activation. In particular, we found that TERT non-canonical activity induces reprogramming of kidney epithelium into stem-like cells that resemble those observed in Wilms tumor, a pediatric renal malignancy characterized by abnormal proliferation of kidney cells showing features of stem cells. In this study, we aimed to decipher the molecular mechanisms targeted by TERT non-canonical activity that lead to cellular reprogramming. Forward that goal, we used sophisticated genetic approaches in vivo that allow cell sorting of kidney epithelium upon TERT-induced reprogramming. We subsequently performed high-throughput sequencing of RNA extracted from epithelial cells collected either at an early or a late time point of reprogramming. Comparison of transcriptomes of reprogrammed vs. quiescent kidney epithelial cells allowed us to determine the molecular signature underlying TERT-induced kidney epithelium activation. Moreover, our kinetic approach allowed us to discriminate potential master regulators of the reprogramming process versus effectors of this process. Indeed, while more than 1500 genes were deregulated at a late time point of reprogramming, only 60 genes were targeted at an early time point of reprogramming. We found striking up-regulation at early time point, that is maintained over time, of a gene encoding a protein with unknown function that we named TETARG-1 (Telomerase Target-1). Subsequent in-silico analysis allowed us to identify the pathways that are modulated upon TERT-induced reprogramming. Together, those data highlight genes and pathways whose disturbance might represent critical steps in both Wilms tumor initiation/progression and in the initial steps of reprogramming of highly differentiated and quiescent cells into stem-like cells.

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T-3071

BIOMIMETIC SURFACE FUNCTIONALIZATION FOR IMPROVING DIRECT NEURONAL REPROGRAMMING PROCESS

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After the advent of direct reprograming technology, this phenomenon has been received considerable attention in the field of tissue engineering and regenerative medicine. However, there are a number challenges of such application are yet to be solved for clinical settings. One key challenge would be to generate highly mature and functional neuronal cells. Given that an in vivo-like extracellular environment can provide optimal biochemical and biophysical cues in guiding cell reprogramming, the present study describes a potential platform for generating highly functional, therapeutic induced neuronal (iN) cells by engineering reprogramming niches in vitro. Herein, we report an engineered cell culture platform by functionalizing surfaces with mixture of proteins that exist in brain ECM. We observed that our engineered extracellular matrix (eECM) promoted conversion of fibroblasts to iN cells at a higher efficiency compared to the single ECM component-coated groups. The iN cells produced by eECM showed highly upregulated neuronal gene expression, and displayed more mature neuronal morphology with electrophysiological functionalities. Finally, transplantation of the iN cells cultured on eECM in a mouse model of ischemic stroke resulted in significant improvement in the locomotive behaviors of the diseased animals, suggesting great therapeutic efficacy. To conclude, we reveal the great potential for eECM in generating iN cells with high functionality and therapeutic efficacy, moving translational medicine one step closer to successful autologous cell therapy.

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T-3073

ROLE IN RNA METABOLISM OF PROCESSING ON RNA HELICASE DDX6 DURING IPS REPROGRAMMING

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Previously, we have reported that small non-coding RNA, RNY1 (112 nt) which plays a major role during iPS reprogramming. However, it was unclear the relationship between them. Therefore, we focused on ribonucleoprotein, RO60 (TROVE2), which binds RNY1 and investigated the mechanism during iPS reprogramming. We immunoprecipitated the RO60 protein complexes and analyzed them using Mass spectrometry. As a result, it was identified the DDX6 protein which is one of the proteins constituting the processing body (P-body) controlling RNA metabolism. Interaction between these proteins were predicted by computational simulation, and it was found that

they were bound via hydrogen bonds. Using these recombinant proteins, it was also revealed that they bound in vitro experiment. We observed the behavior of DDX6 proteins during iPS reprogramming by immunocytochemistry, and found that 86% of DDX6 proteins co-expressed in OCT4-positive cells. In addition, it was also revealed that iPS reprogramming efficiency dramatically decreased in fibroblasts of CRISPR/Cas9 treated to DDX6 locus. Next, we examined that the relationship between miRNAs and DDX6 using the luciferase assay (pmirGLO-TGFBR2_3'UTR, target of hsa-miR-302b-3p). Surprisingly OSKM and siRNY1 treated fibroblasts remained luciferase activity. Furthermore, we also revealed that RNA degradation of mesenchymal genes such as TGFBR2, RHOC, and CDKN1A in RNY1-knockdown cells hardly occurred during iPS reprogramming using BRIC-qPCR. We could suggest that DDX6 protein metabolized the mesenchymal RNA molecules during iPS reprogramming, and may control its reprogramming.

T-3075

REGULATION OF CELL REPROGRAMMING IN 3D MICROENVIRONMENT INTERACTIONS

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After the introduction of induced pluripotent stem cells (iPSCs), various methods to reprogram the somatic cells into the pluripotent state have been investigated. Along with transducing agent and supplemented chemical substances to improve reprogramming, cell culture substrates were highly modified. A recent study has been shown that chemically defined PEG hydrogel enhances reprogramming by promoting mesenchymal-to-epithelial transition (MET) as well as epigenetic remodeling. However, cell/microenvironment interaction on cell reprogramming was not defined. In this study, we look at various 3D microenvironments to find the possible candidate to improve reprogramming cells significantly and investigate its interaction that is involved with. Selected microenvironments were used to encapsulate the reprogramed cells by photopolymerization system, and analyzed its pluripotency, MET, and epigenetic state. Throughout many hydrogel systems, hyaluronic acid hydrogel microenvironment highly improves reprogramming efficiency by regulating pluripotency, MET, and epigenetic plasticity. These results show that this simple system may produce more purified reprogrammed cells using 3D microenvironment hydrogel.

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T-3077

AN INTEGRATION-FREE METHOD FOR THE GENERATION OF PUTATIVE INDUCED PLURIPOTENT STEM CELLS DERIVED FROM AGED DOGS

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Canine induced Pluripotent Stem Cells (ciPSCs) can provide great potential for regenerative veterinary medicine. There have been several previous reports on generation of canine somatic cell-derived iPSCs. However, there are no reports on the derivation of integration-free canine iPS cell lines, so far. To generate integration-free iPSCs from aged dog's fibroblasts, canine adult fibroblasts (CAFs) were reprogrammed using RNA-based strategy that takes advantage of the non-integrating and self-replicating Venezuelan equine encephalitis (VEE) virus. The VEE RNA replicon expresses four reprogramming factor ORFs (OKS-iG; hOct4, hKlf4, hSox2 and hGlis1). Using this RNA system, CAFs were co-transfected with VEE-OKS-iG RNA replicon and B18R mRNA during 4 hr. After 4 hr, the transfection medium was replaced to the Advanced-DMEM containing 10% FBS and 200 ng/mL B18R protein. Cells were selected puromycin (0.5 μg/mL) from days 2 to 10. On day 11, Advanced DMEM was changed to mouse embryonic fibroblast-cultured medium (MEF CM) containing 20% KSR. The putative ciPSC colonies first appeared between day 15-25. Interestingly, there have been two distinct types of initial ciPSC colonies. They were identified by immunohistochemistry of live cells using TRA-1-60 antibody and also showed clear alkaline phosphatase (AP) activity. To find the optimal culture conditions, the bFGF culture condition was changed to LIF/2i condition after ciPSCs were subcultured. Six days later, dome-shaped colonies appeared and gradually proliferated. Furthermore, analysis of the gene-expression levels suggested that these putative ciPSCs may be in a transition state. Although further studies are needed to characterize the conversion of canine somatic cells to ciPSCs, these results may be useful for developing canine-specific markers to characterize iPSCs in the transition or deterministic phase.

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T-3079

QUIESCENT SECRETORY CELLS IN THE MOUSE PROXIMAL COLON FUNCTION AS RESERVE STEM CELLS TO PROTECT FROM EPITHELIAL DNA DAMAGE

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While most intestinal epithelial cells (IECs) are rapidly renewed, a distinct population of small intestinal IECs remain quiescent and thereby reside as long-lived IECs. Specifically, small intestinal IECs residing at the +4 position are long-lived IECs that are committed to the secretory lineage, and function as reserve stem cells upon massive loss of the intestinal stem cell (ISC) pool. However, the existence of a quiescent IEC counterpart in the colon remains uncertain. We recently reported our establishment of a lineage tracing system for secretory IECs by using the Atoh1-CrePGR; ROSA26-LSL-tdTomato (Atoh1tdTomato) mice (Stem Cell Rep, 2018). By using this system, we conducted a pulse-chase labeling experiment of Atoh1+ IECs, to elucidate the lifetime of those IECs in different regions of the colon. Surprisingly, a distinct population of Atoh1+ IECs in the proximal colon exclusively retained their tdTomato labeling for over 20 days. Those label retaining Atoh1+ IECs were generally post-mitotic, as shown by a BrdU labeling experiment consisted of 1 month labeling period and a subsequent 3-month chasing period. Microarray analysis of those Atoh1+ IECs in the proximal colon revealed that they exhibit enhanced expression of genes required to maintain cell quiescence. To further confirm the region-specific properties of Atoh1+ IECs, we employed the organoid culture system. Intestinal organoids established from the proximal colon were highly sensitive to DBZ-induced secretory-lineage differentiation, compared to those established from other regions of the colon, and showed increased induction of cell cycle arrest leading to a significantly reduced proliferation activity. In contrast, Atoh1+ IECs of the proximal colon in vivo were highly resistant to 5-FU induced DNA damage, as confirmed by the expression of cleaved caspase 3. By using our Atoh1tdTomato mice, we conducted lineage tracing of Atoh1+ IECs under 5-FU induced mucosal injury and found that Atoh1+IECs in the proximal colon can not only survive but can also re-acquire ISC properties to completely reform and repair the damaged colonic crypts. In conclusion, our present results demonstrated that a subpopulation of Atoh1+ IECs in the proximal colon are quiescent IECs that can serve as reserve ISCs in response to DNA damage-induced mucosal injury.

T-3081

REPROGRAMMING OF HUMAN SKIN CELLS TO INDUCED MULTIPOTENT STEM CELLS BY SMALL MOLECULES

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The use of viruses or vector transfection to alter cell fate has been extensively studied. Somatic cells can be converted into many different types of cell relies on forced expression of appropriate transcriptional factors. Recently, by avoiding DNA insertional mutagenesis, small molecules have been utilized to increase the efficiency of lineage conversion and revolutionized the way of cell-fate decision. Mesenchymal stem cells (MSCs) meet with a high-class safety standard and have been attempted to treat dozens of diseases through hundreds of clinical trials due to their multipotency, immunomodularity, and low risk of oncogenicity. However, MSCs remain rare in adult bodies and require invasive treatment to isolate them, which is the main obstacle for autologous MSC-based therapies. Herein, we demonstrated the first chemical method to directly convert human dermal fibroblasts into functional MSC-like cells (induced MSCs, iMSCs). The method uses a defined cocktail of six chemicals, and it can achieve efficient conversion

with an average rate of 38% in 6 days. The pluripotent transcription factor OCT4 was up-regulated in iMSCs to a level similar to bone marrow MSCs as compared to fibroblasts, suggesting that iMSCs may be induced through the action of OCT4. 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 immunomodularity 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%. This finding may greatly benefit stem cell biology, cell therapy, and regenerative medicine.

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T-3083

MELATONIN ENHANCES THE DERIVATION OF EMBRYONIC STEM CELLS IN CLONED MOUSE EGGS USING CRYOPRESERVED OOCYTES

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Pacient-specific pluripotent stem cells (PSCs) can be generated via nuclear reprogramming by somatic cell nuclear transfer (SCNT). However, developmental efficiency of SCNT embryos using cryopreserved (vitrified/warmed) oocytes was very low. The cryopresevation procedure has a negative effect on the quality of oocytes and increases levels of reactive oxygen species (ROS) and apoptotic events. In this study, we found different expression of some apoptosis-related genes in the cryopreserved group by RNA-sequencing. Then, we investigated the effect of melatonin, an agent that reduces apoptosis and ROS production, on the derivation of mouse embryonic stem cell (ESC) lines from cryopreserved SCNT embryos. The addition of melatonin significantly enhanced blastocyst formation rates of the cloned eggs when compared to those of the melatonin-untreated group, and reduced the rate of apoptotic nuclei and ROS levels. Furthermore Melatonin improved embryonic development and implantation rate. More importantly, the addition of melatonin during embryo culture significantly increased the derivation efficiency of embryonic stem cells from cloned eggs (SCNT-ESCs). Based on these results, we conclude that melatonin may mitigate apoptotic-induced developmental decline and provide beneficial effects on the embryonic development of cloned eggs using cytoplasm of cryopreserved oocyte.

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T-3085

REDUCTION EPIGENETIC ERROR IN MOUSE CLONING COULD BE RESCUED BY HDACI COCKTAIL

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Somatic cell nuclear transfer (SCNT) technology has become a useful tool for animal cloning, gene manipulation and research into genomic reprogramming. We currently increased the efficiency of mouse cloning by adding the histone deacetylation inhibitors (HDACis). However, in most cases, the efficiency of animal cloning has been less than 10% because of poorly understood failures in the mechanism of genomic reprogramming. Here, we hope to treat cloned embryos using several HDACi at same time in order to improve the level of epigenetic modification.This project was hoped to significantly improve the efficiency of cloned mouse generation by taking advantage of each HDACi. Also, as a result, we hope to provide new ideas and feasible method for the development of the technology of SCNT.

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T-3087

DISSECTING OCT4'S DNA BINDING IN ESTABLISHING AND MAINTAINING PLURIPOTENCY

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The TF Oct4 employs a unique combination of specific and non-specific DNA interactions and cofactor cooperativity to bind its targets in different chromatin contexts, distinguishing it from other POU family members, despite highly conserved binding domains and binding motifs. These properties make Oct4 uniquely capable of restarting the pluripotency transcriptional program. Both the POU-specific (POUsp) and -homeodomains (POUhd) of Oct4 are shown to be important for DNA binding, have independent functions, and work cooperatively. Preliminary data suggests that DNA recognition by the POUhd is reduced in the presence of Sox2 on some motifs. To further examine this observation, we first tested the importance of the entire POUhd as well as single amino acids involved in DNA recognition, among a subset of POU/HMG composite motifs in the presence or absence of Sox2. Out of five point- and two truncation mutants, each mutant's DNA binding was reduced to a range of around 5-15% that of wild-type activity on the motifs tested, except Q146A and K117A which behaved like wild-type. When bound in the presence of Sox2, K117E was rescued to approximately 80% that of wild-type binding on the two "perfect" POU/HMG motifs. Mutant N143A was also partially rescued by Sox2 to around 30% that of wild-type but only on the imperfect UTF1 motif. Still, the partially rescued binding by the presence of Sox2 was effective in iPS generation only in the presence of c-Myc. When maintenance of pluripotency was tested, only K117A, Q146A, and partially K117E were capable of generating sustainable colonies. Suggesting that despite the reported differences in de novo Oct4 binding sites between ES cells and the early time points of reprogramming, Oct4 may in fact employ similar mechanisms for pluripotency in the two systems, which may be primarily cofactor-driven. Interestingly, mutation or removal of the POUhd reduced reprogramming efficiency to 19 and 28% that of wild-type at early time points, respectively, whereas mutation or removal of the POUsp or Oct4 entirely did not generate any early colonies, indicating that the POUhd is active yet dispensable for even rapid reprogramming. These data agree with earlier observations that POUhd binding is altered by the presence of Sox2, at the same time shedding new light on Oct4 cofactor-driven dynamics.

LATE BREAKING ABSTRACTS

T-4001

THE GLOBAL PICTURE OF CELL-TYPE COMPOSITION IN THE STROMAL VASCULAR FRACTION PREPARED FROM MOUSE ADIPOSE TISSUE

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Mesenchymal stem cells (MSCs) are a promising cell source for regenerative medicine because of their immunomodulatory and trophic properties. Although the heterogeneity of MSCs have been extensively discussed, the global picture of cell-type composition in MSCs has not been clarified. In this study, we used a newly developed high-throughput single-cell RNA-seq method, Quartz-Seq2, to analyze gene expression of freshly prepared stromal vascular fraction obtained from mouse inguinal adipose tissue. Dimensionality reduction and clustering of single-cell transcriptome data showed the clear separation of 11 clusters. Among them, we identified two types of CD34 positive MSCs, CD34+/Efemp1+ MSCs (cluster 1) and CD34+/Col15a1+ MSCs (cluster 2). Pdgfra and Sca1 were expressed in both population, whereas Cd90 was expressed at a higher level in cluster 2 than in cluster 1, and Cd105 was not strongly expressed in either cluster. Neither pluripotent markers (Pou5f1, Nanog, and Sox2) nor skeletal and cardiomyogenic markers (Tnnt2 and Myog) were detected. To investigate the difference between the two MSC clusters, we analyzed differentially expressed genes. CD34+/Efemp1+ MSCs were characterized by the expression of growth factors. On the other hand, CD34+/Col15a1+ MSCs were characterized by the enriched expression of genes that encodes extracellular matrix proteins including collagens. Collectively, our transcriptome analysis showed that the MSC population is divided into two clusters, suggesting that there is less heterogeneity of MSCs than expected. We will discuss these two MSC clusters in detail.

T-4003

YAP AND MRTF-A, TRANSCRIPTIONAL CO-ACTIVATORS OF RHOA-MEDIATED GENE EXPRESSION, ARE CRITICAL FOR GLIOBLASTOMA TUMORIGENICITY

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The role of YAP and MRTF-A, two transcriptional co-activators regulated downstream of GPCRs and RhoA, in the growth of glioblastoma cells and in vivo glioblastoma multiforme (GBM) tumor development was explored using human glioblastoma cell lines and tumor initiating cells derived from patient derived xenografts (PDX). Knockdown of these co-activators in GSC-23 PDX cells using shRNA significantly attenuated in vitro self-renewal capability. Orthotopic xenografts of the MRTF-A and YAP knockdown PDX cells formed significantly smaller tumors and were of lower morbidity than wild-type cells. In vitro studies used PDX and glioblastoma cells to examine functional responses to sphingosine 1-phosphate (S1P) demonstrated that YAP signaling was required for cell migration and invasion whereas MRTF-A was required for cell adhesion and both YAP and MRTF-A were required for proliferation.

T-4005

A POSITIVE-FEEDBACK LOOP BETWEEN NESTIN AND NRF2 IS REQUIRED TO UPREGULATE THE NRF2-MEDIATED ANTIOXIDANT RESPONSE

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Abnormal cancer antioxidant capacity contributes to impair chemotherapy. Thus, modulation of intracellular oxidative stress status is emerging as an anti-cancer treatment. Nestin, a class VI intermediate filament protein, is widely upregulated under pathological conditions and during cancer progression. Our previous studies showed that Nestin could influence the intracellular redox status by altering mitochondrial dynamics and that Nestin-knockdown cells were more sensitive to oxidative stress. However, the molecular mechanisms through which Nestin protects cells from oxidative damage remain unclear. Here, we identify a feedback loop between Nestin and Nrf2 and show that it is critical to the maintenance of redox homeostasis in non-small cell lung cancer (NSCLC). In detail, Nestin knockdown promotes oxidative stress-induced apoptosis due to decreased Nrf2-mediated antioxidant response. Mechanistically, the ESGE motif of Nestin directly interacts with the Kelch domain of Keap1 and competes with Nrf2 for Keap1 binding, leading to Nrf2 escaping from Keap1-mediated degradation and translocating into the nucleus, subsequently promoting antioxidant enzymes generation. Increased expression of Nestin blocks the binding between Nrf2 and Keap1 to inhibit the proteasome-mediated degradation of Nrf2. Finally, we show that several antioxidant response elements (AREs) in the Nestin promoter are responsible for its induction via Nrf2. Taken together, our results indicate that Nestin functions as a highly specialized cytoskeletal stress protein that promotes cellular redox homeostasis and drug resistance in NSCLC.

T-4007

PRE-INJECTION OF MSC FACILITATES THE SURVIVAL OF TRANSPLANTED CELLS IN AMI HEART

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Coronary artery disease (CAD), remain the major cause of morbidity and mortality worldwide. Stem cell therapies have been investigated as potential treatment of severe CAD and HF. Recent evidences reflected the MSC could modulate the host immune responses against the transplanted cells. We hypothesize that pre-transplantation systemic administration of hiPSC-MSC can induce immunomodulation and enhance the local engraftment of direct intramyocardial injection of

hiPSC-MSC. A mouse model of AMI had been used. iPSC derived MSC had been pre-infused intravenously one week before the LAD ligation and cells transplantation. The animals will be randomized into (1) culture medium (MI group, n=10); (2) intramyocardial transplantation of 10x105 hiPSC derived MSC alone (hiPSC-MSC or hESC-MSC alone group, n=8); (3) IV 5x105 hiPSC-MSC 1 week before local transplantation of 10x105 hiPSC-MSC (hiPSC IV group, n=8); (4) IV 5x105 hESC-MSC 1 week before local transplantation of 10x105 hESC-MSC (hESC IV group, n=8); (5) IV 5x105 hiPSC-MSC 1 week before local transplantation of 10x105 hiPSC-derived cardiomyocytes (iPSC-CM IV group, n=10); and (6) IV 5x105 hiPSC-MSC 1 week and immediately prior to local transplantation of 10x105 HES derived cardiomyocytes (HES-CM IV group, n=10). Those transplanted cells had been labeled by DiR viable stain for cell tracking. The fluorescent intensity showed that the labeled MSC injected had been persistently retained on the peri-zone of AMI hearts (MSC-IV group), But only a limited intensity of red fluorescent had been found in the saline group (MSC group). When the iPSC-derived cardiomyocytes (CM-IV group) were used, higher red fluorescent signal had also been found in the peri-infarct area of AMI hearts compared with the saline group. The normal ejection fraction had been 75% 3.20 in sham ICR mice. In MSC-IV group, the ejection fraction of left ventricle (LVEF) had been significantly increased (64.0%35.94 vs 38.33%36.81 in MI group) and also higher than the MSC group (53.67%31.53). For the cardiomyocytes treatment the LVEF had also been increased (CM-IV and CM group, 54.80%3 8.69 and 45.80%3 6.10 respectively) when compared with MI group. As conclusion, pre-injection of iPSC derived MSC could enhance the transplanted cell retention on AMI heart and improve the cardiac function of AMI heart.

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T-4009

DONOR-DEPENDENT VARIATION OF HUMAN UMBILICAL CORD BLOOD MESENCHYMAL STEM CELLS IN RESPONSE TO HYPOXIC PRECONDITIONING AND AMELIORATION OF LIMB ISCHEMIA

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With the rapidly growing demand for mesenchymal stem cell (MSC) therapy, numerous strategies using MSCs for different diseases have been studied and reported. Because of their immunosuppressive properties, MSCs are commonly used as an allogeneic treatment. However, for the many donors who could potentially be used, it is important to understand the capacity for therapeutic usage with donor-to-donor heterogeneity. In this study, we aimed to investigate MSCs as a promising therapeutic strategy for critical limb ischemia. We evaluated MSCs from two donors (#55 and #64) and analyzed the capacity for angiogenesis through in vivo and in vitro assays to compare the therapeutic effect between different donors. We emphasized the importance of intra-population heterogeneity of MSCs on therapeutic usage by evaluating the effects of hypoxia on activating cellular angiogenesis in MSCs. The precondition of hypoxia in MSCs is known to enhance therapeutic efficacy. Our study suggests that sensitivity to hypoxic conditions is different between cells originating from different donors, and this difference affects the contribution to angiogenesis. The bioinformatics analysis of different donors under hypoxic culture conditions identified intrinsic variability in gene expression patterns and suggests alternative potential genetic factors ANGPTL4, ADM, SLC2A3, CDON as guaranteed general indicators for further stem cell therapy.

T-4011

OXYGEN LEVELS REGULATE CELL FATE OF MOUSE DENTAL EPITHELIAL STEM CELLS VIA RHOA SIGNALING

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In recent years, it has become clear that oxygen level regulates stem cell fate, but its mechanism is not clear. In this study, we investigated the effect of oxygen level on dental epithelial stem cells (DESCs) using mouse incisors as a experimental model. First, we investigated the differences in surrounding environment, intracellular organelles and metabolic activity between detnal epithelial stem cells with low frequency of division and actively proliferating transit -amplifying (TA) cells. Surrounding blood vessels of DESCs were fewer and the distance was further than those of TA cells. Mitochondria in DESCs was fewer and smaller than that in TA cells. The expression of oxidative phosphorylation markers in DESCs were weaker than those in TA cells. These results suggested that DESCs were in a low oxygen environment compared with TA. Next, when the mouse incisors were cultured in a hypoxic environment, activation of RhoA signal, elevation of epithelial stem cell marker Sox2, suppression of cell division were observed. Conversely, inhibition of Rho signal induced translocation of Yap / Taz into nucleus, and promoted cell division. Together, these results indicated that the surrounding oxygen level controls cell fate of DESCs via RhoA signal.

T-4013

IMPROVED SURVIVAL AND INTEGRATION OF HUMAN PLURIPOTENT STEM CELL DERIVED RETINA IN CAT SUBRETINAL SPACE WITH SYSTEMIC IMMUNOSUPPRESSION

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The conceptual approaches to restoring vision impacted by degenerative retinal diseases or trauma have been enriched by the introduction of 3-dimensional (3D) retinal tissue, which brings incremental improvements in restoring vision. Compared to Retinal Pigment Epithelium (RPE) layer repair approaches, which can benefit from a simple cell suspension-based delivery into the subretinal space, neural retina repair needs to preserve structure and photoreceptor directionality to restore visual functions. To develop bioprosthetic approaches to neural retina repair, we derived 3D human retinal tissue (retinal organoids) from human embryonic stem cells (HESCs) and investigated delivery, survival and integration of laboratory-grown retina in a large-eye animal model. Human embryonic stem cell-derived retinal tissue was introduced into the subretinal space of wildtype cats using a transvitreal approach following a pars plana vitrectomy (n=6 eyes). Per os (orally introduced) prednisone was given orally at an anti-inflammatory dose for the duration of the study. Cats received either no systemic cyclosporine immunosuppression (n=3 grafts) or continuous systemic cyclosporine (n=3 grafts) starting from seven days before transplantation and then continuously. Eyes were examined by fundoscopy and spectral domain optical coherence tomography (SD-OCT) for adverse effects due to subretinal graft presence or/and surgical procedure and monitored regularly by fundoscopy and SD-OCT. Cats were euthanized 5 weeks following grafting, and immunohistochemistry on retinal sections was performed using human-specific antibodies (HNu, Ku80, SC121), axonal, synaptic, retinal cell type-specific markers and lymphocyte, microglia/macrophage markers. We report substantial improvement in the survival of xenogenic retinal tissue grafts in cat subretinal space with systemic immunosuppression protocol and initial evidence of structural and axonal/synaptic integration. Further refinement of the surgical delivery approaches coupled with improved design of bioprosthetic retina is expected to incrementally improve the methods of retinal tissue replacement enabling development of more realistic approaches of restoring vision.

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T-4015

CHARACTERZATIONS OF PROGENITOR T CELLS DERIVED FROM HUMAN PULRIPOTENT STEM CELLS UNDER FEEDER-FREE CULTURE CONDITIONS

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Recent outstanding clinical results demonstrated by engineered T cells, including chimeric antigen receptors, have already facilitated further researches that broaden their applicability. One such direction is to explore new T cell sources for allogeneic “off-the-shelf” adoptive immunotherapy. Human pluripotent stem cells (hPSCs) may serve as an alternative cell source for this purpose due to its unique features of infinite propagation ability and pluripotency. Several challenges to produce such off-the-shelf cell banks include development of a defined hPSC differentiation culture conditions and evaluation assays. Although the current feeder-dependent T-cell differentiation culture system is efficient and reproducible, the use of poorly defined components from the feeder cells may hinder the utility and contribute to cost and ultimately compromises the impact of PSC-based T cell immunotherapy. In this study, we demonstrate generation of progenitor T cells from human PSCs under feeder-free conditions and characterize the differentiating cells. hPSCs propagated under a feeder- and serum-free condition were induced to differentiate into the hematopoietic lineage by forming embryoid bodies and sequential addition and removal of morphogens. The differentiation cultures efficiently generated HPCs from multiple PSC lines. PSC-HPCs were seeded onto a plate coated with a notch ligand and induced to differentiate into progenitor T cells (PSC-proT) without a feeder layer. By 14 days after differentiation, HPCs differentiated into PSC-proT expressing CD5 and CD7 while expanding approximately 500-fold relative to the input cell number. We also performed surface antigen expression screenings of PSC-HPCs and identified subsets with higher T-cell potential. Transcriptome analyses using PSC-HPCs, -proTs, human bone marrow CD34+ cells (hCD34+), and progenitor T cells differentiated from the hCD34+ identified differentially expressed genes between cell types that could be exploited for further improvement. Collectively, the differentiation cultures

presented in this study together with transcriptome analysis and high-throughput screening of cell surface markers will become a powerful platform to accelerate the development of hPSC-based immunotherapy.

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T-4017

DIFFERENTIAL MRNA PROCESSING DURING MOUSE SKELETAL MUSCLE STEM CELL ACTIVATION

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Muscle stem cells, also called satellite cells (SCs), are muscle precursor cells that reside between sarcolemma and basal lamina of muscle fibers in adult skeletal muscle. In uninjured muscle, SCs are maintained in a quiescent state. Upon injury, they have the ability to proliferate and differentiate into multinucleated muscle fibers to regenerate muscle and demonstrate self-renewal capability to maintain stem cell pool. To elucidate the landscape of transcriptomes of quiescent and activated SCs, we have performed ultra-deep RNA-sequencing and discovered that there is widespread intron retention in transcripts that are specifically expressed in quiescent SCs. Among the myogenic regulatory factors, Myod1 pre-mRNA transcripts are accumulated in quiescent SCs and MYOD protein expression is independent of transcription during early satellite cell activation. We have previously reported that Dek is targeted by miR-489 for the maintenance of SC quiescence. Interestingly, the phosphorylated form of Dek is involved in mRNA splicing and it is rapidly expressed during SC activation for proper intron removal. Intron retention is also observed in other quiescent stem cells in mice and human. Our data suggests that intron retention is a feature of quiescent stem cells.

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T-4019

EVOLUTION AND CELL-TYPE SPECIFICITY OF HUMAN-SPECIFIC GENES PREFERENTIALLY EXPRESSED IN PROGENITORS OF FETAL NEOCORTEX

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Understanding the molecular basis that underlies the expansion of the neocortex during primate, and notably human, evolution requires the identification of genes that are particularly active in the neural stem and progenitor cells of the developing neocortex. Here, we have used existing transcriptome datasets to carry out a comprehensive screen for protein-coding genes preferentially expressed in progenitors of fetal human neocortex. We show that fifteen human-specific genes exhibit such expression, and many of them evolved distinct neural progenitor cell-type expression profiles and levels compared to their ancestral paralogs. Functional studies on one such gene, NOTCH2NL, demonstrate its ability to promote basal progenitor proliferation in mice. An additional 35 human genes with progenitor-enriched expression are shown to have orthologs only in primates. Our study provides a resource of genes that are promising candidates to exert specific, and novel, roles in neocortical development during primate, and notably human, evolution.

T-4021

IN VIVO IMAGING OF TRANSPLANTED DOPAMINGERIC NEURONS FOR PARKINSON'S DISEASE THERAPY

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Parkinson's disease is a devastating neurodegenerative disease, affecting ~10M people worldwide. The loss of dopaminergic (DA) neurons in the substantia nigra pars compacta leads to alterations in the activity of the neural circuits that regulate movement. Recent clinical studies reported the effective restoration treatment on Parkinson's disease (PD) patients using cell-based therapy. Thus, there is a great need to establish effective method to monitor the safety and efficacy of the grafted cells in vivo. Here, we aim to use in vivo diagnostic imaging agents to determine the fate of transplanted cells which could enable proof of safety, efficacy and mode of action of cell therapies. In this project, dopamine transporter expression was measured using [18F] FBCTT to quantify

survived dopamine cells. Striatal re-innervation and dopamine D2/3 receptor occupancy was measured with [18F] Fallypride to quantify the functional restoration of dopamine release by these transplanted neurons. We have longitudinally assessed the ability of hESC-derived immature DA neurons to reinnervate the 6-hydroxy-dopamine-lesioned (6-OHDA) PD rat model, quantifying engraftment with positron emission tomography (PET) at different stages post-transplantation. Amphetamine induced behavioral test was performed at the same time. We found that the behavioral test result correlated well with PET imaging result. Comparing with the behavioral test, which can reach a sutured evaluation capacity easily; PET imaging using our current probes demonstrated its sensitivity. The immunohistochemistry data demonstrated that the transplanted immature DA neurons are successfully maturing in vivo and displaying markers of dopaminergic neuron (TH) and mid brain dopaminergic neurons (GIRK, A9, FOXA2, EN1) with very low proliferation. These results proved that our molecular neuroimaging probes are of great value in the clinical application.

T-4023

DEVELOPMENT OF ORGANOID-BASED DRUG METABOLISM PREDICTION MODELS

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Liver and intestine are the principal organs responsible for metabolizing drugs, and the cytochrome P450 (CYPs) gene superfamily comprises a group of hemoproteins that catalyze the oxidative metabolism of a wide variety of drugs, carcinogens, and endogenous biomolecules. Although in vitro assay platforms such as primary hepatocyte or immortalized liver-derived cell lines have been developed to evaluate the CYP-inducing potential of drug candidates for drug development, several limitations have been suggested such as discrepancies between in vivo and in vitro assays. Recent advances in understanding stem cell physiology and culture technology have enabled the establishment of organoids derived from various organs and tissues. Organoids derived from specific organs can reproduce the normal physiology of the organ in a similar manner to the in vivo environment. In this study, we aimed to develop in vitro platforms to investigate drug metabolism and toxicity based on intestinal organoids and liver organoids from tissue resident stem cells. The expression and activity of CYP subtypes were measured in the organoids and confirmed to be similar to CYPs in vivo. We observed the changes of CYPs expression and activity according to the differentiation status of the organoid. Particularly, it was confirmed that CYPs expression and activity were significantly increased by the differentiation of liver organoids. Next, we observed that the activity of gemcitabine was changed under the co-culture platform of liver organoids and pancreatic tumoroids. These results show that organoids can be effective tools for predicting pharmacokinetics and pharmacodynamics of novel drugs before entering clinical trials.

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T-4025

DIFFERENTIATION OF FUNCTIONAL ISLETS FROM HUMAN IPS -DERIVED ENDOCRINE PROGENITOR CELLS.

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Islets of Langerhans play a major role in blood glucose control in vivo. Islet transplantation is an effective treatment for severe-insulin-dependent diabetic patients. However, the shortage of donor pancreas poses a major challenge for this therapy. In order to obtain a large number of pancreatic islets, methods for producing pancreatic islets from pluripotent stem cells such as ES / iPS cells have been studied. We aimed to establish a culture system for the purpose of forming functional pancreatic islets in vivo from human iPS cells. As a result, we have established a culture system that can make endocrine cell clusters with pancreatic islet-like structures. Those islet-like cell clusters secreted human c-peptide in response to glucose concentrations in vitro and vivo. However, our culture system still has many limitations such as cost, reproducibility and safety. For the purpose of improving differentiation efficiency and ensuring safety in clinical applications, we aimed to separate endocrine precursor cells. To identify endocrine progenitor specific markers, we performed microarray analysis to compare the gene expression pattern in Ngn3 positive and negative cells. As a result, we identified a novel pancreatic endocrine progenitor cell surface marker. We found that the endocrine precursor cells sorted by using antibody against the new marker formed the pancreatic islet-like structures more efficiently than from unsorted cells. Characterization of islet like cell clusters derived from purified cells is now underway and the results will be presented.

T-4027

EFFECT OF STRESSES ON THE PLURIPOTENT STEM CELLS SELF-RENEWAL AND NEURAL DIFFERENTIATION

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Stress Granules (SGs) are dynamic ribonucleoprotein aggregates, which had been previously observed in different types of cells subjected to environmental stresses such as oxidative stress and heat shock. Thus, these granules and considered a part of the stress response program that is known to play an essential role in regulating the cellular processes. It is known that pluripotent stem cells (PSCs) are highly sensitive to oxidative stress, indicating the importance of the stress response program in regulating stem cell fate. In this study we compared the effect of oxidative (sodium arsenite (SA) and hydrogen peroxide (H2O2)) and thermal (heat shock (HS)) stresses on SG formation in iPSCs. The aim was to establish whether these granules have a role in regulating self-renewal and differentiation. We found that SA and HS, but not H2O2, induce SG formation in induced PSCs (iPSCs). The analyses of these granules showed that they are canonical SGs, because (i) they contain the well-known proteins components of SGs (ii) they were found in juxtaposition to processing bodies (PBs); and (iii) they were disassembled after the removal of the stress conditions. Consistent with the SG data, SA and HS, but not H2O2, promote eIF2α phosphorylation in iPSCs forming SGs. An initial screening for pluripotent marker proteins recruited to SGs confirmed that LIN28A and L1TD1 were SG markers and identified DPPA5 as a novel pluripotent marker that was weakly recruited to SGs. Also, we had successfully reprogrammed skin fibroblasts from healthy individuals as well as Parkinson's disease (PD) patients into hiPSCs and differentiate them into neuro progenitor cells. These cells would be used in the future to compare the effect of different stresses on iPSCs and differentiated neurons. Altogether, our results introduce new aspects of how PSCs responds to adverse environmental conditions.

T-4029

DEVELOPMENT OF PERINEURONAL NETS IN CULTURES OF HUMAN NEURONS AND GLIA

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Perineuronal nets (PNNs) play important roles in the regulation of synaptic plasticity and neural regeneration, and may be involved in the pathogenesis of CNS disorders. Although numerous studies have demonstrated their importance in animal models, the development and function of PNNs in the human nervous system is poorly studied. Here we employ human neuronal and glial cells derived from iPS cells or immortalized lines of fetal neural progenitor cells to assess whether the formation of human PNNs can be recapitulated in vitro. iPS cell lines were generated from skin fibroblasts from 2 healthy control donors and 6 amyotrophic lateral sclerosis (ALS) patients with the H46R mutation in superoxide dismutase 1 gene (SOD1). The iPS cells were differentiated to motor neurons through modifications of the protocol reported by Du et al. (2014) and/or astrocytes. ReNcell VM cells were obtained from a commercial supplier and differentiated into putative dopaminergic neurons and astrocytes according to accompanying protocols. A panel of biochemical and functional assays were used to confirm differentiation into neurons and astrocytes. Expression of the principal PNN components - chondroitin sulfate proteoglycans and the link proteins Crtl1 and Bral2, was detected in iPS cell-derived cultures after about 50 and 60 days of differentiation by qPCR and immunocytochemistry, respectively. Expression in ReNcell VM -derived cultures was more rapid. Both neurons and astrocytes produced components of the PNNs although their expression patterns and timings differed. There were no differences between iPS cell-derived cultures from ALS patients and healthy donors.

T-4031

DEVELOPMENT OF SELECTIVE CYTOTOXIC VIRAL VECTORS FOR CONCENTRATION OF UNDIFFERENTIATED PLURIPOTENT STEM CELLS IN CARDIOMYOCYTES DERIVED FROM HUMAN IPS CELLS

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Innovative application of cell therapy products (CTPs) derived from human pluripotent stem cells (hPSCs) in regenerative medicine are currently being developed. Undifferentiated hPSCs possess tumorigenic potential, thus examining the residual undifferentiated hPSCs in CTPs is inevitable for the clinical use of hPSCs-derived CTPs and the establishment of sensitive methods for detection of residual hPSCs has been required. The detection limits of the methods currently available are known to be more than a ratio of 1/10^5 (0.001%, undifferentiated hPSCs/differentiated cells), which could be sufficient to detect hPSCs in a CTP containing cells less than 1 x 10^5. However, if the CTP contains cells more than 1 x 10^5, it is currently impossible to detect a

trace amount of hPSCs as impurities. For example, we cannot detect 100 undifferentiated iPSCs in a CTP that contains 1 x 10^8 iPSC-derived cardiomyocytes. Here, we show a novel approach to conquer the limit, using adenovirus and adeno-associated virus (AdV and AAV)-based selective cytotoxic vectors. We constructed AdV and AAV vectors that possess a suicide gene, iCaspase 9 (iCasp9), regulated by CMV promoter that is known to be dormant in hPSCs, for selective expression of iCasp9 in differentiated cells. As expected, AdV/CMV-iCasp9 and AAV/CMV-iCasp9 had cytotoxicity in immortalized cardiomyocytes, but not in iPS cells (iPSCs). AdV/CMV-iCasp9 and AAV/CMV-iCasp9 also induced apoptosis of iPSC-derived cardiomyocytes, and qRT-PCR analysis revealed that the expression levels of undifferentiated cell marker genes, such as LIN28, NANOG, and OCT3/4, were increased in the residual survived cells. These results indicate that the cytotoxic vectors concentrate cells expressing undifferentiated cell markers in iPSC-derived products and could become biological tools for overcoming the detection limit of 0.001%.

T-4033

MODELING DONOHUE SYNDROME PATHOGENESIS AND THERAPEUTICS USING PATIENT-DERIVED IPSCS AND ISOGENIC INSULIN RECEPTOR-DEFICIENT hESCS

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Donohue syndrome (DS) is an extremely severe autosomalrecessive genetic disorder caused by mutations of insulin receptor (INSR). DS patients are frequently characterized by metabolic abnormalities, dysplasia, eventually infant mortality due to inactive insulin receptor and dysfunction of insulin signaling pathway. However, the pathogenesis of DS is not fully understood partly due to lack of disease models. Here, we derive integration free-induced pluripotent stem cells (iPSCs) from a DS patient without genetic complementation and generation of isogenic INSR-deficient human embryonic stem cell (hESC) lines. DS cellular phenotypes are recapitulated in iPSCs/ESCs and their adult stem/progenitor cell derivatives. RNA sequencing and protein spectrum analysis further reveal the underlying mechanism. Our models serve to facilitate the discovery of novel disease features and mechanism, as well as lay a foundation for novel therapeutic strategies to treat DS.

T-4035

DIFFERENTIATION OF DOPAMINERGIC NEURONS FROM IPSCS AND SCNT-HESCS FOR PARKINSIN'S DISEASE MODELING

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Parkinson's disease (PD) is the second most common neurodegenerative disease. PD is characterized by bradykinesia, rigidity and tremor, and is the result of degeneration of dopaminergic neurons in the substantia nigra par compacta. There are many alternative disease models for PD, but most models are limited to animal models. Therefore, these animal models have difficulties in mimicking human disease. Human Pluripotent Stem Cells (PSCs), which can be subdivided into Human Embryonic Stem Cells (hESCs), induced Pluripotent Stem Cells (iPSCs), and Somatic Cell Nuclear Trasnfer (SCNT)-hESCs, can be used as influential tools for disease modeling because of their pluripotency and differentiation potential. Here, we compared differentiation potentials of pluripotent stem cell lines, such as iPSCs and SCNT-hESCs from PD patients. In this study, iPSCs and SCNT-hESCs have similar differentiation patterns. The both cells showed expression of distinct differentiation markers in each stage, such as progenitor, development and maturation. Each stage makers, pax6, maker of progenitor, Tuj-1, LMX-1A and Nurr-1, maker of developing and maturation, were confirmed by western blotting methods, and dopamine secretion was confirmed by LC-MS method. Dopaminergic neurons differentiated from PD patient iPSCs and SCNT-hESCs were showed distinct loss of dopamine secretion comparing to dopaminergic neurons from normal iPSCs and SCNT-hESCs. The differentiation potential of these cells is different, both iPSCs and SCNT-hESCs can be useful tools for PD modeling.

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T-4037

SINGLE CELL SEQUENCING REVEALS AN INTERMEDIATE EXPRESSION STATE ON THE PATH TO IPSCS THAT EMERGES UPON SOMATIC PROGRAM SHUTDOWN

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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, which impedes the molecular analysis of the underlying mechanisms. 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, with limited appearance of alternative cell identities over the course of reprogramming. We found that cells transitioning to the pluripotent state first silenced the defining genes of the starting cell program, which induced an intermediate state that enabled the transient expression of markers from different lineages, often in the same cell. This intermediate state only arose in a subset of cells after substantial time of OSKM induction, and precedes the upregulation of mesenchymal-to-epithelial transition (MET), which then allows for the induction of pluripotency gene expression. To confirm that the intermediate state characterized by the silencing of critical fibroblast genes is indeed a faithful reprogramming intermediate, we performed additional experiments: First, we identified surface markers in the scRNA-seq data specific to cells in the intermediate state and demonstrated that cells expressing this surface marker have a higher propensity to progress toward the iPSC state compared to those that don't. Second, we demonstrated that known reprogramming intermediates are enriched for cells displaying the intermediate state. Third, in an experiment where reprogramming is dramatically enhanced by the expression of the pluripotency transcription factor Esrrb together with OSKM, the intermediate cell state was established in the majority of cells very early in the process (which normally only occurs late in reprogramming and in few cells), consistent with the conclusion that the intermediate state enables the transition towards pluripotency. Together, our work offers insights into the step-wise sequence of events triggering the induction of pluripotency, and identifies the intermediate expression state as a critical reprogramming step.

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T-4039

OPTIMIZATION OF REPROGRAMMING CULTURE CONDITION FOR THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM COL1A1 4F2A-OCT4-GFP MICE WITH HIGH EFFICIENCY

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A reprogrammable transgenic mouse strain, called Col1a1 4F2A-Oct4-GFP, was bred for the present study. Because the somatic cells of this mouse strain contain only two copies of each Yamanaka factor, these animals are inefficient at producing iPSCs (approx. 0.005%) under traditional culture conditions. With an optimized culture condition, the iPSC production rate of MEFCol1a1 4F2A-Oct4-GFP was increased approximately to 8%. Further, promotion of cell proliferation by serum supplementation did not enhance iPSC production. Inhibition of TGF-beta in the serum by SB431542 neither affected the growth rate of MEFCol1a1 4F2A-Oct4-GFP nor promoted iPSC production. However, the use of gammaSNL cells to serve as feeders for iPSC production resulted in a 5-fold higher rate of iPSC production than the use of gammaMEFICR feeders. Interestingly, the use of SB431542 with the gammaMEFICR-adopted system could eliminate this difference. RT-PCR-based comparative analysis further demonstrated that TGF-beta expression was 10-fold higher in gammaMEFICR than in gammaSNL. Consistent with previous reports, mesenchymal to epithelial transition was found to participate in the initial steps of reprogramming in the specific context of MEFCol1a1 4F2A-Oct4-GFP. Moreover, we found that the initial seeding density is one of the pivotal factors for determining a high efficiency of iPSC generation. The iPSCs efficiently generated from our MEFCol1a1 4F2A-Oct4-GFP resembled mESCs in aspects of teratoma formation and germline transmission. Depending on the culture condition, our Col1a1 4F2A-Oct4-GFP mouse system can generate bona fide iPSCs with variable efficiencies, which can serve as a tool for interrogating the route taken by cells during somatic reprogramming.

T-4041

DIRECT REPROGRAMMING OF HUMAN SOMATIC CELLS INTO INDUCED NEURAL STEM CELLS WITH NON-INTEGRATING SYNTHETIC MESSENGER RNA

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Neural stem cells (NSCs) are a prominent cell source for understanding neural pathogenesis and for developing therapeutic applications to treat neurodegenerative disease because of their regenerative capacity and multipotency. Recently, a variety of cellular reprogramming technologies have been developed to facilitate in vitro generation of NSCs, called induced NSCs (iNSCs). However,the genetic safety aspects of established virus-based reprogramming methods have been considered, and hence, non-integrating reprogramming methods have been developed. Here, we successfully generated expandable iNSCs from human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) via transfection with in vitro transcribed (IVT) SOX2 mRNA. This is a genetically safe reprogramming method that utilizes properly optimized transfection conditions for efficient reprogramming. We confirmed that generated UCB-MSC-derived iNSCs (UM-iNSCs) possess multi-potent self-renewal capacity and can differentiate into three neuronal lineages. Additionally, we transfected human dermal fibroblasts (HDFs) with IVT SOX2 mRNA. SOX2 mRNA-transfected HDFs exhibited neural reprogramming with similar morphologies and NSC-enriched mRNA levels in comparison to human embryonic stem cell-derived neural stem cells, but they had a limited proliferation ability. Our study demonstrated that human UCB-MSCs can be used for direct reprogramming into iNSCs through transfection with single-factor-encoding IVT mRNA, which provides an integration-free reprogramming tool for future therapeutic application.

T-4043

CELLULAR GLUTATHIONE AS A MARKER PREDICTING STEM CELL FUNCTION

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Glutathione is the most abundant non-protein thiol in cells, functioning as an antioxidant and a redox regulator. In stem cells (SC), redox status controls the cellular activities of self-renewal or differentiation. Recently, we showed that the dynamics and heterogeneity of glutathione levels in SC can be monitored by FreSHtracer, a real-time glutathione probe. In the present study, we developed the glutathione parameters (GP) using FreSHtracer, for estimating stem cell function. GP consist of the glutathione mean level (GM), the glutathione heterogeneity (GH), and the glutathione regeneration capacity (GRC) of SC. When we measured GP of human umbilical cord mesenchymal SC (hUC-MSC) at low and high passage numbers (P4 vs P9), the P4 cells exhibited a higher GM but a lower GH and GRC than P9 cells, indicating that the replicative senescence process modulates GP of SC. Notably, the mitochondrial GP was more sensitive to the passage number than the whole cell GP, implying that mitochondria is the major site occurring the senescence-mediated redox change in SC. When comparing the GP and the in vitro stem cell function between seven different UC-MSCs, the GH results revealed a significantly negative correlation with their CFU-F activity. Moreover, the GM and the GRC exhibited a strong positive correlation with their migration and their regulatory T cell-inducing activities, respectively. Thus, our data suggest that cellular glutathione can be used as a marker for the monitoring of SC function.

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T-4045

A MODULAR ASSEMBLY OF SPINAL CORD-LIKE TISSUE ENDOWS TARGETED TISSUE REPAIR IN A COMPLETELY TRANSECTED RAT SPINAL CORD MODEL

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Tissue engineering based nervous tissue construction is holding promise in providing organoids with committed differentiation and therapeutic potentials. The aim of this study is to assemble a functional spinal cord-like tissue (SCLT) in vitro by simulating the white matter and grey matter composition of normal spinal cord using rat neural stem cell-based tissue engineering technique, and to evaluate whether the pre-built SCLT would execute targeted tissue repair in a completely transected rat spinal cord model. The integrated SCLT, assembled by the white matter-like tissue (WMLT) module and the grey matter-like tissue (GMLT) module, shared phenotypic

similarities to the rat spinal cord. Functional maturation of the SCLT, represented by synaptic transmission and myelin sheath formation, was detected in the WMLT and GMLT, respectively. Organotypic co-culturing with the dorsal root ganglion (DRG) or muscle cells showed that the SCLT embraced spinal cord organogenesis potentials to establish a structure touch with the targeted cells, respectively. Transplantation of the SCLT into the completely transected site of spinal cord resulted in a significant motor function improvement of the paralyzed hindlimbs in rats. Additionally, targeted spinal cord tissue repair was achieved by the modular design of SCLT as evidenced by an increased remyelination of regenerating nerve fibers in the WMLT area and an enlarged innervation in the GMLT area. More importantly, the pro-regeneration milieu had facilitated the forming of neuronal relay by the donor neurons allowing the conduction of descending and ascending neural inputs. The application of the SCLT is expected to provide a novel therapeutic approach for the structural and functional repair in completely transected spinal cord injury.

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T-4047

ENGINEERED TISSUE FOLDING BY MECHANICAL COMPACTION OF THE MESENCHYME

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Many tissues fold into complex shapes during development, defining the geometry of emerging stem cell and tissue niches. In vitro control over this process would represent an important advance for tissue engineering, by enabling spatial niche arrays with an engineered developmental "history" encoded in the local ECM composition and mechanical properties. We use embryonic tissue explants, finite element modeling, and 3D cell-patterning techniques to show that mechanical compaction of the extracellular matrix during mesenchymal condensation is sufficient to drive tissue folding along programmed trajectories. The process requires cell contractility, generates strains at tissue interfaces, and causes patterns of collagen alignment around and between condensates. Aligned collagen fibers support elevated tensions that promote the folding of interfaces along paths that can be predicted by modeling. We demonstrate the robustness and versatility of this strategy for sculpting tissue interfaces by directing the morphogenesis of a variety of folded tissue forms from patterns of mesenchymal condensates. These studies provide insight into the active mechanical properties of the embryonic mesenchyme and establish engineering strategies for more robustly directing tissue morphogenesis ex vivo.

T-4049

BIOFABRICATION OF HUMAN ARTICULAR CARTILAGE: IN SITU BIOPRINTED HUMAN STEM CELLS TRIGGER NEOCARTILAGE INTEGRATION IN AN EX VIVO OSTEOCHONDRAL MODEL

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Cartilage injuries cause pain and loss of function, and if severe may result in osteoarthritis (OA). 3D bioprinting is now a tangible option for the delivery of bioscaffolds capable of regenerating the deficient cartilage tissue. Our team has developed a handheld device, the Biopen, to allow in situ additive manufacturing during surgery. Given its ability to extrude in a core/shell manner, the Biopen can preserve cell viability during the biofabrication process, and it is currently the only biofabrication device tested as a surgical instrument in a sheep model using homologous stem cells. As a necessary step toward the clinical translation, we now aim to demonstrate that we can successfully biofabricate long lasting human cartilage. In the present study we analyzed the efficiency of our approach to combine the immediate adhesion provided by in situ biofabrication with the chondrogenic capacity of a stem-cell-laden biomaterial. We specifically used human derived mesenchymal stem cells (hADSCs), isolated from the Infra-Patellar Fat Pad of donor patients affected by OA, which represent the source of cells for the future clinical application. We then

developed an ex vivo osteochondral (OC) model from human condyles of the same patients. We generated a cartilage defects by exciding 25 mm3 of tissue and performed the in situ biofabrication to evaluate the behavior of the damaged site. We analyzed three groups: i) In situ: using the Biopen we extruded hADSCs laden in GelMA/HA (Gelatin Methacrylate/Hyaluronic Acid) and crosslinked directly in the defect. ii) Ex situ: a bioscaffold of the same components was fabricated in a mold of the same size of the defect and implanted with and without fibronectin glue. iii) Control: defect left empty. The samples were maintained in a perfusion bioreactor and stimulated via a chondrogenic cell culture media for several weeks. A comprehensive characterization including immunohistology, confocal microscopy, second harmonic generation, light sheet imaging, and mechanical tests demonstrated that our strategy drives both chondrogenic differentiation and integration within the host tissue. Our in situ biofabrication approach represents an innovation with important implications for customizing cartilage repair in patients with cartilage injuries and osteoarthritis.

Funding Source: Arthritis Australia, Victorian Orthopaedic Research Trust, John Loewenthal Foundation for Surgery (RACS), Australian Research Council Centre of Excellence Scheme, St Vincent's Hospital (Melbourne) Research Endowment Fund.

T-4051

ATTEMPTS TO ESTABLISH SCREENING SYSTEM FOR IDENTIFICATION OF DRUGS TARGETING HUMAN UTERINE ENDOMETRIAL CANCER STEM-LIKE CELLS

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Uterine endometrial cancer is the most common malignancy of the female genital reproductive tract. It has been recently reported that cancer stem cells (CSCs) are involved in the pathogenesis of some types of endometrial cancer. Few studies, however, have reported a possible therapeutic strategy using endometrial CSC-selective drugs, partly because there is a lack of cell culture and drug screening system employing an endometrial cancer cell line that contains CSCs stably and reproducibly. We here found that, among several endometrial cancer cell lines, HHUA most stably and reproducibly contained CSC-like cells that exhibited side population (SP) phenotype characteristic of stem cells. SP cells of HHUA showed more proliferation, colony forming and invasive activities together with a higher expression of multidrug resistance (MDR) proteins such as MDR1 than non-SP cells (also referred to as main population [MP] cells) of HHUA. Furthermore, HHUA-SP cells formed a larger tumor with a higher expression of Ki67, a proliferation marker, and Zinc Finger E-Box Binding Homeobox 1 (ZEB1), which is known to promote progression of various types of cancer through epithelial-mesenchymal transition (EMT), than HHAU-MP cells did when transplanted into immunodeficient mice. Based on the concept of drug-repositioning, we chose 14 candidate drugs with potentials for negatively affecting various CSC behavior and tested their inhibitory effects on HHUA-SP cells to identify and repurpose the drugs as endometrial CSC-selective drugs. Among them, we found that sorafenib, a multikinase inhibitor effective for renal, hepatocellular and thyroid carcinoma, not only inhibited cell proliferation, induced apoptosis, reduced invasive activities in whole HHUA cells but also inhibited HHUA-SP cell proliferation and invasion and decreased HHUA-SP cell fraction. Moreover, sorafenib reduced the weight of HHUA SP-derived tumor together with a decreased Ki67 and ZEB1 expression. The results of this study suggest that HHUA is a useful endometrial cancer cell line suitable for a possible drug-repositioning screening system for identification of endometrial CSC-selective drugs and that sorafenib may be an effective therapeutic drug for endometrial cancer targeting its CSCs.

Funding Source: This research is partially supported by the Project for Whole Implementation to Support and Ensure the female life (Wise) from Japan Agency for. Medical Research and development, AMED.

T-4053

CHARACTERIZATION OF MURINE HAIR FOLLICLE STEM CELLS UNDER LONG-TERM CULTURE CONDITIONS

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Characterization of hair follicle stem cells (HFSCs) is important for understanding follicle cell homeostasis and differentiation, as well as hair morphogenesis. HFSC isolation methods and the characteristics of those cells have recently been reported, though few reports regarding effective long-term culture methods and techniques for HFSC long-term maintenance in vitro are available. We have developed an HFSC culture method that does not require serum or feeder cells, and utilizes commercially available medium without exogenous factors. In the present study, cells cultured according

to our method were collected after 10 and 48 passages (10p-, 48p-HFSCs, respectively), then examined to determine their characteristics and potential usefulness as HFSCs. CD34+CD49f+ cells (0p-HFSCs) were prepared from adult mouse skin using flow cytometry and cultured in serum-free medium up to 48 passages (1 passage per week). From those, we examined 10p- and 48p-HFSCs to elucidate their characteristics and potential for use as HFSCs with real time RT-PCR, immunocytochemistry, and in vivo patched assays. Both p-10 and p48-HFSCs showed immunopositivity for markers of undifferentiation, as well as vigorous proliferation. However, differentiation of p48-HFSCs into follicle epithelial cells was limited as compared to that of p10-HFSCs. In addition, inhibition of Wnt signaling in p48-HFSCs did not result in a decrease in markers of undifferentiation, whereas a marked decrease of those was noted in p10-HFSCs. Patched assay findings demonstrated that the ability of p48-HFSCs to form follicles was either lost or largely impaired. Our results suggest that regardless of their persistent expression of markers of undifferentiation, the differentiation ability of HFSCs cannot be maintained and altered responsiveness to Wnt signaling also occurs when maintained in long-term cultures.

POSTER SESSION II-EVEN 19:00 – 20:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

T-1002

IMPAIRED FUNCTION OF PLACENTAL MESENCHYMAL STROMAL CELLS FROM PREGNANCIES WITH FETAL GROWTH RESTRICTION

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The extensively branched network of blood vessels in the placenta is key for materno-fetal exchange, and is inadequate in pregnancies complicated by fetal growth restriction (FGR). Pro-angiogenic mesenchymal stem/stromal cells (MSCs) reside in a perivascular niche in the placenta where they likely influence angiogenesis. However, the contribution of placental MSCs to FGR is poorly understood. Here we aimed to determine whether functional differences in placental MSCs exist that may influence normal and pathological placental function. MSCs were isolated from first trimester (n=5), term (n=10) or FGR (n=8) placentae.1) The secretomes of 1st trimester (n=5) and term (n=5) MSCs were determined by Quantibody Cytokine Arrays. First trimester and term MSCs secreted different pro-angiogenic cytokines. In angiogenesis tube formation assays visualised by time lapse microscopy, endothelial cells treated with pooled first trimester MSC conditioned media formed shorter tubes with fewer branching points than those treated with pooled term MSC conditioned media (n=5 replicates, $p<0.001$). This shows that MSC contribute to placental vascularisation differently throughout gestation, which may correspond to different stages of vessel formation and expansion. 2) Similar tube formation assays also showed a significant inhibition of tube length and branching points in wells treated with pooled FGR MSC conditioned media in comparison to pooled term MSC conditioned media (n=5 replicates, $p<0.001$). This indicates that a dysregulation in paracrine factors produced by FGR MSCs may contribute to inadequate vascular development in these placentae. **3)** Term and FGR MSC proliferation was quantified by Alamar blue. MSCs from FGR placentae (n=4 placental replicates) proliferated at almost half the rate of those from normal term placentae (n=5 placental replicates, $p<0.001$), suggesting that deficiencies in the number of MSCs in FGR placentae could further limit their contribution to successful angiogenesis.

Funding Source: Health Research Council

T-1004

COMPARISON OF IMMUNOLOGICAL CHARACTERISTICS OF HUMAN MESENCHYMAL STEM CELLS FROM THE PERIODONTAL LIGAMENT, UMBILICAL CORD, AND ADIPOSE TISSUE

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Mesenchymal stem cells (MSCs) are of therapeutic importance in the fields of regenerative medicine and immunological diseases. Accordingly, studies evaluating MSCs for clinical applications are increasing. In this study, we characterized MSCs from the periodontal ligament, umbilical cord (UC-MSCs), and adipose tissue, which were relatively easy to obtain with limited ethical concerns regarding their acquisition, and compared their immunological characteristics. Among MSCs isolated from the three different tissues, UC-MSCs grew the fastest in vitro. The three types of MSCs were shown to inhibit proliferation of activated peripheral blood mononuclear cells (PBMCs) to a similar degree, via the indoleamine 2,3-dioxygenase and cyclooxygenase-2 pathways. They were also shown to inhibit the proliferation of PBMCs using HLA-G, which was most prominent in UC-

MSCs. Unlike the other two types of MSCs, UC-MSCs showed minimal expression of HLA-DR after activation, suggesting that they pose minimal risk of initiating an allogenic immune response when administered in vivo. These characteristics, the ease of collection, and the minimal ethical concerns regarding their use suggest UC-MSCs to be suitable MSC therapeutic candidates.

Funding Source: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2015M3A9E6028677).

T-1006

PRETREATMENT OF HUMAN ADULT STEM CELLS WITH DIFFERENT INFLAMMATORY SIGNALS IMPROVED THERAPEUTIC POTENTIALS IN A MURINE MODEL OF ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease, but there is no known cure for AD. Recently, there have been great efforts to treat AD using mesenchymal stem cells (MSCs), multipotent stem cells which have immunomodulatory functions. To enhance the efficacy of MSCs, these cells can be primed with small molecules, biological agents and/or biomaterials. However, little is known about the underlying mechanisms of the immunoregulatory functions of primed MSCs for the AD treatment. To evaluate the therapeutic effects of primed MSCs in AD model, MSCs obtained from the Wharton's Jelly (WJ-MSCs) are pretreated with Toll-like receptor 3 (TLR3) agonist or interferon-γ (IFN- γ). Untreated and pretreated WJ-MSCs are injected into the murine AD models and investigated the clinical and molecular characteristics of their therapeutic effects. Compared to the untreated cells, the primed WJ-MSCs exhibited increased inhibitory effects in the clinical symptom score, transepidermal water loss (TEWL), epidermal thickness, and inflammatory cell infiltration. Although there is no difference in the levels of IL-10 and IFN-ɤ in skin draining lymph node (LN), the IL-17 levels were significantly decreased in primed WJ-MSCs group. To identify gene signature for each condition, we next performed transcriptome analysis. Compared to the controls, IFN- γ treated WJ-MSCs showed 2,540 genes that were differentially regulated ($p < 0.001$). In contrast, only 268 genes were changed in TLR 3 agonist-treated WJ-MSCs ($p < 0.001$). We further identified the core regulatory pathways for those preconditioned WJ-MSCs. Taken together, we showed that priming with TLR3 agonist or IFN-γ of hWJ-MSCs promotes immunomodulatory function through the regulation of Th17 immune response for human AD.

Funding Source: Funded by NRF-2016R1D1A1B03935929, NRF-2015K1A4A3046807, and KHIDI-HI15C0925

T-1008

MIGRATION OF HUMAN WHARTON'S JELLY DERIVED MESENCHYMAL STEM CELLS INTO THE RAT BRAIN IS POSSIBLE VIA INTRA THECAL INJECTION

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Optimizing the delivery route is very important to maximize the efficacy of stem cell therapy. Out of the various delivery routes to the brain, the intra-thecal route is convenient for brain surgery can be avoided. The major objective of this study was to optimize the procedure of intra-thecal injection and to investigate the distribution of human Wharton's jelly-derived MSCs (WJ-MSCs) injected via intra-thecal route in a rat model. In order to optimize the procedure of intra-thecal injection, we injected 500 µL of trypan blue dye into lumbar 2-3 of 4 week-old Sprague Dawley (SD) rats. Fifteen minutes after injection, trypan blue dye was detected in the lumbar, thoracic, cervical cord tissues, cerebellum, and also the ventral side of the rat brain. However, trypan blue was not detected in the dorsal side of the brain or the lateral ventricles. Afterwards, we performed intra-thecal injections of ferumoxytol (MRI compatible nanoparticle) labeled 1×106 of WJ-MSCs (passage 6). After MSC injection, experimental animals were sacrificed at 3 different time points: 0, 6 and 12 hours. A control group (vehicle) was also included in the study. Utilizing real-time polymerase chain reaction, the distribution of WJ-MSCs in various organs was quantified. Overall, at 0 and 6 hrs, a significant number of WJ-MSCs was not detected in all of the organs (brain, liver, heart, lung, kidney, and spleen). However at 12 hrs, 2.4x104 WJ-MSCs were detected in the brain which indicated the possible time it takes for MSCs to migrate towards the brain. While we did not observe the distribution of MSCs past 12 hrs the results of this study signify that it is feasible to deliver MSCs to the brain via the intra-thecal route.

Funding Source: This study was supported by grants from Basic Research Program through the National Research Foundation of South Korea (NRF) funded by the Ministry of Education (NRF-2017R1D1A1B03035940)

T-1010

CEREBROSPINAL FLUID FROM ALZHEIMER'S DISEASE PATIENTS AS AN OPTIMAL FORMULATION FOR THERAPEUTIC APPLICATION OF HUMAN MESENCHYMAL STEM CELLS IN ALZHEIMER'S DISEASE

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Mesenchymal stem cells (MSCs) have emerged as one of the promising treatment options for Alzheimer's disease (AD), since these cells have shown widespread effects such as: reduction of beta amyloid (Aβ) levels, modulation of neuroinflammation, enhancement of endogenous neurogenesis, and also improvement in the behavioral performance. Although many preclinical studies have investigated the efficacy of MSCs in AD, how MSCs actually change following exposure to the AD environment has not been studied extensively. In this study, we investigated the potential of AD patient-cerebrospinal fluid (CSF) samples to be used as a formulation of MSCs and its application in AD therapeutics. When wharton's jelly mesenchymal stem cells (WJ-MSCs) were stored in the CSF of AD patients, the stemness of WJ-MSCs was preserved, showing unaltered cell surface marker expression and differentiation potential. The viability of hypothermic stored WJ-MSCs in AD CSF were also similar to that of WJ-MSCs stored in media formulation. Furthermore, although the expression levels of various genes remained unaltered after CSF storage, expressions of certain genes that were altered signified the therapeutic potential of CSF formulation for AD therapy. Overall, these findings suggest that the CSF from AD patients can be used as an optimal formulation of MSCs for the treatment of AD. Moreover, this use of CSF as an optimal formulation of MSCs can not only be extensively applied to various neurological diseases, but also improve the overall efficacy of MSC therapy.

Funding Source: This research was supported by the Ministry of Health & Welfare (Korea Health Industry Development Institute (KHIDI): HI14C3484, Korea Health Technology R&D Project: HI14C2746), Republic of Korea.

T-1012

HUMAN PLACENTAL MESENCHYMAL STEM CELLS PROTECT HUMAN ENDOTHELIAL CELLS FROM DAMAGE INDUCED BY HIGH GLUCOSE LEVEL

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Diabetes mellitus (DM) is characterized by hyperglycemia, insulin resistance, and insulin deficiency. It is a metabolic and vascular syndrome associated with aberrations in both micro- and macrovasculature. DM has adverse long-term effects that affect many organ systems. DM is associated with abnormalities of angiogenesis which may result in several clinical manifestations of diabetes. These are associated with the pathogenesis of vascular abnormalities of the kidneys, retina, reduced wound healing, increased risk of transplanted organ rejection, and reduced coronary collateral formation. A confusing feature of the abnormal angiogenesis is that excessive and insufficient angiogenesis can arise in different organs in the same patient. Despite significant therapeutic advances, DM remains an unmet medical need. In addition to the conventional therapies, there is an increased demand of alternative treatment options. One of the therapeutic approaches is to protect endothelial functions from damage by high level of glucose; thus maintaining normal angiogenesis. We investigated the ability of our in-house developed MSCs isolated from chorionic villi of human term placenta (pMSCs) to protect endothelial cell functions from damage by increased concentrations of glucose. Endothelial cells were cultured with increased concentrations of glucose. Proliferation, angiogenesis (capillary network) and flow cytometric characterisation of endothelial cells were examined in the presence and absence of different treatments of pMSCs. The anti-proliferative effect of increased glucose concentration was reversed by pMSCs. In addition, impaired endothelial cell capillary network formation (angiogenesis) in high level of glucose environment was also restored by pMSCs. pMSC also reduced the expression of pro-inflammatory cytokines in endothelial cells. We show for the first time that pMSCs protect endothelial cells from damage induced by high level of glucose. Therefore, our results suggest that pMSCs could be a potent candidate for diabetes therapies by preventing complications associated with diabetes.

ADIPOSE AND CONNECTIVE TISSUE

T-1014

CHARACTERIZATION OF ADIPOCYTES DIFFERENTIATED FROM PRIMARY HUMAN DERMAL FIBROBLASTS OF NORMAL AND DIABETIC PATIENTS

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The sharp rise in the incidence of diabetes and obesity has reached an epidemic state world-wide. Obesity is one of the risk factors for onset of type 2 diabetes, it also can affect the cardiovascular health leading to onset of cardio-metabolic disease. The expansion of white adipose tissue in obese individuals can affect both lipid and glucose metabolism. Diabetic patients often suffer from increase adipose tissue that are defective in glucose metabolism. Therefore, it is essential to examine the cellular and molecular changes in adipocyte of human diabetic patients. Since obtaining human adipocytes from patients are challenging we aimed to establish protocols for differentiation of human skin fibroblast cells from normal and diabetic patients to adipocytes. Our main objective was to characterize these adipocytes and determine whether they maintain the diabetic phenotypes after differentiation. In our current study, we successfully differentiated primary human skin fibroblast cells from normal and diabetic patients into adipocyte after 10 days culture in differentiation media. Oil red O staining showed a significant accumulation of lipid droplets in the differentiated cells. Real time PCR of non-differentiated skin fibroblast showed a significantly higher mRNA level of Adiponectin, PPAR gamma and CEPB Beta of diabetic patients compared to normal. After differentiation the level of Adiponectin mRNA in diabetic cells further increased however, there was no difference between the CEBP beta mRNA in diabetic as compared to normal human differentiated adipocytes. Furthermore, we observed a significant increase in FABP mRNA level in diabetic patient accompanied by a significant decrease in GLUT-4, insulin receptor and insulin receptor substrate 1 mRNA. Overall our data illustrate that adipocytes differentiated from skin fibroblasts of diabetic patients maintain the characteristics of the disease state.

Funding Source: This research was made possible by a grant by QNRF, NPRP07-208-3-046.

T-1016

THE SPRY4 TRANSIENT KNOCKDOWN EFFECT IN HUMAN ADIPOSE-DERIVED STEM CELLS ENHANCES OSTEOGENIC DIFFERENTIATION

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Byoung ju Kim - *CHA University, Seongnam, Korea*
Alvin Bello - *CHA University, Seongnam, Philippines*
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The MAPK signaling pathway has been widely investigated for its role in stem cell proliferation and differentiation and one of its protein antagonists, Sprouty 4 (SPRY4), has been found to be related with ERK 1/2 phosphorylation. In this study, we determined the effect of the knocking-down SPRY4 gene on the tri-lineage differentiation of human adipose-derived stem cells (hASCs). First, we determined that siSPRY4 treatment reduces proliferation rate but increases phosphorylation of ERK1/2 in hASCs. Interesting, siRPY4 treatment exhibited varying effects on the tri-lineage differentiation. Gene (qPCR) and protein analysis (Western Blot and cytological staining) results showed that siSPRY4 treatment enhanced osteogenic differentiation, decreased chondrogenic differentiation while leaving adipogenic unperturbed. Moreover, siSPRY4 treatment in hASCs enhanced in vivo tissue regeneration as indicated by the increased bone formation. Overall, our results suggest that SPRY4 is possibly a critical molecule in regulating osteogenic differentiation of hASC by enhancing ERK1/2 phosphorylation of the MAPK signaling pathway and promoting hypertrophy in chondrogenesis. Indeed, transient knockdown of siSPRY4 is a potent tool in regenerative medicine via the enhancement of osteogenic differentiation and bone formation in hASCs.

T-1018

SUBSTANCE-P, AS AN INJURY-INDUCIBLE MESSENGER, IDENTIFIES ENDOGENOUS HEALING MECHANISM RECALLING BONE MARROW STEM CELLS TO BE ENGAGED IN THE TISSUE REPAIR

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Hyun Sook Hong - *Kyung Hee University, Seoul, Korea*

Tissue injury may create a specific microenvironment, which brings up the systemic participation of reparative stem cells in the repair process. Previously, we identified a new role of substance-P (SP) as an injury-inducible messenger to mobilize bone marrow stromal cells, namely mesenchymal stem cells (BMSC or MSC) from the marrow to the blood, home to the injured tissue, and be engaged in the tissue repair in the alkali-burn corneal

injury model. This elucidates endogenous healing mechanism recalling BMSC to the wound site. In addition to SP's BMSC mobilizer function, SP also mobilize endothelial precursor cells (EPC) from the bone marrow to the peripheral blood. We explored SP's dual positive roles in the orchestration of the tissue repair in a variety of the animal models such as the spinal cord injury, acute myocardiac infarction, stroke, radiation-induced BM injury and gastrointestinal injury. At the early stage of wound healing, anti-inflammatory effect of SP seems to play positive roles in the reduction of inflammation-induced secondary cell death and the creation of favorable microenvironment for the engraftment of incoming stem cells. At later stage, endogenous stem cells such as BMSC and EPC mobilized by SP finally participate in the tissue repair as reparative stem cells.

Funding Source: This work was supported by NRF2016M3A9B4917320 and HI13C1479

MUSCULOSKELETAL TISSUE

T-1020

EX VIVO POLARIZED MONOCYTE/ MACROPHAGES SHOW DIFFERENTIAL EFFECTS WITHIN HUMAN JOINT EXPLANTS OF LATE STAGE OSTEOARTHRITIS

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Monocytes/macrophages (Mϕs, as a heterogenous population) are immune cells that promote inflammation and subsequent tissue regeneration in wound repair and infection. Dysregulation of Mϕ balance between inflammatory versus homeostatic subtypes is found in many degenerative diseases. Knee osteoarthritis (KOA) is a disease of cartilage degradation and joint inflammation mediated by significant peripheral Mϕ infiltration. Ex vivo Mϕs polarized into regenerative phenotypes have been studied pre-clinically and clinically as therapeutic agents in liver cirrhosis, stroke, and renal transplant. We aim to investigate the effects of ex vivo polarized human Mϕs within a human joint model as a proof-of-concept towards developing Mϕ-based therapies for KOA. We optimized a human explant culture of cartilage and synovium tissue from end-stage KOA total knee replacements to simulate an arthritic, human model to study interactions with human Mϕs. Inflammatory or homeostatic CD14+ Mϕs (isolated from healthy peripheral blood and ex vivo polarized using established cytokine protocols or co-cultured with mesenchymal stromal cells) are co-cultured with the KOA joint explants. Cartilage and synovial tissue and conditioned medium (CM) harvested at day 2 and day 7 provide readouts on Mϕ-mediated modulation of inflammation (cytokine/chemokine gene expression in tissue by qRT-PCR, immunoassay in CM) and cartilage degradation (proteoglycan loss by dimethylmethylene blue in CM, safranin-O histology; protease inhibitor levels by immunoassay in CM.) Our results show that inflammatory Mϕs upregulate catabolic extracellular matrix (ECM), pro-inflammatory, chemotactic genes and simultaneously downregulate anabolic ECM genes in cartilage; conversely, homeostatic Mϕs upregulate anabolic ECM gene expression (N=5, >2-fold change). Inflammatory Mϕs decrease, while homeostatic Mϕs increase the secreted levels of protease inhibitors in CM (N=3). Importantly, ex vivo polarized Mϕs, especially homeostatic Mϕs, maintained their phenotype when exposed to OA synovial fluid, and co-cultured with OA joints, and did not revert to an inflammatory functionality. Additional studies in a dynamic in vivo murine OA animal joint will confirm our in vitro observations and lend credence to developing Mϕ-based therapies for OA.

Funding Source: The Arthritis Society, University Health Network Arthritis Program Seed Grant, Krembil Foundation Grant, Mount Sinai Fellowship, Ontario Graduate Scholarship Program, Institute of Biomaterials and Biomedical Engineering

T-1022

HOPX: A KEY FACTOR IN HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELL PROLIFERATION AND ADIPOGENIC DIFFERENTIATION

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Ageing, osteopenia and osteoporosis have been the major causes for poor bone health worldwide. Current treatments including usage of drugs, reduce the symptoms of bone loss such as fractures, but do not compensate for the loss of bone completely. To develop new therapeutic strategies for these bone issues, the basic molecular mechanisms of how bone cells reproduce and make bone needed to be explored. Bone cells (osteoblasts) are derived from bone-marrow derived mesenchymal stromal cells (BMSC), which is also the precursors for fat cells (adipocytes) and cartilage cells (chondrocytes) in the marrow. Therefore, this study aims to study the underlying molecular mechanisms and cues for the mesenchymal stromal cells to either reproduce or diverge into other lineages. The bHLH transcription factor, TWIST-1 was found to

be highly expressed by BMSC and plays an important role in BMSC self-renewal and differentiation. However, many of the underlying mechanisms mediating TWIST-1 regulation of BMSC growth and differentiation still remain poorly understood. In order to identify novel TWIST-1 gene targets involved in BMSC proliferation and osteogenic differentiation, microarray analysis was performed to compare the gene expression profile of BMSC which express either endogenous or enforced expression of TWIST-1 during growth culture condition or undergoing osteogenic differentiation. One novel differentially expressed gene was HOPX. HOPX encodes for a homeodomain-only protein homeobox, important in cardiogenesis. Currently, no known function of HOPX has been identified during BMSC growth or differentiation. We aim to determine whether HOPX is a novel target of TWIST-1 in BMSC and thus possibly be involved in mediating the effects of TWIST-1 on cell proliferation and fate determination. Our data demonstrates that TWIST-1 negatively regulates HOPX expression in BMSC. Moreover, reduced HOPX gene expression in BMSC lead to an increase in the adipogenic potential of these cells and decreased proliferation rate. Thus, our results suggest that HOPX could have an important function in BMSC proliferation and adipogenic differentiation. Further studies would help understand the pathways involved in these processes and therefore facilitate the invention of new therapeutic strategies for the abovementioned bone issues.

T-1024

NEGATIVE REGULATION OF SMOOTH MUSCLE GENE EXPRESSION BY H19 LONG NON-CODING RNA IN HUMAN MESENCHYMAL STEM CELLS

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Loss or dysfunction of smooth muscle cell (SMC) affects multiple tissues and organs in the body. Thus, cells and technologies capable of generating functional SMCs are of great interest. In this study, we continue our effort to investigate new molecular regulators of smooth muscle genes during differentiation of SMCs derived from mesenchymal stem cell (MSC). Long non-coding RNAs (lncRNAs) have been recognized by their diverse functional characteristics in several disorders including cardiovascular diseases. We recently identified several lncRNAs involved in TGFbeta1-induced differentiation of human MSCs into SMCs. To further characterize the role of SMC-related lncRNAs, H19 has been selected as the first target to investigate the novel mechanism involved in modulating the differentiation and phenotypic switch of SMCs. Up-regulation of H19 expression was observed at 48 hours after TGFbeta1 treatment of human MSCs, which co-expressed SMC-specific genes. Knockdown of H19 expression by synthetic small interfering RNA (siRNA) oligos in turn down-regulated KLF4 mRNA expression and enhanced the expression of alpha-SMA, calponin and myocardin on both days 2 and 7. The comprehensive H19-mediated lncRNA-mRNA network has also been established during SMC differentiation using RNA-sequencing (Illumina HiSeq X Ten). Among identified transcripts, a novel lncRNA named lncRNA_000093 (1418 bp) has been confirmed to be up-regulated post H19-siRNA treatment. LncRNA_000093 may act as a competing endogenous RNA via putative binding sites of miR-34 and/or miR-449 on myocardin 3'UTR (analysed in silico by TargetScanHuman 7.1). The potential responsive elements between H19 and lncRNA_000093 are under investigation using CRISPR-Cas9-induced endogenous gene cleavage with two H19 single guide RNAs. These data indicate the novel role of H19 as a negative regulator in myocardin-mediated transcriptional regulation of smooth muscle genes.

T-1026

MKX AND SCX PROTECT TENDONS FROM FIBROSIS THROUGH DKK3 INTERACTION

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Tendons connect muscles to bone and transmit the force generated by muscles to the skeleton. They are highly prone to injury and overuse. Replacement of adult damaged tissues by fibrotic scars is a consequence of this limited regenerative capacity, leading to impaired biomechanical properties. Scleraxis (Scx) and Mohawk (Mkx) emerged the last decade as pivotal actors in tenogenisis and tendon healing. Still, the precise cellular and molecular mechanisms played by these transcription factors remain poorly understood. Transcriptomic analysis of Scx and Mkx Ko mice tendons revealed several categories related to muscle structure and development as the most enriched GO terms. Further investigation using qRT-PCR and western blot showed the up-regulation of MyoD in the KO mice. In-vitro characterization of tendon stem progenitor cells over-expressing MyoD showed a switch into a myofibroblastic phenotype instead of a skeletal muscle lineage transition. Analysis of neonatal tendon injury model brought in-vivo evidence of Scx and Mkx roles in fibrosis. This role is in partly mediated by Dkk3, a candidate selected from the Scx and Mkx common protein interactome as revealed

BIFC assay. CRISPR Cas9 knock-out of this gene led to the up-regulation of collagen III (Col-III) and α-smooth muscle actin (α-SMA) opening potential therapeutic avenues to treat tendinopathy.

T-1028

POLARIZATION OF THE INFLAMMATORY RESPONSE BY ADIPOSE TISSUE DERIVED STROMAL CELLS IN MINIPIGS IRRADIATED MUSCLES INJURIES

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High local dose irradiation of the skin leads to a specific syndrome, the cutaneous radiation syndrome composed of several symptoms such as poor revascularization, extensive inflammation of skin and underlying muscles and fibrosis. Subcutaneous injections of autologous adipose tissue derived stromal cells (ADSC) have previously shown favorable effects on skin wound healing in a minipig model of gamma acute local irradiation (50Gy). However a persistent muscle fibrosis remained, being a consequence of radio-induced muscles inflammation. Thus, based on the pro-myogenic and immunomodulation potential of ADSC, a new protocol combining subcutaneous and intra-muscular injections of ADSC has being evaluated. Six minipigs were locally irradiated (gamma ray; 50 Gy) and three controls animals received the vehicle (phosphate-buffer-saline) whereas three animals received 3 injections of 75x106 ADSC (intra-muscular and subcutaneous injections). The polarization of the inflammatory response was evaluated by CD80, CD68 and C206 immunostaining as well as IL1-6-10 and TGF-beta detection (western-blot and in situ hybridization) and the regeneration pathway of irradiated muscle were analyzed by SCA1, Pax7 and Myf5 detection (western-blot) 76 days after irradiation. Muscle regeneration pathway activation and macrophage (M2) polarization of the inflammatory response after ADSC intramuscular injections were highlighted in opposition to a prominent fibrosis observed without inflammatory response anymore in PBS group. To conclude, after local irradiation, intramuscular injections of ADSC favour biological processes involved in muscle regeneration especially inflammatory response. Work is on-going to further investigate this key process and to prevent subcutaneous muscles fibrosis in cutaneous radiation syndrome.

CARDIAC TISSUE AND DISEASE

T-1030

SINGLE-CELL TRANSCRIPTOME ANALYSIS OF EARLY MOUSE CARDIOGENESIS AND PERTURBATION UPON HAND2 LOSS

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Sub-specification of cardiac progenitor cells (CPCs) to distinct lineages is a highly dynamic, asynchronous process that is challenging to interrogate with bulk tissue modalities. In this work, we compiled a high-resolution transcriptome framework of CPC specification events at mouse embryonic day (E)7.75, E8.25 and E9.25 using single-cell RNA sequencing. We leveraged advances in droplet-based microfluidics to acquire ~18,000 transcriptomes, which enabled us to capture and computationally identify diverse and rare cardiac subtypes. Our analysis identified unexpectedly extensive subtype specification of cardiac progenitors as early as E7.75 and revealed their progressive differentiation paths. We harnessed this framework to investigate the consequences of genetic perturbation on individual cells during development, using embryos lacking the essential transcription factor, Hand2. While most progenitor populations were similar in the Hand2 mutant compared to wild type, the anterior heart field (AHF) progenitor compartment and its descendent cells fated to be outflow tract (OFT) and right ventricle (RV) were transcriptionally dysregulated as early as E7.75. Mutant AHF cells showed dysregulation in retinoic acid signaling, which regulates anterior/posterior patterning of cardiac progenitors. Ordering the OFT/RV progenitors in pseudotime revealed that Hand2 loss resulted in delayed and disrupted differentiation of outflow tract-fated cells, whereas right-ventricle cells were appropriately specified but failed to migrate and form the right-ventricle chamber. Importantly, these mutant populations upregulated Upp1, a newly identified marker of posterior second heart field derivatives such as the atria and sinus venosus, suggesting that the observed differentiation and migration defects are due to a fate

switch to a more posterior identity. This work provides unprecedented insight into the initial specification of cardiac progenitor cells during development and reveals mechanisms of disrupted cardiac development at the single-cell resolution, answering long-standing questions of cardiogenic regulatory networks. Moreover, this study presents an approach for dissecting the effects of genetic perturbation in distinct progenitor compartments in any complex, developing tissue.

T-1034

LONG-TERM ELECTROPHYSIOLOGICAL MATURATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTES

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The comprehensive in vitro proarrhythmia assay (CiPA) initiative recommends the use of multi-electrode array (MEA) technology and human iPSC derived cardiomyocytes (hiPSC-CMs) for preclinical screening of arrhythmia risk. Currently, the gold standard for iPSC-CMs functional studies is primarily conducted around 30 day post-differentiation. Here we show that while cardiac marker expression is robust at day 30, the electrical maturity of hiPSC-CMs recorded via micro-gold MEA system is limited. Human cardiac fibroblasts derived iPSCs were optimized for 90% cardiomyocyte production using the feeder free monolayer protocol. Beating cultures at day 30 post-differentiation expressed cardiac makers namely, α-actinin, connexin43 (Cx43) and troponin I (Trop I) with higher sarcomere and gap-junction organization when compared to day 10. Furthermore, the kinetics of calcium transients of day 30 hiPSC-CMs was consistent with an embryonic/neonatal phenotype. Asynchronously beating constructs at 32 days post- differentiation showed field potentials (FP) activity in 63329% of the electrodes. As the constructs matured their percentage increased to 9039% and 95311%, at day 34 and 37, respectively. Fourty day old preparations attained syncytium showing 100% activation with average beating frequencies ranging between 20-32 bpm with a ~10 fold increase in average peak-to-peak amplitude (5.53 1 mV). The average peak-to-peak amplitude of 32, 34 and 37 day old constructs was 1233 28 μV, 1593 21 μV and 4643 82 μV, respectively. Additionally, T-wave peaks were predominant in 40 day old constructs. The average FP duration and RR interval across the array was 66533 ms and 263235 ms, respectively. Overall, synchronous beating frequency, increase in FP amplitude, and defined T-wave observed for 40 day old constructs demonstrate electrophysiological stability and maturity. The long-term electrophysiological recordings had no apparent detrimental effect on the health of the constructs when examined for Cx43 and Trop I localization. Overall, our study highlights the electrical plasticity of hiPSC-CMs over time and emphasizes the significance of the electrophysiological maturation state for drug screening and functional studies.

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T-1036

EFFICIENT CARDIAC REGENERATION THERAPY BY CELL CYCLE ACTIVATION OF IPSC-DERIVED CARDIOMYOCYTES

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Cardiac replacement therapy using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) is a promising strategy to cure heart diseases. We have previously reported that engrafted iPSC-CMs proliferate for three months after direct injection into the myocardium of immunodeficiency mouse hearts with acute left anterior descending artery (LAD) ligation. Here we analyzed the cell cycle phase of iPSC-CMs with the aim of enhancing engraftment efficiency after transplantation. We established an iPSC line constitutively expressing Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI), which allows visualization of the cell cycle phase: Cells in a state of S/G2/M phase (proliferating stage) emit green fluorescence, cells in a state of G0/G1 phase (non-proliferating stage) red fluorescence. Next, we compared the engraftment ability between S/G2/M phase iPSC-CMs and G0/G1 phase iPSC-CMs, three months after transplantation. Cell cycle activated (S/G2/M phase) iPSC-CMs showed significantly higher engraftment efficiency than those in inactivated (G0/G1 phase). High-throughput screening of drugs for their ability to activate the cell cycle in iPSC-CMs was performed to elucidate the function of the cell cycle and engraftment efficiency. Out of 4032 initially screened compounds 82 candidates emerged, which promote S/G2/M phase iPSC-CMs. Second screening identified a group of promising compounds showing i.e. an increase in iPSC-CMs in S/G2/M phase from 2.8% to 39.8% via flow cytometry analysis. By EdU assay we could observe enhanced DNA synthesis in those iPSC-CMs implying an increased proliferation ability. In accordance, cell number was increased upon cell cycle activating drug treatment. In vivo, those drug-treated iPSC-CMs showed enhanced graft size after transplantation into mice ischemic heart. Altogether, these data demonstrate that cell cycle plays a key role in the effectiveness of cardiac cell therapy using iPSC-CMs, and its modification might lead to novel therapeutic treatments.

T-1038

EXPRESSION OF TELOMERASE IN HUMAN CARDIAC MESENCHYMAL STEM CELLS ENHANCES CARDIAC REGENERATION AFTER MYOCARDIAL INFARCTION

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We recently characterized a sub-population of human cardiac fibroblasts co-expressing Platelet Derived Growth Factor Receptor-Alpha (PDGFRα)+ and CD90+ (Rα+cMSCs) and have demonstrated their multipotent progenitor potential in addition to showing their cell number and function decreases with age/disease. A deeper understanding of this progenitor population could forge novel therapeutic strategies to repair/regenerate the injured heart. This study aims to test human telomerase reverse transcriptase (hTERT) over-expression for enhancement of Rα+cMSC regenerative capabilities. We isolated human Rα+cMSCs from non-diseased young (2-10yrs), old (54-64yrs) and diseased (54-64yrs) left ventricular (LV) samples (N=4/group), purifying by fluorescence-activated cell sorting. Lentiviral-mediated over-expression of hTERT in Rα+cMSCs induced telomerase activity and increased telomere length. In growth curve analysis, hTERT over-expression rescued Rα+cMSC age-related decline and extended self-renewing capacity from >3 to >8 months. Compared to Rα+cMSCs, hTERT+Rα+cMSCs possessed greater cell cycle activity assessed by Ki67+ and BrdU+ (both, 2-fold) and showed less starvation-induced apoptosis in young and old hearts (AnnexinV+, 50% & 60%, respectively). hTERT+Rα+cMSCs had significantly enhanced endothelial cell (CD31+, 7738% vs 4836%; $P<0.05$) and cardiomyocyte (cTnT+, 1834% vs 831%; $P<0.05$) differentiation compared to Rα+cMSCs. RNA sequencing identified increased cell differentiation, proliferation, survival and angiogenesis related genes in hTERT+Rα+cMSCs. Transplantation of 5x10^6 hTERT+Rα+cMSCs in athymic-rats (N=10/group) four weeks after induced myocardial infarction (MI) significantly increased LV ejection fraction (LVEF, 3232%) and reduced scar area (1431%) compared to Rα+cMSCs (LVEF, 2632%, $P=0.06$; scar, 1732%, $P=0.27$) or vehicle controls (LVEF, 2532%; scar, 2032%; $P<0.05$). Moreover, hTERT+Rα+cMSCs treatment results in decreased myofibroblast activation, increased proliferation of both cardiomyocyte and interstitial cell fractions and increased angiogenesis. In conclusion, hTERT over-expression rejuvenates Rα+cMSCs from aged and diseased hearts. Transplantation of hTERT+Rα+cMSCs is a novel therapeutic strategy to enhance cardiac function after MI.

T-1040

THE POSITIVE FORCE-FREQUENCY RELATIONSHIP IN STEM CELL BASED ENGINEERED HUMAN MYOCARDIUM IS RELATED TO METABOLIC MATURATION

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Non-failing adult myocardium exhibits a positive force-frequency relationship (FFR). To our knowledge, engineered human myocardium (EHM) generated from human embryonic (hESC) or induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CM) and

fibroblasts interspersed within a collagen hydrogel represent the only engineered human heart muscle model with a near-physiological positive FFR. Here, we asked whether electromechanical conditioning would further advance functional maturity of EHM and, if so, whether transcriptome analyses would provide insight into the underlying mechanisms. EHM were exposed to mechanical preload, and either no electrical conditioning (control) or chronic, 3 Hz field stimulation (2 V/cm, 5 ms duration) for 32 days. Electromechanically conditioned hESC-EHM and hiPSC-EHM generated a more positive FFR at physiologically-relevant field stimulation frequencies between 1-3 Hz, a marked right shift in [Ca2+]EC50 (hiPSC-EHM: 0.7530.03 vs. 1.3330.04 mM, $p<0.001$; hESC-EHM: 0.6730.07 vs. 1.3430.07 mM, $p<0.001$), and decreased spontaneous beating (hiPSC-EHM: 5538 vs. 1833 bpm, $p<0.05$; hESC-EHM: 5334 vs. 1836 bpm, $p<0.001$); all being features of advanced maturation. RNA sequencing of hESC-EHM and human adult heart ventricle (n=3/group) suggested significant modulation of mitochondrial metabolism in response to electromechanical stimulation. In particular, expression of nuclear genes encoding subunits of mitochondrial complex I and III correlated highly with transcriptome patterns of the adult heart. This finding was further validated by pharmacological inhibition of complex I with rotenone (0.1 nM to 1 microM), revealing a concentration-dependent blunting of the positive FFR. In summary, electromechanical conditioning further enhances functional maturation of EHM. The development of a hallmark feature of healthy adult myocardium, the positive FFR, depends in the maturation of respiratory chain function.

T-1042

GLOBAL TRENDS OF CLINICAL TRIALS FOR HEART FAILURE BY CELL THERAPY

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Definitive treatment of heart failure is an important thesis of regenerative medicine in the cardiovascular field. An autogenous bone marrow-derived product from Korea and an autologous myoblasts-derived product from Japan have been approved by corresponding national authorities and available in the market so far. In this study, we examined the clinical research trends related to cell therapy products in the heart failure field based on data obtained from the ClinicalTrial.gov website. Although this website does not provide comprehensive results of clinical trials, it offers information on prospective clinical trials, including work in progress, and thus allows chronological analysis of the data. We selected 210 studies for cell therapy related to the field of heart failure treatment from ClinicalTrial.gov. The United States, which manages the ClinicalTrials.gov, had about half of them, ranking the top of the countries, followed by China and Germany. Classifying with the cell source used, the studies used bone marrow-based cells occupied one third of the whole. Although the use of bone marrow as the cell source had been initially predominant, the use of cardiomyocyte and adipose tissue increased since 2009 and 2011 respectively, conversely the use of bone marrow decreased. Classifying with the origin (autologous or allogeneic) of cell source used, the studies used autologous cells occupied more than 70% of the whole. But, since 2014 the allogeneic cells have been actively utilized. Thus, the results showed a shift in the clinical translational trend in cells used in such research from bone marrow to other cell sources and from autologous to allogeneic cells occurred in this period. There were 14 studies advanced to Phase III, most of which used autologous bone marrow. It is inferred that all of them would have shown certain effectiveness in previous early phases. So, we speculated that other cell sources or other households are being examined in new studies, due to difficulties derived from donor cell variability, for example. The use of ClinicalTrials.gov as the sole data source can yield a perspective view of the global clinical translational trends.

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T-1044

CULTURE-FREE ON-SITE IMPLANTATION OF ALLOGENEIC ADIPOSE-DERIVED HUMAN MESENCHYMAL STEM CELL SPRAY BOOSTS CONVENIENCE OF REGENERATIVE THERAPY IN CARDIOVASCULAR DISEASE

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Although remarkable progress has been made in myocardial regeneration therapy, how to deliver cells to damaged heart less invasively and more effectively without cardiac tissue destruction may be crucial in the cellular therapy for heart failure. We hypothesized that culture-free on-site implantation of allogeneic Adipose-Derived Human Mesenchymal Stem Cell (hADSC) using surgical fibrin glue spray might have impact on the recovery of cardiac function in porcine

ischemic cardiomyopathy. Most of the hADSC isolated from human fat tissue expressed CD73 and CD90, and were negative for CD31 and CD45, as measured by flow cytometry. In graft preparation, fibrin and thrombin solution were prepared using a Manufactured Combi-Set. ADSC (1x108/graft) were mix with fibrin solution before implantation, and then they were sprayed over the infarct area of the myocardium to deliver cells. ELISA analysis revealed that ADSC graft secreted various cytokines such as VEGF and HGF in vitro. We induced myocardial infarction in porcine model and divided into the ADSC-transplanted groups (A group, n=6) and the control (C group, n=6). At 4 weeks after the transplantation, US revealed that A group had significantly greater ejection fraction compared with the C group (54.931.5, and 41.032.6%, P<0.05 respectively) with improvement longitudinal strain and LV reverse remodeling assessed by MRI. NH3 positron-emission tomography depicted that coronary flow reserves were 1.5 to 2 times greater in the A group. Histological analysis revealed that the graft had survived for at least four weeks and that the significant reduction in hypertrophy, interstitial fibrosis, and higher vascular density in the A group. The mRNA levels of VEGF, b-FGF and SDF-1 in infarct border and remote area were significantly higher in group A. Culture-free on-site implantation of allogeneic ADSC by using fibrin glue spray method could easily deliver allogenic cells to epicardium with adequate functional and histological recovery, suggesting that this new cell delivery method have some impacts on cell-based therapy in clinical scenario.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

T-1046

HYPOXIA-DEPENDENT MITOCHONDRIAL FISSION REGULATES ENDOTHELIAL PROGENITOR CELL MIGRATION, INVASION, AND TUBE FORMATION

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Tumors undergo uncontrolled, excessive proliferation, leading to a hypoxic microenvironment. To fulfill their demands for nutrients and oxygen, tumor angiogenesis is required. Endothelial progenitor cells (EPCs) are the main source of angiogenesis because of their potential to differentiate into endothelial cells. Therefore, understanding the mechanism of EPC-mediated angiogenesis in hypoxia is critical for the development of cancer therapy. Recently, mitochondrial dynamics has emerged as a critical mechanism for cellular function and differentiation under hypoxic conditions. However, the role of mitochondrial dynamics in hypoxia-induced angiogenesis remains unclear. In this study, we demonstrated that hypoxia-induced mitochondrial fission accelerates EPC bioactivities. We first investigated the effect of hypoxia on EPC-mediated angiogenesis. Cell migration, invasion, and tube formation were significantly increased under hypoxic conditions; expression of EPC surface markers was unchanged. Mitochondrial fission was induced by hypoxia in a time-dependent manner. We found that hypoxia-induced mitochondrial fission was triggered by DRP1; specifically, phosphorylated DRP1 at Ser637, a suppression marker for mitochondrial fission, was impaired in a hypoxia time-dependent manner. To confirm the role of DRP1 in EPC-mediated angiogenesis, we analyzed cell bioactivities using Mdivi-1, a selective DRP1 inhibitor, and DRP1 siRNA. DRP1 silencing or Mdivi-1 treatment dramatically reduced cell migration, invasion, and tube formation in EPCs, but the expression of EPC surface markers was unchanged. In conclusion, we identified a novel role of mitochondrial fission in hypoxia-induced angiogenesis. Therefore, specific modulation of DRP1-mediated mitochondrial dynamics is a potential therapeutic strategy for EPC-mediated tumor angiogenesis.

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T-1048

TRANSDIFFERENTIATION OF FIBROBLASTS TO ENDOTHELIAL CELLS IN VIVO

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We discovered that optimal activation of innate immunity triggers changes in the expression and activity of epigenetic plasticity that is required for reprogramming, a phenomenon which we term transflammation. More recently we have shown that innate immune signaling is also required for transdifferentiation of fibroblasts into endothelial cells (iECs) in vitro. Whether such transdifferentiation occurs in vivo in response to ischemia is controversial. Here we assessed this question in a murine model of peripheral arterial disease. Using fibroblast lineage tracing mice (Fsp1-Cre: R26R-EYFP) and quantitative FACS analysis, we found that fibroblast derived endothelial cells (YFP+CD144+) started to appear in the ischemic hindlimb as early as 7 days after femoral artery ligation, and increased up to 28 days when the blood perfusion is recovered to 80% of normal level. FACS Sorted YFP+CD144+ cells have similar endothelial specific gene expression as endogenous YFP-CD144+ cells, whereas fibroblast specific gene

expression is downregulated compared to YFP+CD144- cells. Importantly, injection of dexamethasone, an innate immune inhibitor, blocked this transdifferentiation, hindered angiogenesis and impaired perfusion as examined by FACS, immunostaining and Laser Doppler. Our results suggest that transdifferentiation of fibroblasts to endothelial cells contributes to angiogenesis in hindlimb ischemia and is mediated through innate immune signaling. We observe that resident Fsp1+ cells represent a pool of cells that can undergo transdifferentiation toward endothelial phenotype to enhance vascularity, improve perfusion and repair ischemic tissues.

HEMATOPOIESIS/IMMUNOLOGY

T-1050

OBLIGATE ROLE FOR THE SERINE/THREONINE KINASES, ROCK1 AND ROCK2, IN HEMATOPOIETIC STEM CELL RENEWAL AND MULTI-LINEAGE BLOOD CELL GENERATION

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Hematopoietic stem cells (HSC) continuously undergo cycles of proliferation and renewal throughout human life. This regeneration is essential for the maintenance of the blood cell lineages. Mutations that alter these processes can cause primary immunodeficiencies and malignancies. The Rho-family GTPases are critical to regulating the proliferative capacity of HSC by modulating the actin cytoskeleton. As seen with conditional deletion of *RhoA*, perturbations to the cytoskeletal reorganization required for successful mitosis lead to cell cycle arrest and cell death. The serine/threonine kinases, Rock1 and Rock2 are direct mediators of RhoA responses. However, parallel studies elucidating a role for these enzymes in HSC function in vivo have not been performed. To address this question we used conditional gene targeting to delete both the Rock1 and Rock2 alleles in adult mice. $Rock1^{flox/flox}$, $Rock2^{flox/flox}$, or $Rock1^{flox/flox}$: $Rock2^{flox/flox}$ mice were crossed to the Rosa26-Cre ERT2 line to globally delete the floxed alleles with tamoxifen. Loss of either allele had no impact on hematopoiesis, suggesting functional redundancy between the two kinases for sustaining this homeostatic process. In contrast, deletion of both genes caused severe cytopenia and an absence of all mature leukocyte subsets. Likewise, lymphoid organs displayed abnormal architecture, reflecting the low cellularity. Examination of the bone marrow compartment revealed a dramatic reduction in the frequency and number of the $Lin^{-}Sca1^{+}c\text{-}kit^{+}$ (LSK) progenitor population. Adoptive transfer of the few LSK cells recovered from the double conditional knockout mice failed to generate leukocytes when transferred to congenic donors, suggesting the defect was cell intrinsic. Likewise mutant bone marrow could not reconstitute irradiated wild-type hosts. Mixed bone marrow chimeras revealed wild-type cells had a survival advantage to those lacking Rock1 and Rock2. Cellular analysis revealed deletion of both Rock1 and Rock2 led to mitotic arrest of LSK cells. Akin to the $RhoA^{-/-}$ phenotype, we observed an accumulation of cells in the S phase of cell cycle, indicative of impaired cytokinesis, and heightened apoptotic activity. Our data reveal a fundamental role for this RhoA-Rock pathway in sustaining HSC homeostatic proliferation and survival.

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T-1052

GASTRIC CANCER CELLS DERIVED EXOSOMES CAN AFFECT THE IMMUNOMODULATORY FUNCTION OF MSC THROUGH CONSTANT ACTIVATION OF NFKB BY PKM2

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Bone marrow or adipose-derived MSCs homing to tumor tissues are educated by tumor cells and become tumor MSCs (T-MSCs), which is an important part of the tumor immune microenvironment(TME). T-MSCs can secrete large numbers of inflammatory factors and increase the proportion of inhibitory immune cells, which plays an important role in tumor progression. In our previous study, we found that gastric cancer cells-derived exosomes(GC-exosomes) can activate the NF-κB signaling pathway of naïve MSCs, and G-exosomes treated MSCs(GC-exosomes-MSCs) had the similar function of T-MSCs. GO analysis found that the proteins in tumor exosomes were mainly associated with anaerobic metabolism. Therefore, we plan to investigate whether GC-exosomes can activate the NF-κB signaling pathway through changing the metabolic phenotype of MSCs, and further transform the immunomodulatory function of MSCs. The tumor exosomes were isolated and purified by ultrafiltration. The effect of GC-exosomes on the metabolic phenotype of MSC was detected by Agilent Seahorse XF Cell Energy Phenotype Test. T-exosomes-MSCs could then up-regulate the expression of IL-6, IL-8, CCR2, inhibit the proliferation of T cells, promote the activation of $CD4^{+}$ T cells, and regulate the homing of macrophages. GC-exosomes couldswitch T-MSCs towards a more glycolytic phenotype (similar to Warburg effect in tumor cells).The expression of anaerobic metabolism-related protein the M2 isoform of pyruvate kinase(PKM2) and other enzymes were obviously up-regulated.PKM2 could then translocate to the nucleus of GC-exosome-MSCs(gradually increased from day 3). PKM2 could bind to the transcription factor p65 of NF-kB signaling pathway and constantly promote the activation of NF-kBsignaling pathways. Our study suggests that in MSCs gastric cancer cells derivedexosomes can promote

the expression and translocation to nucleus of PKM2, which binds to p65, and ultimately trigger the constant activation of NF-kB signaling pathway and transform the immunomodulatory function of MSCs.

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T-1054

DEVELOPMENT OF HEMATOMIR TECHNOLOGIES FOR EX VIVO HEMATOPOIETIC STEM CELL EXPANSION WITH AN INDUCED HDR GENE EXPRESSION

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Autologous hematopoietic stem cells (HSCs) transplantation could be used to treat many inherited hematopoietic disorders. However, it requires gene-edited HSCs to be expanded ex vivo with preferentially higher engraftment capability and higher rates of homology-directed-repair (HDR). Here, we report the development of HSC specific HematoMiR technologies that target HSC quiescence factors to achieve ex vivo HSC expansion and induction of HDR pathways. About 60 genes genes with loss of function in HSCs have been reported to have at least 1.5 fold expanded stem cell pool in vivo. We identified and designed HematoMiRs with eight unique seed sequences targeting majority of these 60 genes. Lineage negative cells enriched for HSCs from mouse bone marrow were isolated and treated with designed HematoMiRs. After 5 days of treatments, HSC count and HSC frequency, and gene expression were analyzed. We have found that HematoMiR-2 and HematoMiR-5 increased murine LSKCD34^{low} HSC population approximately 3 fold. In addition, HematoMiR-2 and HematoMiR-5 treatments led to robust downregulation of target HSC quiescence modulators including CDKIs. These were associated with the progression of HSC cell cycle from G_0 into G1/S phases of cell cycle; with an increased expression of HDR and S-phase associated genes. Besides, HematoMiR-5 treated HSCs demonstrated better engraftment into immunocomprimized animals. Furthermore, HematoMiR-5 treatment increased human HSC expansion as determined by analysis of CD34+, CD90+, CD133+ populations by flow cytometry. Overall, these studies suggest that HematoMiR-5 treatment could be utilized in procedures involving HDR mediated gene editing and ex vivo HSC expansion by targeting quiescence factors.

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T-1056

METTL3-MEDIATED N6-METHYLADENOSINE IS REQUIRED FOR MURINE HEMATOPOIETIC STEM CELL DIFFERENTIATION IN VIVO

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Hematopoietic stem cells (HSCs) are a rare population of cells characterized by their ability for life-long self-renewal and lineage-specific differentiation to all types of hematopoietic cells. HSC function is regulated by complex cell-intrinsic and cell-extrinsic pathways, but the mechanisms that control HSC differentiation and self-renewal are not fully understood. For the first time in vivo, we show that RNA methyltransferase METTL3, the required catalytic enzyme for N6-methyladenosine (m6A) RNA modification, regulates the balance between HSC differentiation and self-renewal. We conditionally deleted METTL3 in adult HSCs in vivo and found that loss of METTL3 disrupted hematopoiesis and expanded the HSC pool, without impacting HSC quiescence. METTL3-depleted HSCs are severely functionally compromised, both in colony-forming potential and long-term multi-lineage competitive reconstitution activity. Analysis of HSCs after transplantation revealed that their primary functional defect stemmed from poor differentiation. Furthermore, we found that in vivo deletion of METTL3 from myeloid cells did not cause a functional defect, suggesting a preferential role for METTL3 in regulating HSC activity, rather than general hematopoietic cell maintenance. Using a novel protocol for low cell number methylated RNA-Seq analysis, we were able to characterize the m6A methylome in HSCs with as few as 2,000 cells. This analysis revealed thousands of m6A mRNA targets including key HSC regulators, such as cMyc. Unexpectedly, RNA-Seq analysis of METTL3-depleted HSCs revealed almost no differentially expressed genes, but significant alterations of relative abundance of isoforms of many alternatively spliced genes. This suggests that m6A in HSCs primarily impacts target alternative splicing and translation, rather than transcription efficiency or transcript stability. All together, we present the first in vivo evidence that METTL3-mediated m6A methylation is specifically required for HSC differentiation and serves as a previously unexplored epitranscriptomic mechanism for regulating HSC function.

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T-1058

ASSESSMENT OF IMMORTALIZED ADULT HUMAN ERYTHROID CELL LINES PROPOSED FOR THERAPEUTIC USE

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Global demand for blood transfusion has led to investigation of in vitro red blood cell (RBC) generation as a solution. Following the first generation of immortalised adult human erythroid cell (BEL-A) lines, we have investigated the clinical potential of such lines. Produced from adult CD34+ cells using lentiviral doxycycline (DOX)-inducible HPV16-E6/E7, such lines are erythroblastic and undergo normal maturation to enucleated reticulocytes (10-35% at d14), with no change in performance over extended culture of the line. Characterisation of BEL-A2 showed them to be similar to in vitro reticulocytes from primary CD34+ cells and this approach was proposed for in vitro production of RBC for transfusion. We performed additional characterisation of E6/E7 immortalised lines in order to assess the risk for therapeutic use. Two lines, BEL-A2 and SC7P1, were differentiated for 14d, phenotyped by flow cytometry and residual proliferative capacity analysed to assess their potential tumorigenicity. After 14d differentiation, cells were replaced into expansion media (+DOX) for an additional 3-14d, there was no evidence of any proliferation, the majority of cells were dead by d14+3. In CFU assays no growth was observed from >250,000 cells/condition. However, analysis of CD36, CD235a, Integrin α4 and Band 3 showed that at d14 the phenotype of ffi1% (0.2 to 5%) of differentiated cells overlaps with that of the undifferentiated cells. Leukofiltration gives a 4-log depletion of nucleated cells, thus a 'unit' of 1012 RBC could contain 106 nucleated cells that cannot be phenotypically distinguished from proliferating, undifferentiated cells. Genetic analysis of E6/E7 immortalised cell lines (G-banding/SNPs) from multiple time points during derivation showed abnormal karyotypes with multiple trisomies. Abnormalities seen in the polyclonal population mainly arose during derivation of the line, but changes continued to evolve during maintenance making the populations unpredictable. The RBC product would be enucleated but genetic instability of the immortalised lines and potential for residual nucleated cells in the filtered product carries significant risk, so this approach requires further technical development before it is suitable for generation of clinical RBCs, however these lines are a very valuable research tool.

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T-1060

T-CELLS EMERGE FROM AGM-LIKE VASCULAR ORGANOIDS DURING IN VITRO DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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The process of T-cell generation in the adult haematopoietic system initiates with the migration of bone marrow derived hematopoietic precursors to the thymus. By contrast, the generation of T-cells during early development is less well understood. We recently described a hematopoietic differentiation system that generates aorta-gonad-mesonephros-like hematopoietic precursors from pluripotent stem cells. In this system, embryoid bodies transferred to adherent culture at day 7 form complex organoid-like structures containing a mixture of stromal cells, endothelium and haematopoietic cells. Our analysis of these organoids revealed robust expression of NOTCH ligands on endothelial and stromal populations, providing an environment permissive for the endothelial-hematopoietic transition that initiates definitive blood cell development. Given the key role of NOTCH signalling in directing T-lymphocyte differentiation, we hypothesized that these developing haemogenic organoids might also support T-cell development without the requirement for exogenous mouse stromal layers or exogenously added DLL ligands. Indeed, culturing day 8 organoids in medium supplemented with IL7 permits the emergence of cells with T-cell lineage characteristics. Imaging analysis indicates that T-cell progenitors develop within a CD31+ vascular lumen whilst time lapse photography shows the movement of these early lymphoid cells along these structures. Flow cytometry analysis shows that the earliest T-cell precursors are CD90+CD144(vascular endothelial cadherin)+. These differentiate into CD5+CD7+ Pre-T-cells by day 24, and progress to become CD4+CD8+ by day 35. Single cell and bulk RNA sequencing has been used to confirm the identity of these early T-cell progenitors and to examine

lineage relationships between emerging lymphoid cells. Importantly, preliminary experiments suggest that organoid derived T-progenitors can colonize the thymus and generate CD4+CD3+ and CD8+CD3+ cells. In conclusion, our system enables T-cell production in the absence of exogenous NOTCH1 ligands and without the need for purification of CD34+ progenitors by FACS. As such, our T-lymphopoietic organoids provide a scalable platform for generating T-cells for both research and for potential clinical applications.

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T-1062

METABOLIC PATHWAYS THAT MEDIATE EXPANSION AND HOMEOSTASIS OF HEMATOPOIETIC LINEAGES

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Due to the rarity of hematopoietic stem cells (HSCs), their differentiation into progenitors and then on to mature cells must occur with proliferation, leading to an expansion of each subsequent downstream population. This process, known as transit amplification, is required to produce a sufficient population of mature cells. Transit amplification, however, must be balanced with the survival of mature cells to maintain homeostasis and prevent hematological malignancies. How this transit amplification and subsequent homeostasis is controlled is not well understood but may involve metabolic switches. Various groups have demonstrated that hematopoietic expansion requires a switch from glycolysis to oxidative phosphorylation, but what induces this switch, as well as the specific carbon sources that fuel the switch, remain unclear. To address these questions, we employed mice conditionally deficient in mitochondrial pyruvate import or fatty acid oxidation and examined the requirements of these pathways in hematopoietic lineage homeostasis. Within two weeks of deletion of the mitochondrial pyruvate carrier 2 gene (Mpc2), peripheral neutrophils and monocyte numbers declined dramatically in bone marrow chimeras. Defects in other hematopoietic lineages, such as lymphocytes, were not observed. Over time after the ablation of MPC2 a progressive recovery of myeloid cells was observed. This recovery was associated with increased proliferation seen in Mpc2-/- granulocyte macrophage progenitors (GMPs) and common monocyte progenitors (cMoPs) relative to their wild type counterparts immediately following genetic deletion. Further studies will seek to elucidate the mechanism by which mitochondrial pyruvate promotes the myeloid lineage and to determine if myeloid cells switch to another metabolic pathway in order to recover from mitochondrial pyruvate ablation. Additionally, the role of fatty acids and glutamine in lineage-specific transit amplification and homeostasis will be examined.

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PANCREAS, LIVER, KIDNEY

T-1066

COMPARATIVE ANALYSIS AMONG MESENCHYMAL STEM CELLS AND SIMVASTATIN IN THE TREATMENT OF RENAL INDUCED ISCHEMIA/REPERFUSION INJURY IN RATS

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Kidney transplantation has been the gold standard treatment for most patients in end-stage renal disease. However, it is not free from risks such as delayed graft function (DGF). The aim of this study is to perform the ischemia / reperfusion model to produce lesions as observed in the DGF condition after renal transplantation and analyze the possibility of alternative treatments such as mesenchymal stem cells (MSCs) adipose tissue-derived and simvastatin. Wistar rats were divided into five groups: Control (ischemic and without any treatment), Sinv (ischemic and treated with simvastatin), SC (ischemic and treated with local infusion of MSCs), SC+ Sinv (ischemic and treated with combination of simvastatin and infusion of MSCs), and the S / I-SC + Sinv group (non-ischemic and treated with simvastatin and MSCs). The process of renal injury induction was performed by right nephrectomy and ischemia of the left renal artery through the clamp for 60 minutes. Blood samples were collected for serum creatinine and NGAL protein analysis. Histopathological analysis of renal tissue were made after 30 days of study. Serum

creatinine results showed an elevation of these levels during the first 15 postoperative days, with subsequent reduction and return to baseline, being significant only for the SC + Sinv group. The NGAL protein showed a significant difference compared to healthy animals, however, it was not significant in the differentiation of ischemic processes. The histopathological analysis resulted in lower tubular necrosis scores for the SC + Sinv group in relation to the isolated treatments, being equivalent to the S / I-SC+ Sinv (non-ischemic) group. Therefore, we concluded that the association of MSCs and simvastatin has a beneficial effect in the treatment of renal tissue damage by reducing serum creatinine levels and ameliorating tissue necrosis. In continuity to this research, it will be used extracellular vesicles derived from MSCs and endothelial progenitor cells (EPCs), obtained by ultracentrifugation and characterized by flow cytometry, Light Scattering and electron microscopy, to evaluate its beneficial effects in the vasculogenesis process and of the inflammatory response in the treatment of kidney injury.

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T-1068

S100A8/S100A9 IS INVOLVED IN HOMING OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN LIVER FIBROGENESIS

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In our previous studies, we have reported that the homing of bone marrow-derived mesenchymal stem cells (BMSCs) to injured liver plays an important role in liver fibrogenesis. S100A8 and S100A9, also named as calgranulins A and B, are small molecular weight proteins and belong to S100 protein family. S100A8 and S100A9 are secreted out of the cells, and are involved in various pathologic processes by forming heterodimer (S100A8/S100A9). It has been reported that S100A8/S100A9 mediate tumor cells migration in many kinds of tumourgenesis. But the role of S100A8/S100A9 in BMSCs migration and liver fibrogenesis is still unclear. In this study, we found that the expressions of S100A8 and S100A9 were increased in methionine-choline-deficient and high fat diet (MCDHF) induced liver fibrogenesis. The results of immunofluorescence showed that S100A8 and S100A9 are produced by the nonparenchymal cells in the injured liver. *In vitro*, S100A8/S100A9 induced BMSCs migration in a dose-dependent manner. The results of filamentous actin (F-actin) stain and high content analysis showed that the fiber number, total fiber area, mean area per fiber and fiber alignment were increased in S100A8/S100A9-treated BMSCs, indicating that S100A8/S100A9 caused the remodeling of F-actin in migratory BMSCs. We also studied the signal pathway involved in S100A8/S100A9-induced BMSCs migration and found that small GTPases of the Rho family were activated in this process. Pull-down analysis showed that active GTP-bound RhoA, Rac1, and Cdc42 were detectable in S100A8/S100A9-treated BMSCs. Furthermore, we employed the inhibitor of RhoA, Rac1 and Cdc42 to confirm this conclusion. Both C3 transferase (RhoA inhibitor), NSC23766 (Rac1 inhibitor), and MLS573151 (Cdc42 inhibitor) blocked the effects of S100A8/S100A9 on BMSCs migration and F-actin remodeling. In conclusion, our results prove that the increased S100A8/S100A9 in injured liver induces BMSCs homing in liver fibrogenesis.

T-1070

ADRENERGIC RECEPTOR AGONISTS INDUCE THE DIFFERENTIATION OF PLURIPOTENT STEM CELL-DERIVED HEPATOBLASTS INTO HEPATOCYTE-LIKE CELLS

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Current induction methods of hepatocytes from human induced pluripotent stem cells (hiPSCs) are neither low cost nor stable. By screening a chemical library of 1,120 bioactive compounds and known drugs, we identified the α1-adrenergic receptor agonist methoxamine hydrochloride as a small molecule that promotes the differentiation of hiPSC-derived hepatoblasts into ALBUMIN+ hepatocyte-like cells. Other α1-adrenergic receptor agonists also induced the differentiation of hepatocyte-like cells, and an α1-receptor antagonist blocked the hepatic-inducing activity of methoxamine hydrochloride and that of the combination of hepatocyte growth factor (HGF) and Oncostatin M (OsM), two growth factors often used for the induction of hepatoblasts into hepatocyte-like cells. We also confirmed that treatment with methoxamine hydrochloride activates the signal transducer and activator of transcription 3 (STAT3) pathway downstream of IL-6 family cytokines including OsM. These findings allowed us to establish hepatic differentiation protocols for both mouse embryonic stem cells (mESCs) and hiPSCs using small molecules at the step from hepatoblasts into hepatocyte-like cells. The results of the present study suggest that α1-adrenergic agonists induce hepatocyte-like cells by working downstream of HGF and OsM to activate STAT3.

T-1072

MESENCHYMAL STEM CELLS CULTURED IN SERUM-FREE MEDIUM AMELIORATE EXPERIMENTAL RENAL FIBROSIS

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Serum used in culture medium for mesenchymal stem cells (MSCs) presents problems that can hinder using *ex vivo* expanded MSCs for medical treatment. Here we cultured MSCs in a serum-free medium (SF-MSCs) and in a medium containing 10% fetal bovine serum (10%MSCs) and investigated their effects on inflammation and fibrosis. MSC-Conditioned medium suppressed transforming growth factor-β1-induced phosphorylation of Smad2 in HK-2 cells, with no significant difference between the two MSCs. This finding suggests that the direct anti-fibrotic effect of SF-MSCs is similar to that of 10%MSCs. However, immunohistochemistry revealed that renal fibrosis induced by unilateral ureteral obstruction (UUO) in rats was more significantly ameliorated by the administration of SF-MSCs than by that of 10%MSCs. Co-culture of MSCs and monocytic THP-1 cell-derived macrophages using a Transwell system showed that SF-MSCs significantly induced polarization from the pro-inflammatory M1 to the immunosuppressive M2 phenotype macrophages, suggesting that SF-MSCs strongly suppress the persistence of inflammation. Furthermore, the gene expression of tumor necrosis factor-a-induced protein 6 (TSG-6), which inhibits the recruitment of inflammatory cells, was higher in SF-MSCs than in 10%MSCs, and TSG-6 knockdown in SF-MSCs attenuated the anti-inflammatory responses in UUO rats. These findings imply that serum-free culture conditions can enhance the immunosuppressive ability of MSCs and the administration of *ex vivo* expanded SF-MSCs to patients with chronic kidney disease has the potential to be a useful therapy for preventing the progression of renal fibrosis

T-1074

CO-TRANSPLANTATION OF AN ANGIOGENIC BONE MARROW-DERIVED SPHEROID AS AN EFFECTIVE STRATEGY FOR ENHANCING INTRAPORTAL TRANSPLANTATION OUTCOME

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We have applied a strategy for co-transplanting accessory cells that possesses higher angiogenic or paracrine activities to islet transplantation. We recently reported that co-transplantation of bone marrow-derived spheroids (BM-spheroid) formed using 3-dimension culture from BM-derived mononuclear cells (BM-MNCs) enhanced therapeutic efficacy of islet in a marginal mass renal subcapsular islet transplantation model. In the present study, we investigated whether co-transplantation of islets with BM-spheroid can improve intraportal islet transplantation outcome in syngeneic mice and assessed the safety and the feasibility of co-transplantation of islets with BM-spheroid in nonhuman primate (NHP) intraportal islet allotransplantation. The morphology of intraportally transplanted islet, revascularization of islets and iron-labeled BM-spheroids were examined by immunohistochemistry. In mice models, portal-spheroid co-transplantation with islets improved the post-transplant outcomes in terms of glucose tolerance, serum insulin levels, and diabetes reversal rate when compared with islet alone. The area of grafted endocrine tissue and vascularization of individual islets within the graft-bearing liver was significantly higher in the spheroid group compared to the islets alone. Magnetic resonance imaging and intravital microscopy findings revealed the persistence of intraportally infused BM-spheroids in portal sinusoids. In NHPs model, post-transplant blood glucose levels were maintained within the target without administration of exogenous insulin only in the BM-spheroid group. There were few islets in the recipient's liver in the islet alone group, whereas more islets were present in the BM-spheroid group. In NHPs models, iron-labeled BM-spheroids revealed visible hypointense spots in in vivo MRI, and those of tissue ex vivo MRI corresponded with Prussian blue-positive cells. Our results suggest that

intraportal co-transplantation of BM-spheroids presents a promising strategy for improving the efficacy of islet transplantation. In addition, NHP results suggest the clinical feasibility of intraportal co-transplantation of allogeneic islets and autologous BM-spheroids.

T-1076

PURIFICATION OF CELL POPULATIONS BASED ON ENDOGENOUS MIR-LET7A ACTIVITY USING SYNTHETIC MICRORNA SWITCHES

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Type 1 Diabetes is one of the diseases that most affect the world population. The development of cellular therapies by reprogramming cells to treat it, remains as a major concern. However, reprogrammed cells are commonly related to heterogenic cell products, being necessary to use efficient cell-purification methods in order to obtain homogeneous cell products before using them in safe transplants. To solve this problem, in this work we explore a purification method that distinguishes and separates cell populations according to the activity of endogenous microRNAs (miRNA), which is called synthetic microRNA-switches (Miki et al., 2015). For that we validate the miR-Let7a in order to complement the miR-375 activity as pancreatic beta cells markers; so we synthesize as gBlocks the miR-Let7a-BIM-switch (containing the miR-Let7a complementary sequence and encoding the apoptosis inducer protein BIM), miR-Let7a-BFP-switch, miR-375-BFP-switch and we included mRNA-GFP as lipofection control. All synthetic RNAs were synthesized using a MegaScript® SP6 kit. We found that different cell types, like HEK293T and MIN6, have different tolerance to miR-Let7a-BIM-switch (50 vs 5000 ng/mL respectively; TUNEL assay, p positive HeLa cells. Lastly, a combination of miR-Let7a-BIM-switch and miR-375-BFP-switch efficiently enriched (>80%) insulin positive MIN6 cells from a heterogenic cell populations without cell sorting in an *in vitro* model. We confirmed that microRNA-switches have great potential for the detection and purification of living target cells according to the activity of endogenous miRNAs.

T-1078

YAP REGULATES THE DIFFERENTIATION, PROLIFERATION AND FUNCTION OF HUMAN STEM CELL-DERIVED PANCREATIC BETA CELLS

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Stem cell-derived insulin-producing beta cells (SC-β) would provide an inexhaustible source of functional β cells for cell replacement therapies and disease modeling for diabetes. Although directed differentiation protocols into the pancreatic lineage have been described, the molecular underpinnings controlling the terminal differentiation and function of this cell type still remain elusive. Here we report that YAP activity regulates the differentiation and physiological function of SC-β cells. We observed that YAP downregulation precedes the pancreatic endocrine and β cell differentiation of human pluripotent stem cells (PSCs). YAP expression was detected in insulin-producing SC-β cells that do not express NKX6.1, a factor essential for β cell function and monohormonal expression. Pharmacological inhibition of YAP activity during the endocrine and β cell specification stages of PSCs resulted in an increased endocrine progenitor differentiation and enhanced generation of C-peptide/NKX6.1 double-positive SC-β cells. More importantly, YAP inhibition also led to an increased glucose-stimulated insulin secretion (GSIS) of SC-β cells. Interestingly, the overexpression of a constitutively active form of YAP during the last stages of β cell differentiation resulted in an impaired differentiation of C-peptide/NKX6.1 double-positive and dedifferentiation of SC-β cells, blunted GSIS function, and an increase in the proliferation of SC-β cells. Correlating with the potential role of YAP in limiting β cell function, we observed an increased in YAP expression in β cells of human diabetic donors. Pharmacological inhibition of this factor preserved NKX6.1 expression in human donor β cells and other genes that control β cell function in a model of β cell dedifferentiation. Our study lends support to the role of YAP in limiting the differentiation and physiological GSIS function of human β cells.

T-1080

INHIBITION OF WNT/BETA-CATENIN SIGNALING IN HUMAN EMBRYONIC STEM CELL-DERIVED PANCREATIC PROGENITORS RESULTS IN ENDOCRINE CELL HYPOPLASIA

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We have generated a heterozygous human embryonic stem (hES) cell line in which sequences encoding green fluorescent protein (GFP) and mCherry fluorescent protein (mCh) were placed under the regulatory control of the INSULIN and GLUCAGON genes, respectively (INSGFP/w GCGmCh/w). This allows for the exploration of factors that influence commitment to pancreatic endocrine cells via the monitoring of fluorescent protein expression. Using this INSGFP/w GCGmCh/w hESC line in conjunction with a modified previously published pancreatic differentiation protocol, we found, similar to others, that pancreatic endocrine cells expressing INSULIN or GLUCAGON are formed following three to four weeks of differentiation. These endocrine cells display similarities to adult human beta cells in their transcriptional profiles. Previous studies in mice have demonstrated that Wnt/Beta-catenin signaling plays an important role in the formation of both the endocrine and exocrine compartments of the pancreas. For example, deletion of a loxP flanked Beta-catenin gene using Pdx1CRE-late resulted in reduced numbers of Insulin and Glucagon expressing cells. Consistent with this finding, our preliminary studies indicate that inhibition of Wnt/Beta-catenin signaling using either DICKKOPF-1 or a small molecule stabilizer of AXIN2 (IWR-1), results in a marked reduction in the number of INSULIN+ and GLUCAGON+ cells. This effect is most notable when inhibitors are added at the differentiation stage corresponding to early pancreatic progenitors. In contrast, inhibition of Wnt/Beta-catenin signaling at later time points does not appear to influence endocrine cell number. This suggests that the role of Wnt/Beta-catenin signaling in endocrine cell formation from pancreatic progenitors may be conserved between mice and man.

Funding Source: This work was supported by the Juvenile Diabetes Research Foundation and the National Health and Medical Research Council, Australia

EPITHELIAL TISSUES

T-1082

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-1084

CRUCIAL ROLE OF BNIP3-INDUCED AUTOPHAGY IN DIFFERENTIATION AND MAINTENANCE OF SKIN EPIDERMIS

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Autophagy is an important homeostatic process to degrade unnecessary or dysfunctional cellular organelles and to recycle nutrients. Epidermal differentiation is known to be going along with activation of lysosomal enzymes and organelle clearance, suggested the contribution of autophagy in this process. Previously, we have shown Hes1, a downstream effector of Notch signaling, is required for maintenance of the immature status of suprabasal cells by preventing premature differentiation. In this study, we found that Hes1 directly bound to the promoter region of BNIP3 and suppressed its expression. We also found that BNIP3 was expressed in the granular layer of the epidermis, where autophagosome formation is normally observed. Forced expression of BNIP3 in human primary epidermal keratinocytes (HPEKs) elicited induction of autophagy and mitophagy, whereas knockdown of BNIP3 had the opposite effect. Intriguingly, addition of an autophagy inhibitor significantly suppressed the BNIP3-stimulated differentiation of keratinocytes, suggesting that BNIP3 plays a crucial role in keratinocyte differentiation by inducing autophagy. Furthermore, epidermal equivalent of BNIP3 knockdown HPEKs exhibited increased cell death by apoptosis. Therefore, we next focused on BNIP3 functions involved in maintenance of epidermal keratinocytes. Although UVB irradiation in HPEKs resulted in the stimulation of BNIP3 expression, suppression of its expression caused a further enhancement of apoptosis levels and attenuation of autophagy levels. Furthermore, suppression of UVB-induced BNIP3 also conducted attenuation of mitochondrial membrane potential. These findings indicate that BNIP3 is required for the degradation of dysfunctional mitochondria upon UVB irradiation via autophagy. Moreover, we found that BNIP3 expression was stimulated by UVB-induced reactive oxygen species through JNK and ERK1/2 mitogen-activated protein kinase. Addition of autophagy inhibitor resulted in the further stimulation of UVB-induced apoptosis, demonstrating that autophagy plays crucial roles in protecting epidermal keratinocytes from stress-induced apoptosis. Overall, our data provide valuable insights into the crucial role of BNIP3 in the differentiation and maintenance of epidermal keratinocytes.

T-1086

ALTERED EXPRESSION OF MRNA PROFILES IN CHILDREN WITH ADENOID HYPERTROPHY

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The growth of adenoid begins in early childhood with a peak size between 3 and 6 years of age and numerous adverse effects of enlarged adenoids have been described. However, the mechanisms underlying the regulation of benign follicular lymphoid proliferation as well as hypertrophic enlargement are poorly understood. We hypothesized that pathologically enlarged adenoids would lead to altered gene expression. Oligonucleotide-based microarray technology was used to identify mRNAs that may be differentially regulated in hypertrophic adenoids compared to non-hypertrophic adenoids. mRNA from adenoid tissues was extracted, labeled, and hybridized onto independent oligonucleotide-based microarrays. Of the 44,000 transcripts, 1824 transcripts were differentially expressed in hypertrophic adenoids (p-value <0.05), with 44 transcripts fulfilling high stringency criteria. Microarray data were further validated using quantitative RT-PCR techniques and immunohistochemistry. Biological pathways pertinent to the differentially expressed genes were explored and revealed prominent involvement of inflammatory pathways. RNA derived from hypertrophic adenoids confirms the presence of altered expression of functionally relevant gene clusters in children with adenoid hypertrophy. Large-scale genomic approaches may provide further insights into related mechanisms of adenoid hypertrophy.

T-1088

FUNCTIONAL RESTORATION OF SALIVARY GLAND BY TRANSPLANTATION OF SALIVARY GLAND-DERIVED STEM CELL TO RADIATION DAMAGED RAT

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Xerostomia is a side effect of radiotherapy for head and neck cancer, has been treated using various strategies but there is no effective long term therapy. We investigated whether transplantation of the undifferentiated adult rat salivary gland progenitor/stem cells (SGSCs) could treat radiation-induced salivary gland hypofunction. Salivary gland damage was induced by local exposure to ionizing radiation with single dose of 30 Gy in the head and neck region of adult rats. The capacity of SGSCs to cure radiation-damaged salivary gland hypofunction was examined by transplanted the mCherry tagged SGSCs into the irradiated glands at 21 days post-irradiation. The salivary flow rate, amylase activity and histological analysis for restoration of salivary hypofunction were performed at 7, 21 and 42 days post-transplantation. The salivary flow rate and amylase activity were significantly increased in

SGSCs transplanted group (IR+SGSCs) compared with irradiated group (IR). The salivary gland tissue of IR observed numbers of vacuolization, pyknotic nuclei and decreased activities of mucin and mucopolysaccharide compared with control group (Ctrl). However, IR+SGSCs group showed the decreased activities by comparison with IR. Immunohistochemistry for α-amylase, aquaporin 5 (AQP 5) and c-Kit were significantly decreased in IR, while IR+SGSCs was gradually recovered up to Ctrl. Above results were seemed to be very correlated with the survival of mCherry tagged SGSCs detected at 7 and 21 days post-transplantation. This study suggests that the transplanted SGSCs have a capability to improve the recovery of damaged salivary glands, and shows the potentials as a cell therapy for the treatment of the radiation-induced hypofunction in the future.

T-1090

RUNX1 ENHANCER ELEMENT ER1 MARKS TISSUE STEM CELLS IN MOUSE

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Runx1 is required for generation of hematopoietic stem cells from endothelial cells. Between the two promoters of Runx1, there are multiple enhancer elements. One of them which, we call eR1 (270 bp), drives the expression of Runx1 in hematopoietic stem cells. Transgenic mouse (TG) was produced containing eR1 linked to heterologous promoter and GFP. eR1 in TG mouse faithfully induced GFP in hematopoietic stem cells. Interestingly, in this TG mouse, eR1 drives the expression of GFP in tissue stem cells in multiple organs including stomach, intestine, colon, liver, pancreas, lung and mammary gland. Lineage tracing experiments revealed that eR1+ cells are indeed tissue stem cells. In the case of mammary gland, luminal epithelial stem cell was marked by eR1. Consistent with the results of in vivo lineage tracing experiment, organoids were generated from eR1+ cells from these multiple organs. Most cancer cells may be developed from tissue stem cells, and eR1 can be a useful tool to examine the step-wise carcinogenesis by activating and inactivating the genes of interest in eR1+ cells. We crossed the mice carrying eR1-CreERT2 with Runx1F/F or Runx3F/F mice to examine the effect of depletion of Runx genes in mammary gland. When CreERT2 was activated by tamoxifen, eR1+ luminal epithelial cells showed robust expression of ERα and ki67 and the cells multiplied towards the lumen of the gland. These results suggested that elimination of Runx expression in luminal stem cells induces first step of breast cancer carcinogenesis. RUNX1 is known to be significantly mutated in liminal type of human breast cancer.

EYE AND RETINA

T-1092

TWEETS IN THE CLINIC: A CASE STUDY OF OCULAR STEM CELL AND GENE THERAPY RESEARCH

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Reports on gene and stem cell research give hope to patients with currently untreatable genetic eye diseases. News media is known to hype translational research, leaving patients with incomplete information about therapeutic potential and research timelines. Research teams craft press releases to present findings; however, easy online information access and sharing allows for repeated reinterpretation of these findings as they move between newspapers, advocacy organizations, patients and social media. Our research illustrates how the accuracy, quality, and completeness of reports vary by information outlet. These variations affect communication requirements for clinicians and genetic councillors, who help patients choose disease management paths within current clinical realities. To assess potential communication challenges about ocular research, we conducted media and Twitter analyses of gene and stem cell research coverage between 2005 and 2017: (1) we collected and compared the quality of media coverage in Canada, USA, and UK. Our search yielded 95 press releases and 213 news media articles, reporting on gene (n=92) and stem cell (n=216) research outcomes; (2) we analyzed how ocular disease advocacy websites presented items on gene (n=321) and stem cell (n=209) research; and (3) we analysed three high-profile clinical research events on Twitter (4665 tweets) to identify the sources most often shared and to assess the quality of the linked articles. We concluded that news media and press releases: (1) overstate benefits and often omit risks of experimental treatments for ocular diseases; (2) conflate research with treatment or cure; and (3) misrepresent research timelines. Advocacy websites generally presented more balanced reports than newspapers but remained overly optimistic in research representations. Tweets often included links to online science or news outlets that offered more complete reports than traditional news media. However, the Tweets themselves were hyped headlines. Furthermore, the quickest outlets to Tweet novel reports

were the most shared, which limited the range of reports shared via Twitter. We conclude with recommendations to guide clinicians in their communications with patients in light of media and social media content.

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T-1094

PURIFIED RETINAL GANGLION CELLS DERIVED FROM 3D RETINAL ORGANOIDS FROM NORMAL TENSION GLAUCOMA PATIENT-INDUCED PLURIPOTENT STEM CELLS

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We made three-dimensional (3D) retinal organoids from induced pluripotent stem cells (iPSCs) from a patient whose family member suffer from normal tension glaucoma (NTG) for three generations. The purpose of this study is to purify retinal ganglion cells from NTG patient's 3D retinal organoids by a two-step immunopanning method. iPSCs were generated from their skins of NTG patients, and 3D retinal organoids were differentiated and maintained until differentiation day (DD) 100. The retinal ganglion cells (RGC) were purified by a two-step immunopanning method with anti-macrophage antibodies and anti-Thy1 antibodies. The purified RGCs derived from the NTG patient's iPSCs (NTG iPS-RGCs) were plated on dishes coated with poly-D-lysine and laminin, and maintained in serum-free medium with several supplements. Also, we performed a gene screening to identify the candidate causal genes. The NTG iPS-RGCs purified by immunopanning methods attached on to dishes within the first 24 hours and extended neurites. NTG iPS-RGCs could be cultured for approximately three weeks, and immunostained with antibodies against BRN3B (RGC-enriched transcription factor) and SMI-312 (RGC-enriched neurofilaments). The gene screening suggested that the NTG patients in this study have a lower association with known NTG causal genes (e.g. MYOC, OPTN, and WDR36). We could confirm the neurite growth of NTG iPS-RGCs for a long-term period, and novel NTG causal genes were suggested. Exome sequencing is currently in progress. This model is useful for studying the pathology and neurogenesis of NTG patients' RGCs.

T-1096

THE ROLE OF LYSOPHOSPHATIDIC ACID AND AUTOTAXIN IN THE RETINAL PIGMENT EPITHELIUM AND PHOTORECEPTORS

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The human retina is a complex structure of organised layers of specialised cells that support the transmission of light signals to the visual cortex. The outermost layer of the retina, the retinal pigment epithelium (RPE), performs many crucial functions, one of which is to create part of the blood retina barrier. Loss of this critical barrier is implicated in many retinal diseases leading to injury of the retinal pigment epithelium and photoreceptor death. Lysophosphatidic acid (LPA) is a bioactive lipid exerting pleiotropic effects in various cell types, during development normal physiology and disease. Its producing enzyme AUTOTAXIN (ATX) is highly expressed by the pigmented epithelia of the human eye, including the RPE. Using human pluripotent stem cell (hPSC)-derived retinal cells, we investigated the role of LPA in the human RPE and photoreceptors. Human pluripotent stem cell (hPSC)-derived RPE cells express and synthesize functional ATX, which is predominantly secreted apically of the RPE, suggesting it acts in a paracrine manner to regulate photoreceptor function. Liquid chromatography mass spectrophotometry (LC-MS) analysis indicated that the RPE generates extracellular LPA. In hPSC-RPE cells, LPA regulated tight junctions in a dose-dependent manner, causing an increase in OCCLUDIN and ZONULA OCCLUDENS (ZO)-1 expression at the cell membrane, accompanied by an increase in the transepithelial resistance of the epithelium. High concentrations of

LPA decreased phagocytosis of photoreceptor outer segments by the RPE, a critical function of the RPE in vivo. In mouse 661W and hPSC-derived photoreceptors, LPA induced morphological rearrangements by modulating the actin myosin cytoskeleton, as evidenced by Myosin Light Chain phosphorylation and cellular membrane relocation. Collectively, our data suggests an important role of LPA in the integrity and functionality of the healthy retina and blood retina barrier.

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T-1098

A 3D/2D STEPWISE RETINAL DIFFERENTIATION PROTOCOL TO ISOLATE HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED RETINAL GANGLION CELLS FOR FUTURE STEM CELL-BASED THERAPIES

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Stem cell-based therapy using human induced pluripotent stem cells (hiPSCs) is one of the most promising strategy to replace lost retinal ganglion cells (RGCs) in optic neuropathies, such as glaucoma. We already established a protocol allowing the differentiation of hiPSCs in Good Manufacturing Practices conditions into 3D retinal organoids containing any retinal cell type, including RGCs (Reichman et al., PNAS, 2014; StemCells, 2017). The present work displayed the optimized generation and isolation by cell surface targeting of hiPSC-derived RGCs, based on a 3D/2D stepwise differentiation protocol. In 3D retinal organoids, RGCs are generated from 40 days of differentiation, to reach a peak around 70 days. After 56 days of differentiation, 3D retinal organoids were dissociated and cultured onto poly-D-lysin/laminin coated plates to promote RGC axonal growth. Dissociated cells from 56-day-old retinal organoids, expressed specific RGC markers (BRN3A, RBPMS, CD90/THY1) after 7-14 days in 2D conditions. Whole-cell patch-clamp recording revealed that hiPSC-derived RGCs displayed voltage dependent currents (up to 1500pA at +40mV), action potentials triggered by depolarizing current injections (+40pA from the resting membrane potential), and spontaneous spiking in some cells. Dissociated retinal cells expressing CD90 could be sorted by Magnetic-Activated Cell Sorting (MACS), leading to enrichment to 87% of CD90+ cells in the positive sorted fraction. Re-plating of the CD90-sorted cells demonstrated the cell viability after the separation process and the expected expression of BRN3A and RBPMS. Interestingly, MACS of CD90+ cells could be performed on freeze-thawed dissociated retinal organoids, with equivalent results to MACS on not frozen organoid. Our results support promotion of axonal growth of hiPSC-derived RGCs in adherent culture conditions. MACS of CD90+ cells resulted in a significant enrichment of RGCs exhibiting specific RGC electrophysiological profile, which will be of a great utility for future clinical translation. For this purpose, CD90-sorted RGCs will be grafted in an optic nerve crush mouse model, leading to rapid RGCs degeneration, in order to evaluate their potential integration into the ganglion cell layer of the host retina.

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STEM CELL NICHES

T-1100

DECREASED TISSUE REGENERATIVE POTENTIAL OF AGEING HUMAN SKIN CAN BE ATTRIBUTED TO CHANGES IN THE DERMAL MICROENVIRONMENT

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Our previous work showed that pericytes can promote human skin regeneration independently of regulating angiogenesis[1]. We therefore sought to identify novel mechanisms by which they increase keratinocyte proliferation. Microarray analysis on pericytes derivedfrom 3 neonatal foreskin (1 were performed for identifying consistent changes in gene expression with ageing. Gene ontology analysis revealed 25 genes as being consistently overexpressed by pericytes in *neonatal* skin, involved in tissue remodelling and wound repair impacting on cell adhesion and extracellular matrix re-modelling. Notably, two proteoglycans: versican (VCAN) and lumican (LUM) exhibited a decreased expression at mRNA level with increasing age. We subsequently demonstrated that both proteoglycans localized to the dermis of the skin and that their levels gradually diminished with increasing age i.e. neonatal versus adult skin from donors aged 20-65. Adhesion assays revealed that both adult and neonatal keratinocytes demonstrated increased attachment

to Collagen I and III (Col I & III) in the presence of recombinant VCAN and LUM. VCAN and LUM also provided a pro-proliferative signal to both adult and neonatal keratinocytes both in the presence and absence of Col I & III *in vitro*. Interestingly, our data suggests that the G1 domain of VCAN alone promotes keratinocyte adhesion and proliferation, a finding in agreement with previous literature describing that this domain enhances fibroblast adhesion and proliferation. Further, our study demonstrated that recombinant VCAN improved the skin tissue regenerative potential of adult human keratinocytes in 3D organotypic cultures. These data suggest that changes in proteoglycans deposited in the dermis or microenvironment of ageing skin epidermal cells, particularly decreased VCAN, may contribute to the poorer ability of adult keratinocytes to regenerate epidermal tissue. Thus, skin ageing is also a function of the extrinsic cellular and molecular microenvironment of which dermal pericytes are important components.

Funding Source: L'Oreal

T-1102

ATTENUATION OF UVB-IRRADIATED PHOTOAGING BY TIMPS SECRETED FROM NEURAL STEM CELLS

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Ultraviolet (UVB) radiation can cause skin photoaging that is involved in deterioration of the dermal extracellular matrix (ECM) through the activation of matrix metalloproteinases (MMPs). The collagens and elastins, as representative ECM components, are reduced by the MMPs, which are secreted by epidermal keratinocytes and dermal fibroblasts and are also modulated by NFkB pathway. It has been well known that most MMPs could be inhibited by tissue inhibitor of metalloproteinases (TIMPs). In this study, we show that pre-treatment of neural stem cell-derived conditioned medium (NSC-CM) to human dermal fibroblasts attenuated UVB photoaging through the inhibition of the activity of MMP-2 and MMP-9 in vitro and in vivo. In addition, UVB irradiation induced a substantial increase of γ-H2AX, a DNA damage marker, whereas a topical application with NSC-CM after UVB irradiation significantly decreased the expression of γ-H2AX in vivo. Interestingly, we found TIMP-1 and TIMP-2 as main ones of the factors secreted in the NSC-CM by Human cytokine antibody arrays. Pre-treatment with recombinant human (rh) TIMPs or with NSC-CM containing TIMPs antibodies led anti-photoaging effects through the inhibition of MMPs activity in vitro. Taken together, these results suggest the secreted factors TIMP-1 and -2 in NSC-CM contributed to attenuate the photoaging through the inhibition of MMPs in in vitro and in vivo.

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T-1104

IDENTIFYING TISSUE-RESIDENT HEMATOPOIETIC STEM CELLS IN EXTRAMEDUALLARY SITES

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Endothelial protein C receptor (EPCR) has been established as a marker specifically distinguishing hematopoietic stem cells (HSC) that reside in murine bone marrow. In contrast, HSC released into the peripheral blood circulation do not express EPCR. We questioned whether the use of EPCR could be extended to identifying tissue-resident HSC in extramedullary organs. Murine spleen is known to harbor HSC from neonatal to adult stages. As an organ that functions explicitly in blood filtration, it has been uncertain whether these cells represent transitory or endogenous HSC. Studies using parabiosis have now clarified that a large proportion of spleen HSC are in fact endogenous to the tissue. We now make a direct comparison of adult spleen, peripheral blood, and bone marrow HSC, to determine the percentage of EPCR+, and thus tissue-resident HSC, in spleen. Our results confirm that spleen indeed contains a high percentage of EPCR+ HSC comparable to bone marrow. We also extend our investigation to examine whether several modes of extramedullary hematopoiesis lead to an expansion of spleen-resident EPCR+ HSC, or an influx of HSC mobilised into the circulation from bone marrow.

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T-1106

CARDIAC DEVELOPMENT AND REGENERATION: NOVEL 3D POLYMERS AND THE INFLUENCE OF PHYSICAL CUES

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High-purity cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSCs) are a promising tool for drug development and myocardial regeneration. However, myocyte immaturity currently constitutes the main hurdles for their application. Most hiPSC-CMs morphologically, genetically and functionally resemble foetal rather than adult CMs, which hampers their coupling with the patient´s mature myocytes and increases the risk of arrhythmias. We developed a novel versatile platform that allows the concomitant manipulation of cell size and substrate stiffness. In this study, we generated arrays of 3D microwells on hydrogels with varying stiffness (14 kPa, 50 kPa and 100 kPa). This system can force individual hiPSC-CMs to adopt the 3D geometry of an adult CM while at the same time provide the relevant physical cues from the underlying substrate. Single hiPSC-CMs isolated by immunomagnetic selection for SIRPα were plated on microwell arrays and cell maturity was assessed. Cell membrane stiffness was measured using atomic force microscopy with indentation mode. This revealed a significant increase in the stiffness when CMs were confined to the microwells vs their flat controls in the 14, 50, 100 kPa arrays (2-fold, 1.5-fold, and 1.5-fold increase, respectively). This strongly mimics the progression of CMs' stiffness during development towards adult CMs, which is higher than foetal CMs. Interestingly, single cell qPCR revealed that 3D shape induced a differential gene expression pattern as compared to hiPSC-CMs plated on flat hydrogels. Furthermore, the multivariate analysis showed a significant increase in TNNI3/TNNI1 ratio for cells forced to adopt an adult-like shape, indicating a closer resemblance to adult CMs. Staining for MLC2v and MLC2a demonstrated that confining the 3D shape on hydrogels of myocardial-like stiffness (14 kPa) increased the number of single positive MCL2v (95%) CMs as compared with 50 kPa (80%) and 100 kPa (70%) stiffness substrates. Functional assays will be discussed. In conclusion, this is the first demonstration that the sole manipulation of cell shape within a biomechanically-relevant environment can significantly improve the maturation gene expression and morphology of hiPSC-CMs, opening the way for the development of advanced therapies and tools.

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T-1108

PERICYTE IN THE ADULT MUSCLE SATELLITE CELL NICHE: A KEY PLAYER IN STEADY STATE AND RECOVERY.

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Muscle growth and post-injury regeneration are supported by muscle satellite cells (mSCs) that reside beneath the myofiber basement membrane in close proximity to capillaries. We previously showed that pericytes play a key role in the microvascular niche of mSCs during post-natal stages of muscle growth, by promoting post-natal myogenesis through IGF-1 and inducing mSC quiescence ending the accretion phase through Angiopoietin-1. Since 90% of capillaries have pericyte coverage at the end of muscle growth, we investigated the role of pericytes in the adult mSC niche. We used TNAP-CreERT2 mice crossed with R26RDTRstoploxP/stoploxP, Angiopoietin-1loxP/loxP, and R26RmTloxPmG/loxPmG animals to generate respectively conditional models for diphteria toxin-induced muscle depletion of microvascular cells, ablation of microvascular Angiopoietin-1 and fluorescent tracing of microvascular cells. Conditional muscle pericyte depletion induced extensive, presumably ischaemic, muscle necrosis. In contrast, selective Cre recombinase ablation of Angiopoietin-1 gene in TNAP+ pericytes induced release of adult mSCs from quiescence. Following chemical injury, the ablation caused delayed muscle regeneration with persistently cycling Pax7+ mSCs. Selective Type 2 fiber hypotrophy was observed consistently with prominent association of TNAP-expressing microvessels with type 2 fibers observed in TNAP-GFP reporter mice. We conclude that pericytes associated with endothelial cells exert paracrine effects on adjacent myogenic cells that are essential to maintain adult muscle homeostasis and during muscle repair.

Funding Source: Association Française pour les Myopathies (AFM); Agence Nationale pour la Recherche (ANR)

T-1110

ENDOTHELIAL CELLS AND HEPATIC STELLATE CELLS CREATE NICHE FOR HEMATOPOIETIC STEM CELLS BY SECRETING STEM CELL FACTOR IN THE DEVELOPLING LIVER

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The liver is the main hematopoietic organ and the site of hematopoietic stem cell (HSC) expansion and maturation in mammalian fetuses. However, little is known about the nature of the niche that maintains HSCs in the liver during development. Several cell types such as fibroblastoid cells, hepatic progenitors, and cells with characteristics of epithelial-to-mesenchymal transition (EMT) have been suggested to have HSC

supporting activity in vitro. However, direct in vivo functional evidence supporting important roles of these putative fetal liver niche components still needs to be obtained. Recently, based on cell ablation experiments, Nestin+NG2+ periportal stromal cells in the fetal liver have been suggested to be an important cellular component of the niche. However, no systematic evaluation of the source of key cytokines for HSC maintenance has been conducted in the developing liver. Here we genetically dissect the cellular components of the developing liver niche by determining the cellular sources of a key niche factor, stem cell factor (SCF). We observed that rare CD150+CD48-lineage- candidate HSCs are close to sinusoidal endothelial cells throughout the liver. Systematic characterization of Scf expression revealed that that Scf is primarily expressed by endothelial cells and hepatic stellate cells. Conditional deletion of Scf from hematopoietic cells, hepatocytes, NG2+ periportal stromal cells or endothelial cells did not affect HSC frequency or function. Deletion of Scf from hepatic stellate cells depletes HSCs. Strikingly, nearly all HSCs were lost when Scf was deleted from both endothelial cells and hepatic stellate cells in the developing liver. Therefore, HSCs reside in a perivascular niche created by endothelial and hepatic stellate cells that elaborate SCF for their maintenance in the developing liver.

Funding Source: This study was supported by Rita Allen Foundation and New York Stem Cell Foundation.

T-1112

LGR5+ MESENCHYMAL STEM CELLS (MSCS) IN PERIPHERAL BLOOD, THEIR IDENTIFICATION AND FUNCTION

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Peripheral blood-derived mesenchymal stem cells (PB-MSCs) are an alternative source of MSCs, which may be collected with minimal invasiveness. However, the PB-MSCs are relatively rare cell population and their biological roles and optimal culture methods are still under investigation. By comparing the genetic profiles between BM-MSCs (bone marrow derived MSCs) and PB-MSCs, we identified that Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5) is highly unregulated in the PB-MSCs. Lgr5 is a Wnt target gene and a bona fide marker of several adult stem cells in various adult organs, such as stomach, small intestine, kidney, and hair follicle. We found that the expression of Lgr5 was localized in cytoplasm of PB-MSCs. In bone tissues, Lgr5 and BrdU (a cell proliferation marker) was co-localized, suggesting that Lgr5 positive cells are proliferating cells. Trans-well assay showed PB-MSCs migrated more rapidly than those of BM-MSCs. Suppression of Lgr5 expression using siRNA significantly inhibited the migration ability of PB-MSCs. Immunocytochemistry data showed that inhibition of Lgr5 expression decreased actin cytoskeleton number in PB-MSCs and reduced their osteogenic potentials. In contrast, cytoskeletal organization of negative control PB-MSCs was orderly and they have better osteogenic differentiation potential. In addition, gene silencing of Lgr5 inhibited expression of CD146, while unregulated the expression of E-cadherin, which were markers for epithelial-mesenchymal transition (EMT). In conclusion, Lgr5 may be an important biomarker to distinguish PB-MSCs from BM-MSCs. PB-MSCs express higher levels of Lgr5; they migrate more rapidly. Higher Lgr5 expression may be the case for enhanced cell migration ability of PB-MSCs. In the process of MSCs mobilization, cells lose cell-cell contact and cell-extracellular matrix connections, similar to EMT event. Lgr5 may regulate E-cadherin and CD146 expression and promote PB-MSCs migration.

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T-1114

SUBLETHAL IRRADIATION OF MICE IMPROVES LONG-TERM ENGRAFTMENT OF HUMAN MESENCHYMAL STROMAL/STEM CELLS.

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Mesenchymal stromal/stem cells (MSC) are an important multipotent cell population within in the bone-marrow microenvironment. MSC contribute to this microenvironment either in their stem cell state or as differentiated progeny (i.e. osteoblasts or adipocytes). MSC and their progeny are known to play a critical role in maintaining the bone-marrow hematopoietic stem cell (HSC) niche, both through direct and indirect signalling. Recent work has demonstrated a species-specific preference for human MSC when human HSC are transplanted intra-venously into irradiation conditioned immune-compromised mice. These studies were completed with a combination of human HSC and MSC, and did not quantify if irradiation enhanced human MSC engraftment when these cells were transplanted without human HSC. Herein, we quantified the engraftment of human MSC in the bone marrow of NOD-SCID IL2γ-/- (NSG) mice either conditioned or not conditioned with 2Gy irradiation. Human MSC (5x105) were injected intra-femorally into the right femur and transplanted mice were imaged weekly using an In Vivo Imaging System (IVIS) to quantify the bioluminescence emanating from the luciferase labeled human MSC. At 8 weeks, the animals were euthanized, and harvested femurs were fixed overnight in 4%

paraformaldehyde, decalcified in EDTA and paraffin embedded prior to sectioning to enable histological and immuno-histochemical analyses. Irradiation was found to increases the level of MSC engraftment significantly by week 2. IVIS results also suggested that human MSC engraftment persisted significantly longer in irradiated mice. By further understanding how the human MSC engraft long-term in mice, we hope to further optimise the use of MSC within mouse models. This is critical, especially in the development of in vivo model systems that require extended timelines in order to study the impact of human MSC on processes such as human HSC engraftment or human cancer bone metastasis.

NEURAL DEVELOPMENT AND REGENERATION

T-2002

REMYELINATION OF CHRONIC DEMYELINATED LESIONS OF THE SPINAL CORD WITH DIRECTLY INDUCED NEURAL STEM CELLS

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Remyelination is a spontaneous reparative process that occurs following demyelination of the peripheral and central nervous system (CNS). However, in chronic demyelinating diseases of the CNS such as progressive multiple sclerosis (PMS), this endogenous regenerative process is mostly inefficient or impaired. Neural Stem Cell (NSC) transplantation has emerged as a promising therapeutic approach for several CNS disorders. We have shown that NSCs can foster endogenous remyelination by replacing damaged tissue and providing local trophic support to the injured CNS. However, major hurdles still limit the use of conventional stem cell therapies in clinical settings. Induced Neural Stem Cells (iNSCs) directly reprogrammed from skin fibroblasts have partially overcome these limitations by providing a source of autologous tissue-specific stem cells, which has no histocompatibility barriers and can be easily obtained from accessible somatic cells. Here we investigated the potential of mouse iNSCs to promote remyelination after transplantation in a mouse model of focal spinal cord demyelination. We found that a single injection of mouse iNSCs in the lesioned spinal cord of wild type mice led to a successful integration of the graft into the host spinal cord. Transplanted iNSCs were able to promote remyelination by increasing endogenous myelinating cells and partially differentiating into mature oligodendrocytes. When injected in Olig1-/- mice, which lack endogenous remyelinating response after lesion, we found that the direct differentiation of the iNSCs grafts into mature oligodendrocytes was able to remyelinate the injured spinal cord. Our results suggest that iNSCs can be used to adjuvate endogenous remyelinating responses and/or efficiently replace lost cells in the demyelinated CNS. These data further support the use of directly reprogrammed iNSCs as an effective therapeutic tool to enhance remyelination in chronic demyelinating CNS diseases.

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T-2004

NEURAL INDUCTION OF 3-DIMENSIONAL HESC AGGREGATES USING A THERM

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We have generated 3D aggregates of hESC utilising our thermoresponsive poly(N-isopropylacrylamide) (PNIPAM), conjugated to a fibronectin fragment. This system facilitates cell expansion and release and here we investigated the efficacy of using this system for differentiation of hESC towards the neuronal lineage. HESC were differentiated towards the neuronal lineage as either 2D cultures, single aggregate (96 well) cultures, or multi aggregate (6 well) cultures. Following generation of aggregates, neuronal induction was initiated at ffi 300mm aggregate width and passaging performed at day 5 (multi aggregate only), day 10 (all aggregates), and thereafter on a weekly basis (all aggregates). Under neuronal induction, the cell lines demonstrated disaggregation when low shear was applied at a temperature below the lower critical solution temperature, and re-aggregation following re-addition of the polymer. Data demonstrated that multi

aggregate cultures significantly outperformed both single aggregate and 2D cultures in proliferation, width and gene expression, suggesting secreted elements may play a role in enhancing differentiation and proliferation. Single aggregate cultures demonstrated a decrease in aggregate width and a slowing of proliferation starting at passage 1 (p1), in comparison multi aggregates cultures demonstrated these changes between p2- p3. Cell viability remained high across all cell types and conditions. All cell lines demonstrated downregulation of pluripotency genes and proteins (OCT-4, NANOG, DNMT3B) during initial differentiation and upregulation of neuronal-associated genes and proteins (PAX6, SOX1, NCAM, DCX and MAP2).

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T-2006

EFFECT OF INDUCED NEURAL STEM CELLS COMBINED WITH SILK-COLLAGEN HYDROGEL FOR THE FUNCTIONAL RECOVERY OF SPINAL CORD INJURY

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Neural stem cells (NSCs) has tremendous potential therapy for the regeneration of spinal cord injury (SCI). However, a low cell survival rate and uncontrolled differentiation of transplanted NSCs might reduce the effectiveness of successful cell therapy for SCI. Recent strategies aimed at modulating the microenvironments to promote survival and differentiation of transplanted cells using a combination of various biomaterials. We have developed an injectable silk-collagen hydrogel with highly tunable mechanical properties that can encapsulate and protect induced NSCs (iNSCs), which were directly reprogrammed from mouse embryonic fibroblasts, in vivo condition. The purpose of this study was to examine the role of iNSC for the functional restoration after SCI when they combined with optimized concentration of silk-collagen hydrogel. We made contusion models at thoracic spinal cord using adult Sprague-Dawley rats, and the hydrogel was made from a proportion of silk and collagen, which showed optimized stiffness for cell survival and neuronal differentiation, and then mixed to iNSCs with or without laminin and injected into contused spinal cord after SCI in rats. iNSCs without the hydrogel were also transplanted into the injured spinal cord as controls. Twelve weeks after transplantation, transplanted iNSCs mixed in the hydrogel with or without laminin had survived and migrated adjacent to the injured spinal cord, and successfully differentiated into neurons and astrocytes more than iNSC without the hydrogel. As conclusion, we found that the optimized silk-collagen hydrogel increases the survival and differentiation of engrafted iNSCs after SCI, and also be useful in promoting functional recovery of spinal cord injured rats.

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T-2008

PRECLINICAL STUDIES TO ASSESS THE SAFETY AND EFFICACY OF HUMAN EMBRYONIC STEM CELLS-DERIVED ASTROCYTES (AstroRx®) AS A POTENTIAL TREATMENT OF AMYOTHROPHIC LATERAL SCLEROSIS (ALS)

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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 failure. Despite the selective MN death in ALS, there is currently strong evidence that astrocyte dysfunction plays a crucial role in disease progression. This led to the rationale that ALS could be treated by implantation of healthy astrocytes from an external source, to support or replace dysfunctional ALS astrocytes. We developed a GMP protocol for generation of clinical-grade human astrocytes from embryonic stem cells (ESC). The first stage of our protocol is derivation of astrocyte progenitor cells (APC) from hESC. These APCs can be expanded in large quantities and stored frozen as cell banks. Further differentiation of the APCs yields a highly enriched population of astrocytes (AstroRx®). AstroRx® cells express the classical astrocytic markers, such as GFAP, GLAST, GLT-1 and Aquaporin-4. *In vitro*, the cells possess the activities of functional healthy astrocytes, including uptake of glutamate, promotion of axonal outgrowth, protection of MNs from oxidative stress and secretion of the neurotrophic factors BDNF, GDNF, VEGF and IGF-1. In addition, a secretome analysis showed that AstroRx® cells also abundantly secrete several inhibitors of metalloproteases as well as variety

of other neuroprotective factors (e.g. TIMP-1&2, OPN, MIF and Midkine). In animal models of ALS, $hSOD1^{G93A}$ transgenic mice and rats, intrathecal injections of the AstroRx® delayed disease onset, improved motor performance and extended life expectancy compared to sham-injected animals. A nine-month safety study conducted in immuodeficient NSG mice under GLP conditions demonstrated the high safety profile of AstroRx® cells when injected intrathecally to the cerebrospinal fluid. The transplanted cells survived for the entire study duration and did not form teratomas or any other tumors. Biodistribution analysis of transplanted AstroRx® cells showed that the cells efficiently distributed along the brain and spinal cord. These findings demonstrate the feasibility, safety and efficacy of intrathecal injections of AstroRx® as a potential therapy for ALS.

T-2010

INSULIN-LIKE GROWTH FACTOR-1 REGULATES MOTILITY AND MIGRATORY ABILITY OF MOUSE NEURAL STEM CELLS VIA ACTIN DYNAMICS SIGNALING IN THE INJURED SPINAL CORD

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Therapeutic impact of neural stem cell (NSC) transplantation for spinal cord injury is constrained by poor survival and migration ability of grafted NSCs. We have previously demonstrated that survival of NSC grafts rapidly declines after transplantation in the lesioned spinal cord and that combined treadmill locomotor training (TMT) increased the NSC survival in part via insulin-like growth factor-1 (IGF-1) signaling. The current study sought to examine whether IGF-1 receptor (IGF-1R) in NSCs regulate migratory ability of NSCs following transplantation into the injured spinal cord in mice. TMT enhanced the extent of wild type (+/+) NSC migration from the injection site allowing more intimate contacts with host spinal cord circuitry. However, most NSCs derived from IGF-1R haplodeficient (+/-) mice were confined around the injection site regardless of TMT. We also observed that the IGF-1R (+/+) NSCs were highly motile with frequent formation of lamellipodia or filopodia during culture. This motility and migration ability was markedly decreased in IGF-1R (+/-) NSCs. To confirm IGF-1 has a role in motility and migration of NSCs, inhibitors of IGF-1R, PI3-K and ERK resulted in reduced migration of IGF-1R (+/+) NSCs in artificial wound healing assay and boyden chamber migration assay. Furthermore, protein expression of pFAK, Rac 1/2/3, pCDC42 as downstream actin signaling were diminished by inhibiting downstream IGF-1 signaling. Taken together, the induced motility and migration of the NSCs by IGF-1 appears to regulate via actin dynamics signaling.

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T-2012

AGMATINE ENHANCES NEURONAL DIFFERENTIATION OF MURINE NEURAL STEM CELLS IN VITRO

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Stem cell therapy has high therapeutic potential in CNS diseases. Spinal cord injury (SCI) has still needed optimal therapy to cure, so stem cell therapy is hopeful in SCI. In many research, neural stem cell therapy has shown meaningful effect in SCI but the lineage-specific differentiation efficiency of neural stem cell transplanted in vivo has room for improvement. Agmatine has displayed a neuroprotective effect in CNS diseases and improved functional recovery was found in spinal cord injured mice received agmatine-producing neural stem cell. These findings make us expect the synergy effect of combining neural stem cell transplantation and agmatine treatment in SCI. In this study, the effect of agmatine on differentiation of murine nerual stem cells was elucidated in vitro. Neural stem cells was induced to differentiate under favorable condition for differentiation or unfavorable condition for differentiation. Agmatine enhanced differentiation of neural stem cells without lineage-specificity under unfavorable condition. The mRNA expressions of MAP2, dcx, GFAP were increased. Under favorable condition, agmatine strengthened neuronal differentiation. Neuronal markers, MAP2 and dcx were increased but Glial marker, GFAP was decreased in mRNA expression. Oligodendrocyte marker, Olig2 had no difference in mRNA expression with or without agmatine treatment. Based on these results, it is presumed that agmatine enhance differentiation of neural stem cells transplanted in vivo. In next study, this hypothesis will be confirmed in spinal cord injury animal model.

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T-2014

DIFFERENTIAL EFFECTS OF MEK INHIBITORS ON RAT NEURAL STEM CELL DIFFERENTIATION

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MAPK/ERK pathway is important for neural stem cell (NSC) proliferation and differentiation. In the current study, we showed that MEK inhibitors, PD98059, U0126 and SL327, have unexpected differential effects on NSC differentiation. U0126 and SL327, the selective inhibitors of both MEK1 and MEK2, induced neurogenesis without affecting astrocytogenesis. In contrast, PD98059, the selective inhibitors of MEK1, promoted astrocytogenesis without changing neurogenesis levels. siRNA-induced knockdown of MEK2 induced neuronal differentiation, whereas MEK1 knockdown did not affect differentiation. These data indicate that U0126- and SL327-induced neurogenesis may be due to the inhibition of MEK2. Next, to identify the mechanisms underlying PD98059-mediated astrocytogenesis, we synthesized chemicals containing flavone structure, the core structure of PD98059. When we treated NSCs with the chemicals [flavone, compound 1 (2′-aminoflavone), compound 2 (3′-methoxyflavone), and PD98059 (2′-amino-3′-methoxyflavone)], we found that compound 2, like PD98059, increased astrocytic differentiation. Both compound 2 and PD98059 induced phosphorylation of STAT3 which leads to increase astrocyte differentiation. These data suggest that PD98059-induced astrocytogenesis is due to the 3′-methoxy flavone structure. Moreover, the current study implies that cell fate decision of NCSs by MEK inhibitors, is not exclusively mediated by inhibition of MAPK/ERK pathway but also affected by their chemical structures.

T-2016

NEURAL STEM CELL SPECIFIC MICRORNA AS CRITICAL PLAYER IN STEMNESS IDENTIFIED BY GENOME-WIDE-SCREENING

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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 the mechanism by which NSCs proliferate and differentiate for efficient modulation of in vivo neurogenesis as well as derivation of NSCs. MicroRNAs (miRNAs) are small noncoding RNAs controlling gene expression in post-transcriptional control by blocking or degrading mRNA. miRNAs play a role as control mechanisms in accordance with matching target mRNAs. In this study, we first screened out NSC specific miRNAs using real-time PCR based gene array analysis. Then, we conducted real-time PCR analysis to confirm NSC specific miRNAs in comparison to mouse embryonic fibroblasts and other stem cell lines. To evaluate the functional role of the individual miRNAs, we have induced knockdown by anti-miRNA to demonstrate effects on proliferation. In our study, we have identified the new NSC specific miRNA and found its role in NSC proliferation. Overall our finding can provide us insight of potential roles of NSC specific miRNAs in brain neurogenesis and possible usage of the miRNAs for efficient generation of NSCs in vitro.

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T-2018

SYNERGISTIC LOCAL DELIVERY OF GENE RECOMBINANT STEM CELLS AND METHYLPREDNISOLONE AFTER SEVERE RAT SPINAL CORD INJURY

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Stem cells are broadly considered promising therapeutic candidates for various diseases. Gene transfection of stem cells and stem cell-based nerve repair strategies have received extensive attention. Central nervous system diseases, such as spinal cord injury (SCI), always experience complicated dynamic pathology and are mostly incurable due to the limited self-repair capacity. Functional restoration after SCI calls for comprehensive solution for the loss of neurons, absence of extra cellular matrix and severe inflammation in the local lesion cavity, which has ranked among the most challenging projects. This study is aimed to propose an effective solution by developing a composite implantation system and locally deliver the gene recombinant stem cells as well as drugs. Firstly, BDNF gene recombinant mesenchymal stem cells (MSCs) are prepared via a systemic study of non-viral transfection system. On the other hand, the anti-inflammatory drug methylprednisolone (MP) is the only FDA-approved drug for clinical treatment of SCI, but its application has been limited by the systemic toxicity. To overcome this problem, a novel preparation of MP, MP gelatin microsphere (MPGM), is developed in this study for local implantation. Hyaluronic acid is utilized to fabricate a scaffold which is further modified by an adhesive peptide PPFLMLLKGSTR. After investigation of cytocompatibility and nerve repair functions of the scaffold, gene recombinant MSCs and MPGM are encapsulated to construct a composite implant. The composite system exhibits sustained drug release profile for more than 150 h and elicits superior regenerative effect after implantation. Inflammation is alleviated by not only the drug-loaded implant, but also the implant with blank microspheres. Through investigations on different implants, the study also reveals the respective roles of gene modification, MSCs, MP and the scaffold in nerve tissue repair.

T-2020

DELETION OF POLYCOMB PROTEIN EED IN NEURAL STEM CELLS LEADS TO MALFORMATION OF THE DENTATE GYRUS

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EED (embryonic ectoderm development) is a core component of the Polycomb repressive complex 2 (PRC2) which catalyzes the methylation of histone H3 lysine 27 (H3K27) in the process of gene silencing and chromatin condensation. Previous studies have shown that EED plays key roles in the self-renewal, proliferation and differentiation of ESCs. Due to the perinatal death of EED constitutive knockout mice, the function of EED in neurogenesis and brain development remains to be explored. Here we generate an EED cKO mouse line in which EED is conditionally ablated from the brain during neural development. Loss of EED in the brain leads to early postnatal lethality, impaired migration of granule cells, and malformation of the dentate gyrus (DG). We also found that EED is necessary for the maintenance of neural stem/progenitor cell (NSPC) pool, and EED deletion results in defects in neurogenesis in early postnatal DG. Molecularly, down-regulation of Ink4a/Arf (Cdkn2a), a downstream target of EED which is regulated by EED in a H3K27me3-dependent manner, reverses the proliferation defect of EED-ablated NSPCs. Meanwhile, overexpression of SOX11, another downstream target of EED though interaction with H3K27me1 restores neuronal differentiation ability of EED-ablated NSPCs. Collectively, our results suggest that the PRC2 component EED is critical for DG development, which sheds new light on the understanding of intellectual disability of patients with EED mutations.

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T-2022

MOLECULAR BIOSENSORS IDENTIFY CIS- AND TRANS-REGULATORS OF EARLY NEURAL LINEAGE COMMITMENT

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Transcriptional regulatory networks maintain critical levels of tissue-specific transcription factors (TF) and confer unique cellular phenotypes through regulating transcription genome-wide; however, cis- and trans-regulating elements controlling specific cellular phenotypes remain largely unknown. Sox2 is required for pluripotency in embryonic stem cells (ESC), and is persistently expressed in the developing central nervous system and neural stem cell (NSC) population. Sequences surrounding the SOX2 locus are linked to genetic risk variants in neurological diseases and psychiatric disorders; therefore, understanding the mechanism of Sox2 transcriptional regulation will unravel, not only the transcriptional regulatory networks essential for early neural lineage specification, but will also elucidate disease mechanisms. In our present study, we used CRSIPR genome engineering to identity the cis- and trans-regulators of Sox2 involved in ESC transition to NSC. These data reveal that enhancer switching regulates Sox2 transcription during ESC transition to NSC; a downstream NSC-specific proximal enhancer (PE) takes the role of the ESC-specific distal Sox2 control region (SCR, ~100 Kb downstream), and contributes to 45% of Sox2 transcription in NSC. In NSC, the Sox2 locus is surrounded by many discrete distal enhancers (DE) that have enhancer features in neural tissues; open chromatin marks (increased H3k27ac) and binding of neural specific TF. To determine the regulatory roles of these DE regions, we developed an endogenous gene reporter co-expression strategy. The fluorescent biosensor is co-expressed with the endogenous Sox2 gene and reads out altered transcription upon enhancer deletion facilitating the functional validation of a NSC-specific DE (~450 Kb away) that acts coordinately with the PE in regulating Sox2. We also applied this approach to identify trans-regulators of Sox2. We disrupted TF genes using CRISPR that have been shown to interact with SOX2 in NSC. A decrease in biosensor fluorescent intensity was observed within 24 hr of Pax6 and Hes6 gene disruption indicating these proteins are trans-regulators of Sox2 in NSC. Therefore, the biosensor approach will facilitate the discovery of additional novel cis- and trans- regulators of early neural lineage commitment.

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T-2024

NEURONAL DIFFERENTIATION OF DEDIFFERENTIATED CELLS FROM HUMAN MATURE ADIPOCYTES (DFAT)

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Stem cell therapy has been expected to overcome incurable diseases, such as a severe spinal cord injury. To date, adult stem cells are expected to be potential stem cell sources for neuronal regenerative therapy. Dedifferentiated fat cells (DFATs) are fibroblast-like cells from mature fat cells, which have high proliferative activity and multipotent differentiation capacity. We have previously found that neuronal differentiation of canine DFATs was induced by all-trans retinoic acid (ATRA) and basic fibroblast growth factor (bFGF). In this study, we investigated the neuronal differentiation of human DFATs and its characteristics. As previously reported in canine DFATs, human DFATs were treated with ATRA and bFGF. However, ATRA and bFGF failed to induce neuronal differentiation in human DFATs. On the other hand, when Factor X was added to our neuronal differentiation protocol, the mRNA expression of neuron markers increased in a time- and dose-dependent manner. Expression of the neuronal marker proteins was confirmed by an immunocytochemical analysis. On the study with the neuronal function of the cells using whole-cell patch-clamp analysis, the action potential was recorded, which was inhibited in the absence of extracellular Na+. On the Ca2+ imaging study, a high concentration of KCl and the Na+ channel activator veratridine induced an increase in intracellular Ca2+ levels in the Ca2+ indicator Fluo3-loaded cells. Acetylcholine and dopamine, neurotransmitters, also provoked an increase in intracellular Ca2+ levels, which were attenuated by muscarinic receptor and D1 receptor antagonists, respectively. In conclusion, our results provide new insights into the neuronal differentiation of the cells dedifferentiated from adult mature fat, which may contribute to the development of new cell-based treatments for neuronal diseases.

NEURAL DISEASE AND DEGENERATION

T-2026

DIFFERENTIAL GENE EXPRESSION SIGNATURE IN NEURAL PROGENITOR CELLS FROM DISCORDANT TWINS FOR CONGENITAL ZIKA SYNDROME

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Congenital Zika syndrome (CZS) causes early brain development impairment by affecting neural progenitor cells (NPCs). We derived hiPSC - NPCs from three pairs of dizygotic twins discordant for CZS. Following in vitro ZIKV infection with low initial viral burden (0.01 and 0.1 MOI), cells from affected babies show significantly higher ZIKV replication (PFU and viral copies/uL) and reduced cell growth in 2D and in 3D-neurosphere culture. RNA-Seq from NPCs derived from CZS-affected and CZS-unaffected twins, prior to ZIKV infection revealed a different gene expression signature of 64 differentially expressed genes (DEG), including mTOR and Wnt pathway regulators, key to a neurodevelopmental program. The gene with the most significant (p value = 1.45E−07, corrected p value = 0.0058, edgeR exact test) difference in expression is DDIT4L, which is an inhibitor of mTOR signaling. DDIT4L showed an average 12.6-fold lower mRNA level in the NPCs from affected twins compared with non-affected. The top most significantly enriched GO is "regionalization". Among the DEGs belonging to the regionalization GO term are FOXG1 and LHX2, which were down-regulated in affected twins compared with non-affected. Also, NPCs from the CZS-affected group showed increased activity of TSC2 (p value = 0.0056, Student's t test). After infection, cells were treated for 96 h with either 200 nM rapamycin, an mTOR inhibitor, or 100 nM bafilomycin, an indirect mTOR

signaling inductor. Bafilomycin completely inhibited viral release while rapamycin increased viral release in non-affected as well as affected twins' NPCs. Whole-exome analysis in 18 affected CZS babies as compared to 5 unaffected twins and 609 controls excludes a major gene to explain resistance or increased susceptibility to CZS development. Gene expression signatures of neural development genes may contribute to the different susceptibilities to ZIKVBR infection. Our results sheds light into ZIKVBR molecular pathology and provides important new targets for drug development that could ameliorate cellular pathology. In conclusion, our results indicate that CZS is not a stochastic event and depends on NPC intrinsic susceptibility, possibly related to oligogenic and/or epigenetic mechanisms.

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T-2028

LAQUINIMOD AS A REGULATOR OF KYNURENINE PATHWAY IN MULTIPLE SCLEROSIS

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Our research has uncovered that Kynurenine pathway (KP), the canonical route of amino acid tryptophan metabolism, is dysregulated in MS, producing neurotoxic metabolites in levels corresponding to MS severity. Nanomolar Quinolinic acid (QUIN) we showed potently kills oligodendrocytes and neurons. Laquinimod (LAQ) is a drug currently in phase III trials for relapsing-remitting/progressive MS. It's effects on the KP are currently unknown. We investigated if LAQ reduces expression of KP and QUIN levels, and also it's effects on stem cells. LAQ was tested on both cultured human monocytes and cultured mouse primary subventricular zone mouse neural stem/precursor cells. Monocytes were cultured with/without interferon-gamma (IFN-γ), the canonical inducer of KP enzymes including rate-limiting IDO-1. SVZ cells grown as neurospheres, dissociated and plated as a monolayer for experiment. A Multi-caspase kit was used to analyse apoptosis on a Muse cytometer. A p value <0.05 was regarded as statistically significant. LAQ significantly reduced basal IDO-1 expression by 18.44+6.1% compared to control. In IFN-γ-treated monocytes, LAQ increased levels of neuroprotective Kynurenic acid and significantly increased it in monocytes pre-treated for 24 hours prior to 72 hours IFN-γ. LAQ significantly reduced neurotoxic QUIN levels, and completely rescued QUIN levels in LAQ-pretreated monocytes. Rescue of apoptosis was seen in SVZ cells treated with LAQ and 4μM QUIN, restoring to control. LAQ also potentiated the reduced confluency caused by IFN-γ treatment. QUIN secretion may further compromise oligodendrocyte survival and neuronal atrophy. LAQ showed protective effects on both human monocytes and mouse neural stem cells.

T-2030

GENE EXPRESSION VARIABILITY IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases develop when cells in the nervous system begin to lose their structure or function. Many of the diseases are classified as sporadic, meaning that either they are caused primarily by environmental factors or that the genetic component involved is unknown. However, several diseases have strong genetic component which allows to study them using stem cell models. Here we used induced pluripotent stem (iPS) cells that carry a genetic mutation, differentiated them into neurons, and obtained transcriptional profiles at the single cell level. So far, most of the studies on neurodegenerative diseases have used bulk cell populations. While this allows picking up a general picture of the disease state, the details at the single cell level remain concealed. Using single cell measurements, we show that Huntington's disease (HD), as well as other neurological disorders, are characterized by a global increase in gene expression variability. We found several key pathways and genes including mitochondrial, TGF-beta signaling and NFKb which are more heterogeneous in mutant cells. Overall, we suggest that (even) late onset neural diseases may arise as a result of early transcriptional imbalance.

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T-2032

TARGETING MITOCHONDRIA DYSFUNCTION AS A PROSPECTIVE AVENUE FOR DRUG DISCOVERY IN NIEMANN PICK DISEASE TYPE C1

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Niemann-Pick Type C1 (NPC1) is a rare pediatric dementia caused by loss of function mutations of the lysosomal cholesterol transporter NPC1. The disease is characterized by sequestration of cholesterol in the lysosome, and leads to neurodegeneration and death, often in early childhood. NPC1 shares many features with Alzheimer's disease, suggesting common mechanisms of onset and progression. There is no FDA-approved treatment for NPC1, and validation of new therapeutic targets in human neurons is urgently needed. We generated a panel of human induced pluripotent stem cell (hIPSC) lines from patients with NPC1 mutations associated with variable degrees of clinical severity. Our observations in NPC1 hIPSC-derived neurons suggest that cholesterol sequestration triggers strong activation of the autophagy pathway, causing accumulation of fragmented and depolarized mitochondria. NPC1 neurons have increased levels of reactive oxygen species, suggesting mitochondrial failure is a driver of neurodegeneration in NPC1. With this knowledge, we developed a novel drug screen in NPC1 neurons, with a focus on FDA-approved compounds. We used two approaches; 1) a hypothesis-driven screen using mitoprotective compounds, and 2) an unbiased screen using compounds from the NIH Clinical Collections. We optimized high-content screening and fluorometric methods to measure mitochondrial membrane potential and cell viability. Positive hits were selected by dose-dependent rescue of mitochondrial health and viability of NPC1 neurons. We identified a subset of FDA-approved compounds that are known to cross the blood-brain barrier, and we are currently validating the two most promising compounds with the goal of repurposing them for treatment of NPC1 patients. We are examining their effect on autophagy, mitochondrial dynamics, and cholesterol storage in patient-derived neurons carrying the most common human mutation (I1061T), and in isogenic NPC1 knock-out neurons engineered by CRISPR genome editing. Our next goal is to examine pharmacodynamics and in-vivo efficacy of lead compounds through short-term trials in NPC1 I1061T knock-in mice. Our studies illustrate the value of using hIPSCs to generate innovative therapies through repurposing of FDA-approved compounds for NPC1 and related neurodegenerative diseases.

T-2034

PROBING THE ROLE OF SORL1 AND ENDOCYTIC NETWORK DYSFUNCTION IN ALZHEIMER'S DISEASE PATHOGENESIS USING HUMAN NEURONAL MODELS

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The purpose of this study is to determine whether genetic variants associated with AD risk in the SORL1 gene lead to endosomal network dysfunction and cellular AD phenotypes in human neurons. Endosomal abnormalities are documented in post-mortem AD brain tissue and multiple endocytic regulatory genes are associated with increased AD risk in population studies. SORL1 is a vesicular trafficking gene that plays an integral in role trafficking amyloid beta and the amyloid precursor protein through the endocytic network. Loss of SORL1 is documented in AD brain tissue. Our previous studies have used human induced pluripotent stem cell (hiPSC)-derived neurons to show that deficiencies in SORL1 expression induction are correlated with the presence of AD-associated variants in non-coding regions of SORL1. In this work, we hypothesize that AD-associated variants in SORL1 lead to detectable and modifiable endocytic phenotypes in human neurons. In hiPSC-derived neural cells expressing a SORL1 shRNA, we show increased amyloid beta peptides, reduced transferrin recycling, and increased size of Rab5+ endosomes. In collaboration with the UW Alzheimer's Disease Research Center, we have identified AD patients with SORL1 coding variants and are generating hiPSCs from these patients. We will assay hiPSC-derived neurons from SORL1 variant carriers for endosomal phenotypes and use CRISPR/Cas9 gene-editing to correct the variants to determine whether the predicted pathogenic variant leads to endocytic dysfunction in human neurons. This work is significant in that it will investigate a functional genotype-phenotype relationship of genetic variants in the endosomal network, which is known to be disrupted early in AD pathogenesis. Investigating this driver of disease pathogenesis and how it relates to human genetic background is critical in the development of new and precision treatments for AD.

T-2036

A DRUG SCREEN IN HUMAN IPSC DERIVED NEURONS IDENTIFIES CHOLESTEROL METABOLISM AS AN Aβ INDEPENDENT REGULATOR OF TAU PROTEOSTASIS

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that results in loss of neurons and synapses. The dementia associated with AD can be devastating for families and represents an increasing social and economic burden as our population ages. Currently there are no drugs available that can revert or even slow disease progression. Recent developments in disease modeling using human induced pluripotent stem cells (hiPSC) have allowed for the modeling of Alzheimer's disease pathologies in human neurons in a dish. These AD patient derived stem cells recapitulate several pathological hallmarks associated with AD, including increased Abeta production and hyperphosphorylated tau (pTau). We screened a drug library containing >1600 FDA approved drugs for their potency to reduce pTau in hiPSC-derived neurons from an (APP duplication) AD-patient and identified several classes of drugs that reduce pTau, including cholesterol lowering drugs. Cholesterol lowering drugs decrease both pTau and Abeta in a dose dependent manner. Interestingly, we show that APP processing and pTau are both regulated by highly correlated but independent pathways. We map the pathways that regulate cholesterol dependent decreases in pTau. Our data indicates that compared to statins, which inhibit cholesterol synthesis, cellular cholesterol export-inducing compounds decrease AD pathological processes with less adverse effects. Thus, cholesterol export-inducing compounds present an interesting new therapeutic avenue for AD-treatments.

T-2038

REACTIVE OXYGEN SPECIES (ROS) PRODUCED BY NADPH OXIDASE 3 (NOX3) MEDIATE MOUSE CEREBELLAR PROGENITOR AND NEURAL STEM CELL PROLIFERATION STIMULATED BY SONIC HEDGEHOG (SHH)

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NOX3 is a member of the NADPH oxidase family of transmembrane proteins which main function is to reduce molecular oxygen to form reactive oxygen species (ROS). ROS produced by NOX can modulate cell signaling in various physiological processes including proliferation, and migration. We selected an ENU-mutated mouse lineage that lacks motor coordination and have increased proliferation of cerebellar granule cell precursors (GCP) during early postnatal development as well as disorganized Purkinje cell layer. Genetic mapping by polymorphic microsatellite analysis and Next Generation Sequencing, identified the mutation as an A>T transversion at position 190 of the transcribed sequence of Nox3, located on mouse chromosome 17. The mutation causes the substitution of Asp by Tyr at position 64 of the protein. Using in silico analysis, we predict that Nox3eqlb will have only a beta strand formed by aminoacids 58-70 rather than two in the region 59-61 and 65-70 as it occurs in BALB/c. Because Sonic Hedgehog (SHH), secreted by Purkinje cells, is the main mitogen for neuronal cell precursors in the developing cerebellum, our goal was to study the role of NOX and ROS in the control of proliferation of cerebellar GCP and neural stem cells (NSC) stimulated by SHH signaling pathway. Nox3eqlb mice cerebellar NSC and GCP produce higher levels of ROS after 7 days in vitro. Nox3eqlb mice showed increased proliferation of cerebellar NSC until 7 days in vitro when compared to wild type BALB/c. Nox3eqlb cerebellum showed upregulation of SHH pathway members Gli1, Gli2, Gli3 and Cyclin D1, and genes involved in the cell cycle as Cyclin B1, Sox2, Akt1, Cdc25 and Rb1, and downregulation of Cdkn2a and CD133. After inhibition of NOX by apocynin, Nox3eqlb mice showed reduction in the thickness of the external granular layer, decreased expression of Gli2 and Cyclin D1 and increased expression of Gli1 and Gli3 genes and in vitro, Nox3eqlb presented reduction of cerebellar NSC and GCP proliferation. Our results suggest that ROS acts as a second messenger influencing the SHH pathway in the control of cerebellar GCP and NSC proliferation.

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T-2040

THE POTENTIAL OF A HUMAN ADIPOSE-DERIVED STROMAL/STEM CELL PROPATIES TO DIFFERENTIATION OF DOPAMINERGIC NEURAL CELLS

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Parkinson disease (PD) is a neurodegenerative disease that is attributed to a progressive loss of dopaminergic neurons within the substantia nigra. The current therapy for PD mostly relies on symptomatic treatments; therefore, it is desirable that regenerative medicine strategies will be developed for PD. Recent progress in stem cell research has raised hope for the development of stem cell therapies, which can be a valuable tool in regenerative medicine. Human adipose tissue-derived stem cells (hADSCs) are multipotent stem cells that can differentiate into various types of cells, including neuronal cells. Moreover, because of their reduced risk of tumorigenesis, and their hypoimmunogenicity and immunomodulatory effects, hADSCs are an attractive material for cell therapy and tissue engineering. In this study, we attempted to develop a dopaminergic neuronal differentiation strategy from hADSCs. We investigated a dopaminergic neuronal differentiation potential of hADSCs by introducing four transcription factors, Achaete-scute homolog 1(ASCL1), forkhead box protein A2 (Foxa2), LIM homeobox transcription factor 1 alpha (Lmx1a), and Nuclear receptor related 1 (Nurr1). Intriguingly, addition of basic fibroblast growth factor (bFGF) caused a formation of spheroid like cells when introducing Foxa2 or Lmx1a into hADSCs. Nestin expression was observed to be upregulated in these cells, suggesting that these spheroid-like cells were neuronal stem cells. Further induction of neuronal differentiation resulted in the increased expression of Tyrosine hydroxylase (TH), Neurofilament-M (NF-M), and Microtuble-associated protein 2 (MAP2) in these cells. In addition, enzyme-linked immunosorbent assay revealed that dopamine secretion was significantly increased in hADSCs introduced with Foxa2or Lmx1a. These data suggest that the hADSCs introduced with Foxa2 or Lmx1a have the possibility to differentiate into dopaminergic cells via neural stem-like cells. We also try to differentiate into dopaminergic cells from iPSCs derived from hADSCs, and found that they efficiently differentiated into dopaminergic neurons. Our study thus may help developing effective therapy for PD.

T-2044

CELL TRANSPLANTATION THERAPY EFFICACY FOR PARKINSON'S DISEASE: CONSIDERATIONS OF PROCESS AND QUALITY

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Parkinson's disease (PD) is a major debilitating disease as the population of a nation ages. The primary cause is loss of dopaminergic (DA) neurons in the substantia nigra of the midbrain. One approach to therapy is to transplant DA neurons at different stages of development, to replace those lost. With human embryonic stem cells as a starting source, we have systematically developed a process which consists of expansion as cell microcarrier aggregates, differentiation to PAX6 and SOX2 +ve neural stem cells in bioreactors. The differentiation process was further simplified using small molecule inhibitors of ALK5, which increased the yields to above 10 million cells/ml, while reducing the cost of manufacturing and decreased the time of differentiation from 16 to 12 days. Neural stem cells were subsequently harvested for further differentiation to 3 stages prior to implantation: DA progenitors (differentiated in vitro for 16 days (D16)), immature DA neurons (D25), and DA neurons (D35). One million cells were delivered as cell aggregates into mouse models of PD to determine which of these stages provided the best innervation and functional recovery. Both D25 and D35 cells showed neuronal maturation and differentiation towards TH^+ cells and satisfactory behavioral functional recovery ($p < 0.01$) in mice injured by 6-OHDA. D25 cells in particular, showed better innervation in the striatum. In addition, the cells were tracked with a radio-label DATSCAN to monitor long term in vivo dopamine production over 6 months in the PD mice. These findings provide a valuable guideline for developing a seamless bioprocess and quality control of the transplanted cells that can be use in clinical cell therapy for PD.

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T-2046

NEW METHODS FOR STUDYING NETWORK ACTIVITY IN HUMAN INDUCED-PLURIPOTENT STEM CELL (IPSC) DERIVED NEURAL CULTURES

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Neuron cultures derived from induced-Pluripotent Stem Cells (iPSC) are rapidly being used to model circuits in vitro. In these cultures, electrophysiological recordings of populations of neurons has been used to understand how structure influences network dynamics, and the ways in which diseases processes disrupt these networks and consequently alter activity. While methods that use mean firing rate or degrees of synchrony can help to identify differences in networks, they are often insensitive to the complexity of single neuron dynamics and the higher order interactions between neurons that underlie many principles of computation in vivo. One of the major challenges for studying the activity of populations of neurons, both in vitro and in vivo is that the number of possible network states scales exponentially with the number of neurons recorded. In this regard, the benefit In vitro recording methods offer in terms of allowing for the monitoring of large numbers of neurons at high temporal precision, carries with it the consequence of requiring new statistical and computational methods for describing patterns of activity. To address this challenge, we recorded large populations of iPSC-derived neurons in vitro, and then developed approaches to classify and studying network activity. First, we describe strategies for characterizing the spike count statistics of populations of cells, including estimating the entropy of the circuit (a measure of structure). Following this, we use these methods to describe the network topology that generates specific patterns of dynamics. We show that our method can identify differences in circuit structure, for instance the effect of network size and connectivity in generating different kinds of dynamics. Importantly, our methods could serve as a platform for interrogating how different neurological and psychiatric disorders alter network activity.

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CANCERS

T-2048

SEQUENCING BASED PREDICTORS OF PROGRESSION AND UNDERLYING GENETIC PREDISPOSITIONS OF MYELOPROLIFERATIVE NEOPLASMS

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Despite major advances, leukemia stem cell (LSC)-driven progression of myeloproliferative neoplasms (MPNs) remains a major cause of morbidity and mortality. While MPN initiating mutations occur in hematopoietic stem cells (HSCs), MPNs progress as a result of deregulation of stem cell properties, such as dormancy, survival and self-renewal. Because MPN research imparted the first tangible insights into the molecular pathogenesis of cancer, MPNs provide an important paradigm for decoding the evolution of cancer from its inception to progression. Here we present the first comprehensive whole genome sequencing and whole transcriptomic sequencing on samples from patients diagnosed with MPNs that correlates genomic information with biomarker measurements and other phenotype data variables. We performed both transcriptomic sequencing (RNA-seq) and whole genome sequencing (DNA-seq) on more than 100 primary patient samples from various stages of MPN. This dataset consists of samples from all subgroups of the two main categories of MPNs: BCR-ABL1^{+}chronic myeloid leukemia (CML) and BCR-ABL1^{-} MPNs that include polycythemia vera (PV), essential thrombocythemia (ET), myelofibrosis (MF) and secondary Acute Myeloid Leukemia (sAML). The RNA-seq was performed on both bone marrow and peripheral blood sorted for stem (CD34^{+}CD38^{-}), progenitor (CD34^{+}CD38^{+}) and stromal (CD34^{-}) cells. The DNA-seq was performed on CD34+ peripheral blood (90X) and on the corresponding saliva (germline control, 30X). Signaling pathway impact analysis

revealed activation of immune response pathways at the pre-malignant stages of MPNs (PV and ET), and upregulation of corresponding genes, suggesting that earlier infections could play a role in the onset of MPNs. We have also identified biomarkers for progression from the pre-malignant stages towards MF and sAML based on RNA differential gene expression and Editiome analysis and corresponding DNA mutations. We have elucidated a molecular stem cell signature of different stages of MPNs and thus provided insight into the malignant progression of disease in these patients. In this study, we present possible predictors of the progression MPNs by comprehensive DNA and RNA sequencing, coupled with germline control sequencing analysis, of cancer stem cells from patients diagnosed with MPNs.

T-2050

TARGETING MACROPHAGE THAT FORMS A CHEMOTHERAPY-INDUCED NICHE SIGNIFICANTLY REDUCES CANCER STEM CELLS AND TUMORS

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Failure in cancer therapy is often caused by relapse resulting largely from chemo-resistant cancer stem cells (CSCs). Most colorectal cancer patients, after receiving chemoradiotherapy, still harbor treatment-resistant residual tumor cells that are akin to colorectal adenoma. We therefore exploited the APC^{Min} adenoma model that allowed us to study the natural and dynamic interaction between CSCs and their microenvironment in vivo following therapeutic stress. We observed that macrophages were directly recruited from blood vessel to the CSC niche, in response to chemotherapy, playing a role in protecting and further promoting activation of CSCs. Reduction in the $Ly6c^{-}CD11b^{Int}CX3CR1^{+}$ $MHCII^{+}$ population of tumor associated macrophages (TAMs) by celecoxib or clodrosome was correlated with a decline in slow-cycling drug-resistant CSCs and tumorigenesis. Molecularly, we found that COX-2 inhibitor celecoxib suppressed Akt and Wnt signaling, downstream of macrophage-dependent PGE2-EP pathway, thus reduced CSC survival and activation. Taken together, in the naturally formed adenoma reflecting human familial adenomatous polyposis, Chemotherapy-induced recruitment of TAMs to the CSC niche protected and promoted activation of surviving CSCs via PGE_2-EP mediated Akt-Wnt signaling, leading to tumor regrowth. Targeting the TAMs will benefit clinical treatment of colorectal cancer.

Funding Source: Stowers Institute for Medical Research

T-2052

A NOVEL CONSTRUCTION OF LENTIVIRAL VECTORS THAT IDENTIFY AND ELIMINATE TUMORIGENIC CELLS FROM PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are promising sources for cell transplantation therapy. However, incomplete abolition of tumorigenicity, including teratomas and cancers, has raised safety concerns in clinical trials. Importantly, most current strategies focused on "INDIRECT" inhibition of tumorigenicity by reducing the reprogramming-associated oncogenic potential of iPS cells. Due to the intrinsic characteristics of hPSCs, these strategies may not completely eliminate the risk of tumorigenicity. Therefore, to facilitate safe clinical applications, it is necessary to develop innovative safety approaches to "DIRECTLY" eliminate undifferentiated hPSCs.Here we present a novel method for efficiently generating diverse candidates for Tumorigenic Cell-targeting Lentiviral Vectors (TC-LVs). TC-LVs has two different elements, the first element is promoter-subcloning plasmid (pPS) that has a tumorigenic cell-specific promoter that can be easily replaced by a wide range of candidates. The other is lentiviral acceptor plasmid (pLVA) that harbors the recombination cassette upstream of the unit consisting of a fluorescent gene, the 2A sequence, and a suicide gene selected from among several candidates. Our two-plasmid system achieved rapid and simultaneous construction of different TC-LVs with different promoters. To assess the feasibility of our system, we constructed two different types of TC-LVs, which have either a ubiquitously active CA promoter (TC-LV.CA) or a cancer-specific survivin promoter (TC-LV.Surv), upstream of Venus (EGFP) and Herpes Simplex Virus Thymidine Kinase (HSVtk), using the each pPSs and the same pLVA (Venus-2A-HSVtk). Nontoxic prodrug ganciclovir (GCV) exerted remarkable cytotoxicity in HSVtk-transduced hPSCs, and high specificity for undifferentiated cells was achieved using the survivin promoter (TC-LV.Surv). Moreover, GCV treatment completely abolished teratoma formation by TC-LV. Surv-infected hPSCs transplanted into mice, without

harmful effects. Thus, TC-LV can efficiently identify the best promoter and suicide gene for specific and complete elimination of tumorigenic hPSCs, facilitating the development of safe regenerative medicine.

T-2054

MiR-367 IN DIAGNOSIS AND THERAPY OF CHILDHOOD CENTRAL NERVOUS SYSTEM EMBRYONAL TUMORS

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Embryonal tumors of central nervous system (CNS) are the most fatal types of pediatric tumors occurring in young children. Previews studies correlated abnormal expression of the pluripotency factor OCT4A with poor patient survival. Overexpression of OCT4A in embryonal CNS tumor cell lines enhanced aggressive features including metastatic potential and upregulated the expression of miR-367, also involved in pluripotency. In this study, our goal was to evaluate whether inhibition of miR-367 in OCT4A-overexpressing tumor cells can abrogate the pro-oncogenic effects of OCT4A typically observed in aggressive embryonal CNS tumors. Overexpression of OCT4A significantly affected the miR-367 levels in microvesicles produced by four embryonal CNS tumor cell lines, indicating miR-367 as a possible liquid diagnostic biomarker. Moreover, miR-367 silencing in OCT4A-overexpressing tumor cells abrogated the in vitro pro-oncogenic effects of OCT4A by inhibiting cell proliferation, cell invasion and the ability to generate neurosphere-like structures enriched in CD133 expressing cells. We further assessed miR-367 inhibition in intracranial human embryonal CNS tumor xenografts in Balb/C Nude mice by three direct intratumoral injections of a specific miR-367 inhibitor. Treatment with miR-367 inhibitor attenuated intracranial tumor growth and increased overall survival of OCT4A tumor-bearing mice. These findings indicate that aberrant OCT4A expression enhance aggressiveness and stem like traits in embryonal CNS tumor cells partially through miR-367 upregulation, revealing a mechanism that could be further explored to improve diagnosis, prognosis prediction, and treatment of pediatric patients.

T-2056

TARGETING OF MESENCHYMAL STEM CELLS FROM ACUTE MYELOID LEUKEMIA USING IMMUNOMODULATORY DRUGS: A THERAPEUTIC OPTION TO CIRCUMVENT DRUG RESISTANCE AND SURVIVAL OF LEUKEMIC BLASTS

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Heterogeneity and variable survival outcomes of Acute Myeloid Leukemia (AML), suggest that yet undiscovered genes and pathways contribute to AML. Role of mesenchymal stem cells (MSC), an important component of bone marrow microenvironment, in development and progression of leukemia is not yet fully understood. Therefore, it was proposed to culture and characterize MSC from AML bone marrow and modulate their function for improving therapeutic strategies for AML. MSC were successfully cultured from high-risk AML patients (n=9) and characterized for surface marker expression by flow cytometry and ultrastructure morphology. AML-MSC demonstrated heterogenous fibroblastoid cells of abnormal shape. AML-MSC demonstrated characteristic profile of MSC Type II positive and negative phenotypic markers indicating pro-tumor nature. At ultrastructural level, MSC demonstrated increased number of elongated mitochondria, ribosomes, rough endoplasmic reticulum and numerous vesicles near leading edges suggesting transfer of cellular contents in surrounding for cellular communication. To assess MSC supported drug resistance and survival of AML blasts, these cells were co-cultured with HL-60 AML cells and further tested for chemosensitivity to AML therapeutic drugs azacytidine and lenalidomide. MSC were found to be resistant to apoptosis induction by anti-cancer drugs as compared to that shown by HL-60 AML cells. Chemosensitivity of HL-60 cells to azacytidine and lenalidomide decreased when co-cultured in presence of AML-MSC. As evident by laser confocal microscopy, increased survival of HL-60 in presence of MSC could be attributed to transfer of enormous energy in form of large mitochondria from MSC to blasts cells. There was bidirectional transfer of cellular contents between MSC and AML blasts cells. Transdifferentiating AML-MSC Type II into Type I, demonstrated increase in chemosensitivity of AML blasts in vitro coculture assay and reduced tumor volume in 50% of mice, comparable

to that shown by adriamycin in vivo AML xenograft model. Understanding MSC-AML blasts crosstalk and targeting MSC with immunomodulatory reagents may give us leads to manipulate microenvironment niche to control abnormal myelopoiesis in AML and develop targeted immunotherapy.

Funding Source: Indian Council of Medical Research, Government of India; CXCR4 antagonist peptide was a kind gift from Prof Nobutaka Fujii, Kyoto University, Japan.

T-2058

AN INDUCIBLE AND REVERSIBLE GENE KNOCKDOWN MOUSE FOR INVESTIGATING THE ROLE OF TCF7L2 IN WNT SIGNALLING

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In the intestine, canonical Wnt signalling pathway must be tightly regulated to balance stem cell proliferation for homeostasis and yet avoid a hyper-active state which leads to cancer. One level of regulation is the TCF family of transcription factors that act downstream of Wnt signal, because these transcription factors can function as both activators and repressors. However opinions differ on whether Tcf7l2, the most abundantly expressed TCF member in the intestine, has an activatory or inhibitory role in intestinal homeostasis and tumour progression. This has been difficult to resolve because germline Tcf7l2 knockouts are perinatal lethal. To this end, we have established a system in mouse that allows inducible and reversible Tcf7l2 gene knockdown. This system comprises of two transgenic mouse strains, which have been generated in-house by using the CRISPR-Cas9 genome editing technique: (1) a strain that ubiquitously expresses the tetracycline repressor in all tissues and (2) a strain that contains the tetracycline response element in close proximity to the Tcf7l2 promoter. If mouse of appropriate genotypes are treated with doxycycline, the tetracycline repressor binds to the tetracycline response element and represses transcription from nearby promoters in a dose-dependent manner. We will employ this knockdown mouse to study the role of Tcf7l2 in intestinal homeostasis and colorectal cancer. Our mouse is also generally useful to study Tcf7l2 function in other organs or disease settings.

T-2060

DOWNREGULATION OF GATA6 DRIVES THE PHENOTYPIC REPROGRAMMING IN HUMAN HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is a common cancer worldwide with aggressive clinical course. Cancer stem cells in the tumor niche possess tumor-initiating and self-renewal abilities which clinically account for tumor recurrence, metastasis and chemoresistance. Accumulating evidence has highlighted the plasticity of mature hepatocytes in HCC model. Hereby we studied the role of GATA6, a member of the GATA transcription factors, in liver cancer. GATA6 is essential for normal endoderm formation and hepatocyte differentiation. Aberrant expression of GATA6 had been reported in many malignancies particularly in endoderm-derived organs. Given that the liver originates from the endoderm layer, the clinical relevance and functional roles of GATA6 in HCC is worth further elucidation. By analysis of our HCC clinical cohort, GATA6 expression was significantly downregulated in HCC tumor tissues and this was associated with poorer tumor cell differentiation and poorer disease-free survival. With functional assays, silencing of GATA6 in HCC cell lines by a lentiviral-based shRNA approach increased cell proliferation, augmented self-renewal ability by tumorsphere formation assay, and promoted cell migration and invasion *in vitro*. Consistently, suppression of GATA6 enhanced *in vivo* tumorigenicity and self-renewal by subcutaneous injection mouse model on serial transplantation. Furthermore, knockdown of GATA6 fostered aerobic glycolysis in HCC cells through upregulating glycolytic genes expression and promoting glucose uptake. Mechanistically, the key glycolytic enzyme pyruvate kinase M2 (PKM2) was identified as a direct transcriptional target of GATA6 using ChIP-qPCR and luciferase reporter assay, consolidating the involvement of GATA6 in the rewiring of metabolism in HCC. In summary, our findings illustrated that alteration of GATA6 expression regulates oncogenic and metabolic phenotypes of HCC cells. More specifically, loss of GATA6 promotes the oncogenic reprogramming in HCC cells to undergo dedifferentiation and regain stem/progenitor cell properties. GATA6 is a potential druggable target and prognostic biomarker for HCC.

T-2062

DEVELOPMENT OF AN HYDROXYMETHYLGLUTHARYL-COENZYME A REDUCTASE (HMGCR) OVEREXPRESSION SYSTEM FOR THE STUDY OF REPROGRAMMING TO STEM-LIKE STATES IN HUMAN BREAST CANCER

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The rate-limiting enzyme in the mevalonic acid (MVA) pathway, hydroxymethylglutharyl-coenzyme A reductase (HMGCR), is deregulated in tumors, increasing the synthesis de novo of cholesterol, critical for cell survival and proliferation. However, the role of HMGCR in the induction and maintenance of stemness in both transformed and non-transformed cells is still unclear. Therefore, we set out to induce an HMGCR-on phenotype in the breast cancer (BC)-derived cell line MCF-7, to evaluate whether this phenotype facilitates the acquisition of stem-like traits in BC. With this purpose, we developed an HMGCR overexpression model taking advantage of a CRISPR-on system (dCas9-VP160), which includes expression plasmids for guide RNAs (pSPgRNAs) and a plasmid carrying the sequence coding for the dCAS9. Five guide RNAs (gRNAs) targeted to the promoter of the human HMGCR gene were designed with the informatics tools Genome Engineering Toolbox from the Zhang Lab (MIT, Cambridge, MA) and CRISPR-ERA. The gRNAs and the dCAS9 were then co-transfected into MCF-7 cells, and the levels of total HMGCR was assessed by qRT-PCR at 2 days post-transfection. The CRISPR-dCAS9 system increased HMGCR total levels in MCF-7 cells (MCF-7/CR) a x=2,46; sd=0,4-fold ($p<0,05$) when compared to transfection controls (MCF-7/TC). Interestingly, changes in HMGCR levels in MCF-7/CR and MCF-7/TC cells correlated with corresponding changes in the frequency of stem cells (R2=1), as measured by mammosphere formation assay by limiting dilution and statistical analysis with a specialized software (http://bioinf.wehi.edu.au/software/elda). Additionally, the pluripotency markers Oct4 and Nanog were increased in MCF-7/CR cells (x=1,41; sd=0,36 and x=2,87; sd=0,66 -fold, respectively) at the transcriptional level. To further study the relationship between HMGCR and pluripotency, HMGCR expression was assessed by qRT-PCR in the embryonic stem cell line hES9, and found to be increased to levels comparable to those observed in the HMGCRon models (2,25-fold vs. MCF-7 cells). These data suggest that cellular models expressing a HMGCR-on phenotype may offer useful tools for the study of metabolic phenotypes prone to acquire stem-like traits.

CHROMATIN AND EPIGENETICS

T-2064

CHROMATIN 3D STRUCTURE REMAINS UNCHANGED IN ANEUPLOID EMBRYONIC STEM CELLS

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Chromosome mis-segregation during mitosis or meiosis generates aneuploid daughter cells. It brings costs and benefits to cell fitness under different conditions, and elicits both common cellular responses and context-specific phenotypes of aneuploid cells. However, how the extra chromosomes influence the overall fitness of an aneuploid cell population growing in a particular environment is not fully understood. In recent years,

application of high-throughput techniques, such as Hi-C and ChIA-PET, have greatly facilitated studying the spatial structure of chromosomes, which is closely linked to cells' biological processes such as gene transcription, DNA replication and repair process. In this study, we combine high-throughput sequencing and FISH imaging to explore the relationship among aneuploidy, spatial chromatin organization and gene expression. We compare isogenic mouse embryonic stem cells (mESCs) that differ by one or two trisomic chromosomes (Ts6, Ts8, Ts11, Ts15, Ts6+8), which reduces the effect of genetic background when comparing samples from different individuals or cell lines. Compared to wide-type (WT) mESCs, the raw Hi-C interaction matrix of trisomic mESCs shows increased chromatin interactions both within the trisomic chromosomes and between the trisomic chromosomes and other chromosomes. However, the comparison between the column-wise sums of raw and ICE-normalized Hi-C interaction matrices shows that copy-adjusted Hi-C data are independent of copy numbers. We next compare chromatin structures between WT and trisomic mESCs at the compartment and TAD level. A/B compartments remain unchanged between WT and trisomic mESCs. WT and trisomic mESCs also have similar TAD length and TAD numbers and intra-TAD chromatin interaction patterns. Morevover, the FISH imaging shows that trisomic chromosomes stay close to their neighboring chromosomes globally as in disomic cells. These results indicate that one or two extra chromosomes in trisomic mESCs do not affect large-scale chromatin organization of both trisomic and disomic chromosomes, which suggests that the organization of chromatin 3D structure is based on relatively independent units — individual chromosomes.

T-2066

EZ-BP PROTEIN INVOLVES IN THE REGULATION OF EPIGENETIC BIVALENCY TO DETERMINE CELL LINEAGE COMMITMENT

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Bivalent chromatin domains, H3K27me3 and H3K4me3, keep developmental regulatory genes at low expression levels and pose for cell lineage commitment during differentiation. EZH2 is a core protein of PRC2 complex to catalyze H3K27me3 and essential for embryonic development. PRC2 is involved in various biological processes, including differentiation, maintaining cell identity and prolife ration, and stem cell plasticity. EZH2 has been found to be overexpressed in many types of cancers and implicated in the growth and aggression of the tumors. However, the underlying mechanisms of EZH2 regulated tumorigenicity and cell differentiation remain largely unknown. In our study, we demonstrated that EZ-BP orchestrates with EZH2 and RXR in the regulation of bivalent genes expression during differentiation of mesenchymal stem cells (MSC) and mouse embryonic fibroblasts (MEF). Knockout of EZ-BP in MSC and MEF resulted in lineage switch toward adipogenesis and osteogenesis but not chondrogenesis. EZ-BP co-localizes with EZH2 and RXR in the promoters of bivalent genes which keeps lineage-specific genes expression and poses for activation while differentiation. Depletion of EZ-BP leads to an increase of EZH2 and RXR binding which enhances H3K27me3 yet decreases H3K4m3. Mechanistically, depletion of EZ-BP results in downregulation of Gadd45a which enhances DNA methylation and recruitment of EZH2 in the promoters of lineage-specific genes. Thus, we suggest that EZ--BP can work with EZH2 exerts its important biological activity to regulate cell differentiation and cancer progression. Here, we try to figure out the biological functions of EZ-BP and the regulation mechanism on EZH2.

T-2068

DYNAMICS OF 5-CARBOXYLCYTOSINE AND ITS POTENTIAL READERS DURING HUMAN HEPATIC DIFFERENTIATION

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During differentiation the transcriptional profile of a cell must change in order to allow for lineage specification and progression into different mature cell types. Through rearranging patterns of the transcriptionally repressive DNA modification 5-methylcytosine (5mC), DNA demethylation contributes to the regulation of cell type-specific gene expression which is key to establishing and maintaining cellular identity. Oxidation of 5mC by Ten-eleven translocation (TET) proteins generates three oxidized forms of cytosine (oxi-mCs); 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Recognition of 5fC and 5caC by thymine-DNA glycosylase (TDG) and the subsequent incorporation of an unmodified cytosine via the base excision repair (BER) pathway highlights these oxi-mCs as potential indicators of active DNA demethylation. We previously demonstrated that 5caC accumulates during lineage specification of neural stem cells (NSCs) suggesting that such active demethylation pathway is operative in this system. However, it is still unknown if TDG/BER-dependent demethylation is utilised during other types of cellular differentiation. Here we analyse dynamics of the global levels of 5caC and its potential readers during differentiation of human pluripotent stem cells (hPSCs) towards hepatic endoderm. We show that, similar to differentiating NSCs, 5caC transiently accumulates during hepatic differentiation. Interestingly, the expression of Wilms' Tumour protein 1 (WT1), a developmental specific transcription factor and a putative 5caC reader coincides with the 5caC accumulation during early stages of

hepatic differentiation. Collectively, our work indicates that transient 5caC accumulation is a common feature of two different types (glial and hepatic) of cellular differentiation, suggesting that active demethylation by DNA repair may represent a general mechanism of rearrangement of 5mC profiles during lineage specification of post-mitotic cells in mammals and points to a potential role of WT1 in interpreting the oxi-mC, 5caC during hepatic differentiation.

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T-2070

CHROMATIN ACCESSIBILITY DYNAMICS DURING IPSC REPROGRAMMING

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Cell-fate decisions remain poorly understood at the chromatin level. Here, we map chromatin remodeling dynamics during induction of pluripotent stem cells. ATAC-seq profiling of MEFs expressing Oct4-Sox2-Klf4 (OSK) reveals dynamic changes in chromatin states shifting from open to closed (OC) and closed to open (CO), with an initial burst of OC and an ending surge of CO. The OC loci are largely composed of genes associated with a somatic fate, while the CO loci are associated with pluripotency. Factors/conditions known to impede reprogramming prevent OSK-driven OC and skew OC-CO dynamics. While the CO loci are enriched for OSK motifs, the OC loci are not, suggesting alternative mechanisms for chromatin closing. Sap30, a Sin3A corepressor complex component, is required for the OC shift and facilitates reduced H3K27ac deposition at OC loci. These results reveal a chromatin accessibility logic during reprogramming that may apply to other cell-fate decisions.

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ORGANOIDS

T-2074

GENERATION OF INNER EAR ORGANOIDS ENRICHED WITH MECHANOSENSITIVE VESTIBULAR HAIR CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Hair cells are specialised mechanosensitive cells responsible for mediating balance and hearing within the inner ear. They are sensitive to a range of chemical and environmental insults which may cause their deterioration or death and consequently hearing and balance problems. In mammals, hair cells are limited in numbers and importantly they do not regenerate. Human pluripotent stem cells (hPSCs) provide a valuable source for deriving human hair cells to study their development and design therapies to treat and/or prevent their degeneration. In this study we used a dynamic 3D Rotary Cell Culture System (RCCS) as a bioreactor system for deriving inner ear organoids from hPSCs. Analyses of neural differentiation and self-organisation of hPSCs-derived organoids were performed at a transcriptional and protein level through qPCR and immunofluorescence techniques, respectively. Functional properties of hPSC-derived hair cells were investigated by patch clamp recordings and morphological analyses were performed using helium microscopy. The development of inner ear accessory structures, such as the otoconia, which are indicative of a higher anatomical and functional tissue complexity, has been visualised with micro-computed tomography of derived organoids. We have derived a 3D in vitro system for modelling human inner ear development and consequently for generation of hair cells from hPSCs. The hPSC-derived inner ear organoids are of great benefit for developing therapies to enable otic tissue regeneration and also for studying developmental processes occurring within the inner ear.

T-2076

DEVELOPMENT OF AN IN VITRO MODEL OF TASTE STEM CELL REGENERATION

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Radiation therapy for head and neck squamous cell carcinoma (HNSCC) targets actively dividing cells, and whilst effective at reducing the prevalence of malignant cells, taste buds are often irreversibly affected. Taste bud loss is associated with a number of the most significant negative impactors on quality of life following radiation therapy for HNSCC, particularly following treatment for oral cavity carcinoma. Leucine-rich repeat-containing G-protein coupled receptors 5 and 6 (Lgr5 and Lgr6) have been identified as markers for cancer stem cells in the colon, and have more recently been identified on taste stem cells. However, the extent of human taste epithelial stem cell loss and regeneration following irradiation has been difficult to predict, prevent or reverse thereby underscoring the need for a tractable humanized model system. A biopsy in the region of the anterior two thirds and posterior third of the tongue was performed on consenting patients undergoing surgical resection of HNSCC. Circumvallate (CV) papillae from both malignant and non-malignant tissue were identified and dissected, with surrounding epithelium removed. CV tissue was homogenised using mechanical and enzymatic dissociation. Lgr5 positive cells were immunomagnetic bead-selected and cultured within a 3-Dimensional matrix to promote the development of organoids. The expression of stem cell markers (Lgr5, Lgr6, Bmi1, Sox2 and Wnt) and taste receptor family members (T1R1-3, TAS2R, mGluRs and ENaC) were quantified using qPCR and flow cytometry, and visualized using immunohistochemistry. Currently, there are no humanized models of taste stem cell regeneration with which to investigate taste stem cell loss and regeneration following irradiation. The development of a humanized in vitro model of taste stem cell regeneration may be utilized to evaluate the effectiveness of FDA-approved therapies to promote taste stem cell regeneration.

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T-2078

MODELLING PANCREATIC CANCER THROUGH PANCREATIC EXOCRINE ORGANOIDS USING PANCREACULT™ SERUM-FREE MEDIUM

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Despite the prevalence and urgency of pancreatic cancer research throughout the previous decades, a method for the long-term in vitro maintenance of pancreatic exocrine tissue as 3-dimensional (3D) organoids has been described only recently (Huch et al., 2013). Pancreatic exocrine organoids are composed of a polarised monolayer epithelium that retains many of the features of in vivo exocrine pancreatic tissue, and thus can serve as a physiological model system to address diverse research questions related to pancreatic development, stem cell biology, secretory function, and disease modelling. We have developed PancreaCult™ Organoid Growth Medium (Mouse), a defined cell culture medium for the initiation and long-term maintenance of pancreatic exocrine organoids. To establish these cultures, resected mouse pancreatic tissue was enzymatically dissociated to liberate ductal fragments that contain the putative stem cell niche. When embedded into Corning® Matrigel® domes and cultured in PancreaCult™, these ductal fragments formed spherical organoids within 1 week (n = 59 mice). Established organoids were passaged weekly at an average split ratio of 1:25 and maintained in continuous culture for > 1 year. Expanded organoids were also cryopreserved using CryoStor® CS10 for long-term storage. Pancreatic exocrine organoids cultured in PancreaCult™ are composed of cells expressing genes specific for pancreatic stem cells (Lgr5), progenitor cells (Pdx1, Sox9), and ductal cells (Car2, Muc1, Krt19, Cftr). Additionally, we observed that primary and metastatic tumor cells isolated from Kras+/LSL-G12D; Trp53+/LSL-R172H; Pdx-Cre mice and cultured in PancreaCult™ generate tumour organoids that recapitulate the features of the original tumour, thus providing a model system to study ductal pancreatic carcinoma (Boj et al., 2015). Due to the robust growth of pancreatic exocrine organoids in PancreaCult™ and their close resemblance to the in vivo pancreatic epithelium, this

organoid technology can complement or replace other experimental methodologies for studying the exocrine pancreas and could reduce or even eliminate the need for animal experimentation.

T-2080

MODELING AMYLOID BETA AND TAU PATHOLOGY IN HUMAN CEREBRAL ORGANOIDS

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Alzheimer's disease (AD) is the most common cause of late-life dementia and a significant public health threat. AD is characterized by progressive memory and cognitive dysfunction associated with various alterations in the brain, including synaptic abnormalities, neuronal death, brain inflammation and the accumulation of protein aggregates in the form of extracellular amyloid-beta (Aβ) plaques and intracellular tau neurofibrillary tangles (NFTs). However, the role of these abnormalities in human brain cells is not fully understood due to a lack of an appropriate model system. Several studies have supported the usefulness of patient-derived induced pluripotent stem cells (iPSCs) to model diverse aspects of AD. However, the relevance of studies in two-dimensional cell cultures is limited by the fact that AD pathology is intricate and involves diverse cell types, complex tissue structures and cellular pathways. Here, we report the development of a new model to study AD pathology, consisting of cerebral organoids (COs) produced from human iPSCs. Under our experimental conditions, COs grow to form three-dimensional structures containing neural areas with a human cortical-like organization. Analysis of COs by histological and biochemical methods revealed that COs produced from patients affected by familial AD (fAD) or Down syndrome (DS) spontaneously develop pathological features of AD, including the accumulation of structures highly reminiscent to the Aβ plaques and NFTs found in the brain of patients. In addition, increased cell death was observed in the COs containing the largest quantities of Aβ and tau pathology. These pathological abnormalities were not observed in COs generated from several controls, including human iPSCs from healthy individuals, human iPSCs from patients affected by Creutzfeldt-Jakob disease, mouse embryonic stem cells (ESCs) or mouse iPSCs, suggesting that the development of AD pathology in COs is specific for cells coming from patients genetically predisposed to the disease. Our study indicates that, in addition to their potential to model developmental disorders and diseases, COs are useful to study age-related neurodegenerative diseases. This system provides a new and relevant platform for the discovery of novel targets and screening drugs for an effective therapy.

Funding Source: This project was funded by a pilot grant from the University of Texas Brain Initiative Program and from The George and Cynthia Mitchel Center for research in Alzheimer's disease and related brain disorders.

T-2082

UNBIASED IDENTIFICATION OF TUMOUR SUPPRESSOR FUNCTION BY POOLED CRISPR/CAS9 SCREENING IN HUMAN COLON ORGANOIDS

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Prognosis of colorectal cancer (CRC) and prediction of individual therapy responses are hampered by a highly heterogeneous spectrum of somatic mutations. Stem cell-derived organoids allow phenotypic testing, but functional genetic screens have not yet been reported using patient-specific 3D cultures. In order to facilitate unbiased and high throughput genetic testing, we have developed a protocol for pooled lentiviral CRISPR/Cas9 library screening in human colon organoids. To benchmark the performance, we designed a training library to screen for loss-of-function mutations that confer resistance to TGFβ-induced growth arrest. By next generation sequencing, our in vitro experiments show a robust enrichment of positive control gRNAs, confirming both sensitivity and library saturation. Parallel screening using traditional 2D cell lines revealed that existing design algorithms do not reliably predict gRNA functionality in organoids. Using organoid xenotransplantation we have then adapted our protocol to the complex biological selection in the tumor microenvironment. To this end, we screened a custom pan-cancer tumor suppressor library (of > 2500 sgRNAs) using an engineered, premalignant organoid line ($APC^{-/-}$; KRAS-G12D genotype). Analysis of individual tumors and replicate pools from several mice showed a recurring set of tumor suppressors of

both known and unknown function in CRC. We now take advantage of this collection of growth-promoting gRNAs to perform single and multiplex loss-of-function experiments to study their combinatorial effects for tumor progression. Together, we report a powerful organoid platform for pooled CRISPR/Cas9 screening in vitro and within a tissue context as an important step towards patient-specific functional genomics.

T-2084

DISSECTING HUMAN LINEAGE RELATIONSHIPS IN KIDNEY ORGANOIDS DERIVED FROM FLUORESCENT REPORTER INDUCED PLURIPOTENT STEM CELLS

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The capacity to create a model of the developing human kidney in vitro provides a unique opportunity to better understand nephrogenesis at the molecular level. Here we begin to characterize lineage relationships during human nephrogenesis through the generation of complex 3D kidney organoids derived from human induced pluripotent stem cells (iPSCs) that have been genetically modified to permit fate-mapping of specific cell lineages in real-time. Human iPSCs lines suitable for fate-mapping studies were generated by inserting a dual fluorescence cassette (loxP-flanked EGFP and adjacent mCherry reporter genes) within the "safe-harbor" GAPDH locus (GAPDHdual). Upon expression of Cre recombinase, cells switch from constitutive EGFP expression to constitutive mCherry expression. To interrogate the nephron lineage within human kidney organoids and validate the utility of GAPDHdual iPSCs for fate-mapping studies, we used CRISPR/Cas9 to insert the Cre recombinase gene within the endogenous SIX2 locus of GAPDHdual iPSCs (GAPDHdual:SIX2Cre). Following the derivation of kidney organoids from GAPDHdual:SIX2Cre PSCs, mCherry-expressing cells were detected from day 10 of differentiation, coinciding with endogenous SIX2 expression. The proportion of mCherry+ cells increased steadily thereafter with a corresponding loss of EGFP-expressing cells. Importantly, subsequent analysis by immunofluorescence revealed that mCherry+ cells predominantly contributed to ECAD+ epithelial structures characteristic of the developing nephron. In contrast, mCherry+ cells were excluded from GATA3+ collecting duct. This is consistent with the lineage relationships previously demonstrated in murine kidney development. Our findings validate lineage relationships of nephrons during human development and demonstrate the feasibility of using reporter/Cre-driver iPSCs for fate-mapping studies in developing kidney organoids. We are currently using CRISPR/Cas9 to facilitate knock-out of specific genes in GAPDHdual:SIX2Cre iPSCs to interrogate the requirement for specific genes in the formation of human nephrons. We also propose to generate several other Cre-drivers to interrogate lineage relationships of other cellular compartments that remain largely unknown.

T-2086

GENERATION OF LIVER ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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Organoids, a miniaturized three-dimensional (3D) organ-like structure which is self-organized from pluripotent stem cells (PSCs) hold a great potential for both disease modeling and drug screening. Recent studies successfully described the generation of organoids recapitulating actual organ morphogenesis and development such as brain, lung, and intestine. Here 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, 3D liver organoids are superior to hepatocytes which were typically differentiated via 2D condition in terms of their functionality. Our novel approach for robustly generating 3D liver organoids might serve as a useful platform for understanding various liver diseases as well as drug discovery.

TISSUE ENGINEERING

T-2088

DEVELOPMENT OF A NEW EGF - ENRICHED COLLAGEN COATED BIOMATERIAL ASSOCIATED WITH HUMAN KERATINOCYTES FOR USE AS A SKIN SUBSTITUTE

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The replacement of damaged skin areas, such as is found in major traumas and burns, is a challenge for tissue engineering as currently available treatments are insufficient for preventing scar formation and promoting complete tissue healing. Looking for such a skin substitute, the aim of the current study has

been to produce an EGF enriched biomaterial using PDLLA polymer and Type 1 Collagen for use as a scaffold for tissue engineered skin substitutes. For this proposal, scaffolds were constructed by the coaxial electrospinning technique and divided into 3 groups: 1) PDLLA, 2) PDLLA/EGF (coaxial fiber with EGF/albumin solution core) and 3) PDLLA/Collagen (a PDLLA/EGF scaffold with Type 1 collagen coating). Immortalized human keratinocytes (HaCaT) were seeded on top of the scaffolds. The groups were evaluated for cell viability and cytotoxicity on days 1, 7 and 14. As a result, on day 1, cell viability was greater in the PDLLA/Collagen scaffolds with an absorbance of 0.62230.059 in comparison with 0.34930.063 for the control (PDLLA scaffold) and 0.50730.102 for the PDLLA/EGF scaffold. On day 7, the absorbance for the PDLLA scaffold was 0.48630.140, the PDLLA/EGF group was 0.61630.004 and the PDLLA/Collagen was 0.79330.175. On day 14, absorbance for groups 1, 2 and 3 were 0.47430.081, 0.44230.020 and 0.41030.030, respectively. In terms of the biological analysis, the PDLLA/Collagen group showed the best results for cell viability tests up to day 7, with no significant difference on day 14. Cytotoxicity assays through LDH measurements made on days 1, 7 and 14 showed statistical significance ($p<0.05$) when comparing the PDLLA group with the PDLLA/EGF and PDLLA/Collagen groups on day 14. In conclusion, the PDLLA/EGF and mainly the PDLLA/Collagen groups, showed good results for cell growth, with the presence of viable cells. New in vitro studies are currently being performed to evaluate the full potential of this new biomaterial, which could become an option for skin substitute studies.

Funding Source: MCTIC, CNPq and Stem Cell Research Institute.

T-2090

MODELING THE CONGENITAL HEART CONDITION OF PULMONARY ATRESIA WITH INTACT VENTRICULAR SEPTUM USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Congenital heart disease (CHD) is the most common birth defect, affecting about 1% of live births. Pulmonary atresia with intact ventricular septum (PAIVS) is a cyanotic congenital cardiac malformation characterized by an atretic pulmonary outflow and varying degrees of tricuspid valvar and right ventricular hypoplasia. Its genetic basis is ill-defined. Furthermore, despite surgical and/or catheter interventions, right ventricular dysfunction remains an important long-term concern. To gain novel insights of this malformation, blood samples from 4 PAIVS patients and 2 healthy subjects were collected and episomally reprogrammed into human induced pluripotent stem cells (hiPSCs), followed by systematic characterization of single-cell transcriptomes and functional assessment of the patient and control-specific hiPSCs, their derived ventricular cardiomyocytes (vCMs), as well as 2D and 3D engineered cardiac constructs. Comparison of the single-cell transcriptomes of hiPSCs of patients with those of healthy subjects revealed up- and down-regulation of 45 and 53 genes in patients, respectively. Pathway analysis of these differentially expressed genes showed that the TGF beta signaling pathway is the top under-represented pathway, with the left/right asymmetry genes LEFTY1, LEFTY2 and NODAL being significantly downregulated, suggesting their involvement in the development of the disease. Measurement of the developed forces in human ventricular cardiac tissue strips (hvCTS) fabricated from the PAIVS and normal hiPSC-vCMs showed the absence of difference in the magnitude of force generated. However, human ventricular cardiac anisotropic sheets (hvCAS) fabricated from PAIVS hiPSC-vCMs showed a slower action potential upstroke as well as a decrease in conduction velocity when compared with those derived from control. Further systematic analyses would enable us to subtract out changes and effects secondary to anatomical alterations in PAIVS and identify bona fide underlying basis of the condition. Taken together, our findings provide novel understanding of this congenital cardiac malformation from the developmental and functional perspectives.

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T-2092

SOX-9, 6-TRANSFECTED ADIPOSE STEM CELLS TO TREAT OSTEOARTHRITIS

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The treatment modalities currently used to treat osteoarthritis (OA) have several shortcomings, and stem cell therapy has been investigated to address these limitations with respect to the regeneration of articular cartilage (AC). The authors developed SOX-9, 6-transfected human adipose stem cells (SOX-9, 6-ASCs) to treat OA and tested their effectiveness and safety at preventing OA progression when injected intra-articularly (IA) in murine and caprine models. Human adipose stem cell (hASC) transfection efficiencies averaged 92.3%. SOX-6 and SOX-9 protein and mRNA

levels were markedly higher in SOX-9, 6-ASCs than in negative controls. Proteoglycan productions, as determined by GAG/DNA ratios and Safranin-O staining, were similar in SOX-9, 6-ASCs and hASCs treated with TGF-β2 and BMP-7 (positive controls). The mRNA expressions of COL2A1 and aggrecan were 3.5-fold (p = 0.004) and 1.4-fold (p = 0.058) higher, respectively, in SOX-9, 6-ASCs than in positive controls. In rat knee joints injected IA with SOX-9, 6-ASCs, fluorescence imaging showed no signal at 42 days after injection, and a biodistribution study demonstrated the absence of any human DNA from day 14. Furthermore, when nude rats were injected subcutaneously or IA with SOX-9, 6-ASCs, no evidence of toxicity or tumorigenicity was detected. In a surgically-induced goat model of OA, IA SOX-9, 6-ASCs at a dose of 0.6 x 107 best preserved AC and produced significantly better macroscopic and microscopic scores than negative controls in femoral and tibial articular surfaces.Summarizing, SOX-9, 6-ASCs produced chondrogenesis in vitro in a manner comparable to growth factor treatment. Furthermore, IA injection of SOX-9, 6-ASCs lead to the arrest of surgically-induced OA in goats. Accordingly, the present study suggests SOX-9, 6-ASCs offer a potential novel strategy to treat OA.

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T-2094

IN SITU FORMING HYDROGEL FOR OSTEOGENIC DIFFERENTIATION OF HUMAN PERIODONTAL LIGAMENT STEM CELLS

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We prepared a covalently bone morphogenetic protein-2 (BMP2)-immobilized hydrogel that is suitable for osteogenic differentiation of human periodontal ligament stem cells (hPLSCs). BMP2 covalently immobilized on an inectable hydrogel (MC-BMP2) was prepared quantitatively by a simple biorthogonal reaction between alkyne groups on BMP2-OpgY and azide groups on MC-N3. In vivo osteogenic differentiation of hPLSCs in the MC-BMP2 formulation was confirmed by histological staining and gene expression analyses. Histological staining of hPLSC-loaded MC-BMP2 implants showed evidence of mineralized calcium deposits, whereas hPLSC-loaded MC-Cl or BMP2-OpgY mixed with MC-Cl, implants showed no mineral deposits. Additionally, MC-BMP2 induced higher levels of osteogenic gene expression in hPLSCs than in other groups. In conclusion, the injectable in situ-forming MC-BMP2 hydrogel investigated here may be used for noninvasive administration of therapies for debilitating orthopedic conditions.

T-2096

COMPLETE ARTERIO-VENOUS RE-ENDOTHELIALIZATION OF GROWTH-FACTOR PRELOADED RAT AND HUMAN KIDNEY SCAFFOLDS USING HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOTHELIUM

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The bioengineering of a replacement kidney has been proposed as an approach to address the growing shortage of donor kidneys for the treatment of chronic kidney disease. One approach being investigated is the recellularization of kidney scaffolds.Here we present several key advances towards successful re-endothelialization of whole kidney matrix scaffolds from both rodents and humans. Human and rodent kidneys were decellularized with 1% SDS and 0.1% triton-X to obtain kidney scaffolds. Kidney scaffolds were then pre-loaded with vascular growth factors. Human induced pluripotent stem cells (iPSC) were differentiated into endothelial cells in large scale culture and were reseeded in preloaded kidney scaffolds (rat n=3, human n=1) via a novel aterio-venous delivery system. Based on the presence of preserved glycosoaminoglycans within the decelullarized kidney scaffold, we showed a significant increase in iPS-derived endothelial cell adherence and survival after pre-loading of the vascular matrix with VEGF and angiopoietin 1 on slices of human kidney scaffold. Moreover, using a novel simultaneous arterio-venous delivery system, we report re-endothelialization

of the kidney vasculature, including the glomerular and peritubular capillaries, using human iPSC-derived endothelial cells in both rat (n=3, 100% endothelial coverage) and human (80% coverage) decellularized scaffolds after 48 hours, and for prolonged culture in rat scaffolds (13 days, 80% coverage) with endothelial cell proliferation (36% of cells) Here we show the feasibility of re-endothelialization of both rodent and human kidney scaffolds with sufficient iPSC derived endothelial cells . These major advances move the field closer to a human bioengineered kidney.

Funding Source: RECellularizing ORgan Donors for KIDney bioengineering (RECORD KID, Dutch Kidney Foundation Projectcode 15RN02); EU 2007-2013 Seventh Framework Program (Stem cell based therapy for kidney repair; STELLAR; grant number 305436)

T-2098

INVESTIGATING TISSUE TOPOGRAPHIC INFLUENCE ON CELLULAR PHENOTYPE WITH TISSUE ENGINEERED SCAFFOLDS

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Human small intestine possesses a distinct villus-crypt topography in which differentiated cells reside in the villi whereas the proliferative cells reside within the cypts. Each crypt contains multiple stem cells that are interspersed between Paneth cells in a specific pattern along the bottom of these crypts. Paneth cells are a crucial part of the intestinal stem cell niche and, in response to multiple stimuli; they supply the stem cells with cues guiding their activity and maintenance. Both stem and Paneth cell phenotypes are related to their localization in these crypts. However, it is not fully clear why stem and Paneth cells are located at the very bottom of these crypts, and whether such localization is necessary for their maintenance and function. In pursuit of these questions, bioengineered scaffolds containing crypt-villus like topography that replicates key features were fabricated using different biomaterials, including hydrogels. With these bio-engineered scaffolds we are addressing the role of mechanical forces, and extracellular matrix constituents in maintaining crypt function and intestinal tissue renewal. Our ultimate goal is to provide insight into whether their localization is an important factor for the stem cell identity and function.

T-2100

BIOENGINEERED SKELETAL MUSCLE FOR HIGH-THROUGHPUT FUNCTIONAL SCREENING

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In vitro three-dimensional cultures systems are emerging as novel tools with which to study tissue development, organogenesis and stem cell behaviour ex vivo. These tissues promote higher levels of cell differentiation and tissue organisation and can recapitulate tissue-tissue interfaces and mechanical microenvironments of living organs; allowing the study of human physiology in an organ specific context. We recently published a high-throughput micro-tissue screening platform that enables the culture of human cardiac organoids 1. A fundamental advantage of our system is its real-time functional readout; analysing active contractile force in a semi-automated manner. We have now adapted this system to generate human skeletal muscle organoids. After screening approximately 100 conditions, we identified a serum-free, directed differentiation approach to rapidly generate aligned, multi-nucleated myotube bundles from myoblasts. Skeletal muscle organoids express striated desmin and titin, are surrounded by a laminin rich-matrix, have a resident PAX7+ satellite cell population, display a mixture of slow and fast fibre types and contract appropriately to electrical stimuli; recapitulating the features of intact human skeletal muscle. Additionally, skeletal muscle organoids could be used to mimic the effects of exercise. Organoids were treated with an adenovirus encoding a light-sensitive opsin, after a pulse of light, myotubes depolarise, causing muscle contraction. By stimulating the organoids at 0.2HZ for 2hrs a day, we observed the hallmarks of exercise, including an increase in active force and myotube hypertrophy. This approach provides unprecedented access to human skeletal muscle; in order to study human physiology, exercise adaptations and pathological processes. 1 Mills, R. J. et al. PNAS 114, E8372 (2017).

T-2102

3D HUMAN NEURAL CELL CULTURE USING TAILORED POROUS POLYMER SCAFFOLDS

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Cells cultured in a flat, stiff 2D environment often results in cultures with irregular cell morphology and compromised cell-cell interactions. This can be detrimental in the translation of research outcomes from in vitro models, and is particularly evident in the

poor conversion and success of new neurological drug candidates. Conversely neural cells cultured in 3D, typically with aid of a scaffold, have shown improved physiological relevance in terms of terminal differentiation, ion channel function and electrical activity. Porous polymers can be easily manufactured, stored over long term, allow more natural cell migration and give easy access for nutrient and waste diffusion. Here is presented a novel polyethylene glycol diacrylate (PEGDA) crosslinked porous polymeric tissue engineering scaffold with mechanical properties specifically tuned to be comparable to that of mammalian brain tissue. This material is also designed to exhibit enhanced nutrient absorption ability and optical transparency for improved 3D cellular growth and imaging. We have demonstrated the attachment, proliferation and terminal differentiation of human iPSC- and ESC-derived neural progenitor cells (hNPCs) throughout the laminin-coated pores of this scaffold. Here we present phenotypic and functional analyses demonstrating that this material supports the differentiation of hNPCs to a mixed population of viable neuronal and glial cells for periods of up to 4 weeks. This is seen by the RNA upregulation of TUBB3, MAP2, SYP and GFAP as well as positive immunohistochemical detection for TUBB3, NEUN, MAP2 and GFAP. Functional maturity of these cells was tested by calcium imaging after extended differentiation culture periods. This novel construct has potential application as an improved in vitro human neurogenesis model for utility in platform drug discovery programs.

T-2104

ASSESSMENT OF CLINICAL GRADE 3D BIOPRINTED HUMAN ENDOMETRIAL STEM CELL (EMSC) ON MELT ELECTROSPUN MESHES IN A MOUSE MODEL

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Despite considerable advances in stem cell-based tissue engineering, its successful delivery in clinical settings remains challenging. Recently, bioprinting of cells has emerged as a key method for its customization, reproducibility and potential to retain a higher density of cells at the injury site for modulating the immune response and promoting tissue repair. In this study we fabricated 3D printed poly ε-caprolactone (PCL) scaffolds using melt electrospinning (MES) at different temperatures and extrusion speeds using a GMP clinical grade GESIM Bioscaffolder. We also developed an Aloe Vera-Sodium Alginate (AV-ALG) based bio-ink for bioprinting and controlled delivery of eMSCs known for their excellent regenerative capacity in the endometrial lining of the uterus following menstruation. Our team discovered eMSCs and identified a unique marker, SUSD2 to isolate these rare perivascular cells. Herein, eMSCs were genetically labelled with the m-Cherry gene and bioprinted on 3D MES printed scaffolds using optimized AV-ALG bio-ink; 1% ALG and 5% AV. Cell-laden bio-ink constructs (ALG-AV-eMSCs) were crosslinked with 1% calcium gluconate and were subcutaneously implanted in NSG mice (n = 4). The pre and post-implantation mechanical properties of MES scaffolds were assessed by electron microscopy and atomic force microscopy. Our results showed the lowest pore size (144 3 64 µm) and strand thickness (129 3 41 µm) for MES scaffolds at 100°C and speed (20 mm/sec). Bio-ink stability and the expected cellular response were both optimal at 1% for AV-ALG. Tissue integration and immune modulation of the implanted scaffolds were assessed histologically for multinucleated cells and by immunofluorescence for type I, III collagens, and macrophage markers F4/80, CCR7, CD 206. Our results highlight the potential of 3D bioprinted cells in overcoming current limitations of clinical grade cell delivery methods and may find application in unmet medical needs such as pelvic organ prolapse.

T-2106

3D CULTURE OF HUMAN DIRECT REPROGRAMMING NEURAL STEM CELLS WITHIN FUNCTIONALIZED PRP AND RECOMBINANT SPIDROIN SCAFFOLDS

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Three-dimensional (3D) in vitro models of cell culture are necessary for mimicking the extracellular matrix for favorable neural cell growth. There are two main properties that are satisfied by all tissue engineering structures such as biocompatibility and biodegradability. One of such scaffolds is spidroin, a protein of the spider dragline. This protein has been shown to facilitate NSC/NPC adhesion and to enhance neural differentiation due to its non-specific GRGGL sequence, as well. Besides, hydrogels are most suitable for the reconstruction of

the extracellular matrix due to the high water content, biocompatibility and simple tuning. Among those, the most promising hydrogel is platelet-rich plasma (PRP). A number of studies have shown that PRP, due to the presence of different growth factors and thrombocytic exosomes and other signal molecules, provides neurogenesis, axon growth and migration of Schwann cells, which allow considering it as a prospective source of autologous growth factors and a biomimetic scaffold creating the necessary microenvironment, in particular, for peripheral nerve regeneration. The aim of this study was the development of a 3D construct based on neural progenitor cells prepared via direct reprogramming (DrNPCs-01), PRP and anisotropic complex scaffold from recombinant spidroins (rS1/9 + rS2/12) and polycaprolactone (PCL). As a result, we have shown that the two-component matrix we have developed, consisting of a PRP hydrogel-based "liquid" component with a neurobasal medium and an anisotropic rSPCL scaffold, essentially increases DrNPCs' proliferation and neural differentiation, due to complex interaction of PRP components and creation of a 3D biomimetic space from PRP biocompatible with DrNPCs and rSPCL. The anisotropic rSPCL not only provides adhesion of the hydrogel with DrNPCs to the surface, but also has a function of a guiding scaffold which determines the necessary vector of axon growth. The developed construct may by applied both as a conduit for peripheral nerve regeneration and in the creation of a regenerative technology for spinal cord injury treatment.

Funding Source: The study was financially supported by the Russian Science Foundation, Grant No. 16-15-10432.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

T-2108

PUBLIC ATTITUDE ON GENE EDITING OF THE HUMAN EMBRYO

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CRISPR/Cas9 technology is now available to facilitate gene editing, and has recently been applied to embryos in the laboratory in a number of countries. Legislation governing the use of gene editing in human embryos varies across the globe. A 2002 law in Australia prohibits making heritable changes in human embryos, regardless of whether the treated embryo is discarded thereafter. In this study we sought to begin to understand public opinion in Australia about this matter, using a questionnaire given to the public audience attending a Q and A panel of experts during last year's national Science Week. We found majority of the public in support for allowing (i) heritable changes for health purposes (ii) gene editing for clinical trials in both adults and children and (iii) gene editing to be carried out in both human and animal embryos. In contrast, the majority of respondents were not supportive of allowing heritable changes to embryos for cosmetic or non-health related purposes. A recent study in the US found that public are largely in favour of changing law to permit scientists to modify embryos for eliminating genetic disease, provided the technology is used to prevent a child inheriting a genetic disease but not for purposes of enhancing appearance or intelligence. Our findings suggest that public opinion on whether scientists should be permitted to use gene editing technology is inconsistent with current policy. If this is confirmed in a larger survey of the population, we suggest the existing Australian law should be reviewed to allow scientists more freedom to investigate the potential of this technology to improve human health. This is the first study of its kind in Australia to collect public opinion of gene editing of the human embryo.

T-2110

THE PATENT APPLICATION TRENDS OF DISEASE-SPECIFIC CELLS IN INDUCED PLURIPOTENT STEM CELL (IPSC) TECHNOLOGY IN US AND JP APPLICATIONS

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To investigate the patent application trends in disease-specific cell technology related to iPSCs in US applications and JP applications through 2016. We conducted keywords search in claims of published patent applications (applications) filed in USPTO (United States Patent and Trademark Office) and JPO (Japan Patent office) published from January 1, 2006 to December 31, 2016, and extracted the applications on disease-specific cells related to iPSCs. Furthermore, we categorized the extracted applications into 4 fields, genetic engineered cells, patient derived cells, drug screening methods and diagnosis methods, and investigated the target disorder in 4 fields. The number of applications on the iPSCs technology was 1260 in the US applications and 672 in the JP applications, of which about 5% were the applications on the disease-specific cells related to iPSCs. The applications on disease-specific cells were filed by applicants of the United States, Japan, France, Korea and Canada. More than half of the applications on the disease-specific cells were the applications on genetic engineered cells (65% in the US applications and 55% in the JP applications), and the target disorder in the disease-specific cells was neurological disorders (40% in the US applications and 34% in the JP applications). US applicants filed 56 applications in the US applications and 19 applications in the JP applications; most of applications were filed in the

field of genetic engineered cells, and 11 target disorders were the subjects. Although the number of applications was small, JP applicants mainly filed the applications on drug screening methods targeted 4 disorders, FR applicants filed the applications on genetic engineered cells drug screening methods, KR applicants filed the applications on patient derived cells, and CA applicants filed the applications on genetic engineered cells. It has been reported in 2008 that iPSCs were generated from patients with 10 diseases. In this search, the number of the applications on genetic engineered cells were more than half of the total applications on disease-specific cells. From now on, the disease-specific cells combining iPSCs technology and genome engineering will be used for drug development and disease modeling.

Funding Source: This work was supported by Highway Program for Realization of Regenerative Medicine of The Japan Agency for Medical Research and Development (AMED).

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

T-2114

MESENCHYMAL STROMAL CELL CLEARANCE BY ALVEOLAR MACROPHAGES IS CRITICAL FOR THEIR THERAPEUTIC EFFECTS

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Mesenchymal stromal cells (MSCs) are being used to treat a broad range of systemic and organ-specific inflammatory conditions. Yet, the mechanisms underlying their broad and long-ranging therapeutic effects remain unclear, since the vast majority of MSCs die in the lungs shortly after infusion. Therefore we sought to understand how MSC entrapment in the lung and subsequent death lead to anti-inflammatory effects. Tracking the survival of human and mouse MSCs in mouse models lacking various immune cell components, we demonstrate that entrapped MSCs are being cleared by alveolar macrophages. Selective depletion of alveolar macrophages abrogated the therapeutic effects of MSCs in lung inflammation. Our data indicate that the interactions with alveolar macrophages are a key requirement for the immunosuppressive function of MSCs. Unsupervised RNA sequencing analysis revealed that MSCs upregulate alveolar macrophage functions involved in cell clearance and downregulate those involved in driving inflammation. Our studies provide insight into how entrapped and dying MSCs modify the host immune response to therapeutic effect. Understanding this process is crucial for the design of smarter and more efficacious MSC-based therapies.

Funding Source: Tracy Heng is supported by an NHMRC Career Development Fellowship APP1107188.

T-2116

SECRETORY PRODUCTS FROM CHICK BONE MARROW CELLS PROMOTE PROLIFERATION AND OSTEOGENESIS OF HUMAN ADIPOSE-DERIVED STROMAL CELLS

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This study was conducted to confirm the effects of the medium conditioned with chicken bone marrow cells (BMCs) on proliferation and osteogenic differentiation of human adipose-derived mesenchymal stromal cells (hADSCs). Four-day-old white leghorn chicks were used as the BMC donor and the BMCs isolated from the femurs were initially cultured for 3 days in 10%(v/v) fetal bovine serum-containing, DMEM/F12-based media. For conditioning of the medium, BMCs were cultured in a serum-free condition and the conditioned media were collected when the BMCs was reached 100% of confluency, usually 3 days after culture. The conditioned media were then concentrated as 100 times by using centrifugal filter and subsequently provided for hADSC culture. The proliferation of hADSCs was checked every two days after seeding by a cell counting kit-8 (CCK-8) assay, while osteogenic differentiation was evaluated by Alizarin Red S staining. Cell proliferation was significantly promoted in the conditioned medium containing 50 mg/ml concentrates than in the control (no concentrates) medium, which became prominent on day 6 of culture. When osteogenesis was induced with the concentrates, a significant ($p=0.004$) increase was detected compared with the concentrates-free induction. These results showed that the secretary products from chick BMCs stimulate both proliferation and osteogenesis of hADSCs.

Funding Source: This work was supported by the BK21 plus program (Seoul national university, Department of Agricultural Biotechnology) through the National Research Foundation (NRF) funded by the Ministry of Education of Korea

T-2118

PRECLINICAL SAFETY EVALUATION OF INDUCED PLURIPOTENT STEM CELL DERIVED DOPAMINERGIC NEURONS

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Pluripotent-based cell therapies for Parkinson's disease (PD) are planned for clinical trial by several academic groups around the world in the near future. PD is an excellent target for a cell therapy because the therapeutic cell type has been identified, can be differentiated at high efficiency, and previous clinical work using fetal neurons has demonstrated the possibility of long-term (10 - 20 years) improvement of motor symptoms. Members of the GFORCE PD consortium are planning allogeneic therapies using either embryonic stem cells or allogeneic induced pluripotent stem cells. These groups also propose the use of immunosuppression for the first 12 months after cell delivery. We are developing an autologous, induced pluripotent stem cell-based therapy which will not require that the patients be immunosuppressed. Autologous therapies derived from pluripotent stem cells require development of new, bioinformatice diagnostics which allow for preclinical validation of safety and efficacy without the need for animal studies. As part of preclinical development, we have performed whole-genome DNA sequence analysis of 10 patient lines including fibroblasts, iPSC clones and differentiated neurons. We have developed a method to score the potential risks of genetic variants found within the cell banks. Additionally, we have performed a tumorigenicity test using 4 patient cell lines. Dopaminergic neurons derived from patient iPSCs were injected into the putamen of nude rats. A separate group of rats were injected with undifferentiated iPSCs. After 9 months, rats were sacrificed, and histological analysis was performed. In the rats treated with differentiated neurons, <1% of human nuclear antigen expressing cells were proliferating (Ki67+). No tumors were found in over 300 rats injected with differentiated neurons. In contrast, rats injected with pluripotent stem cells had masses of proliferating human cells and cells had migrated throughout the brain. The safety data to be presented will serve a basis for the design of IND-enabling safety studies for clinical trial of patient-specific neuron replacement for PD.

T-2120

EVALUATION OF PERIODONTAL TISSUE REGENERATION BY TRANSPLANTATION OF AUTOLOGOUS ADIPOSE TISSUE-DERIVED MULTI-LINEAGE PROGENITOR CELL IN A CLINICAL STUDY.

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Periodontitis is a chronic inflammatory disease that leads destruction of tooth-supporting tissues (periodontal tissue). Due to unsatisfied efficacy of current periodontal regenerative therapies, the development of a new cell-based therapy has been hoped for. We previously revealed the efficacy of periodontal tissue regeneration by transplantation of adipose tissue-derived multi-lineage progenitor cell (ADMPC) with the beagle dog periodontitis models. The objective of this study was to evaluate the efficacy and safety of autologous ADMPC transplantation for periodontal tissue regeneration in a clinical study. A protocol of the this study was conducted in accordance with the Ministry of Health, Labor and Welfare guidelines on clinical stem cell research and Act on the Safety of Regenerative Medicine. ADMPC was isolated from subcutaneous adipose tissue of 12 periodontitis patients and cultured in a cell processing isolator at Osaka University Dental Hospital. The patients underwent periodontal tissue surgery during which autologous ADMPC was transplanted to the bone defect with a fibrin glue. Until 36 weeks after the transplantation, we performed a variety of clinical examinations including periodontal tissue inspection

and standardized dental radiographic analysis. As a result, ADMPC transplantation clearly induced reduction of probing pocket depth, clinical attachment gain and neogenesis of tooth-supporting alveolar bone. These regenerative effects were observed not only in moderate periodontal bone defects but also in more severe periodontal bone defects. In addition, no transplantation-related side effects were observed in any cases. These results suggest that autologous ADMPC transplantation can induce periodontal tissue regeneration. Further clinical study is required not only to validate the efficacy but also to reveal the indications of the ADMPC transplantation.

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GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-2122

THE CELL FATE COMMITMENT OF THE MOUSE EPIBLAST CELLS DURING GASTRULATION

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During early embryo development, the cells of the epiblast undergo a major transition as they lose pluripotency to acquire the characteristics of the three germ layers known as ectoderm, mesoderm and endoderm. In the mouse embryo, the epiblast cells are either stimulated by high signalling induction in the posterior region, referred to as the primitive streak (PS), of the embryo to become mesoderm and endoderm layers. Conversely, the anterior part is under weak signalling activity and will eventually form the ectoderm. Despite a process well documented over the years, little is known about the molecular regulation underpinning this commitment. Epiblast stem cells (EpiSCs) have been shown to be the in vitro counterpart of the PS at E7.0 based on their transcriptomic signature. Our work is mainly based on the use of these EpiSCs to model the in vivo epiblast cell behaviour. Using this approach, we discovered that these cells can be poised toward a specific lineage depending on Wnt signalling activation. Cells which were never exposed to Wnt eventually commit preferentially to the ectoderm despite remaining pluripotent which aligns with what has been observed in the embryo. Having established EpiSCs as a relevant cell type allows us to further elucidate the molecular regulation involved during gastrulation which is quite tedious to achieve in vivo. Amongst the genes regulated by Wnt activity, Mixl1, a key transcription factor only expressed during gastrulation, was surveyed to a greater extent in order to reconstruct its regulatory network. While it was thought that Mixl1 was only instrumental in regulating gene expression in the PS, our data shows that Mixl1 may be involved in more complex mechanisms. For instance, combined RNA and ChIP sequencing data revealed new interactions with genes expressed in the visceral endoderm as well as in the PS. Our study highlights the importance of selecting the right system to model gastrulation (e.g. the EpiSCs and not the ESCs) and as a result has brought new insights on how gastrulating cells commit to their fate.

T-2124

CHARACTERISING THE ROLE OF TSC22D3 IN GERMLINE STEM CELL FUNCTION

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Transforming growth factor-β1 stimulated clone-22 (TSC22) domain family (TSC22DF) proteins act as pleiotropic cell regulators and contain conserved leucine zipper and tuberous sclerosis complex (TSC)-box motifs. In mouse and human, the family is encoded by four different loci - Tsc22d1, Tsc22d2, Tsc22d3 and Tsc22d4. TSC22DF members are known to be involved in diverse physiological functions including regulation of cellular growth, development, tumour suppression and inflammatory responses. Continual production of spermatozoa over the adult male lifespan is dependent on a population of undifferentiated spermatogonia within the testis that have self-renewal capacity. Regulated maintenance and differentiation of this undifferentiated cell pool is critical for tissue homeostasis and fertility. Previous studies based on a constitutive knockout model have demonstrated that Tsc22d3 (also known as Gilz) plays an essential role in long-term maintenance of undifferentiated spermatogonia and male fertility. However, the mechanisms by which Gilz regulates spermatogonial activity are poorly understood. To better define GILZ function in adult undifferentiated spermatogonia, we developed an inducible Gilz knockout model. Strikingly, acute deletion of Gilz in adults results in rapid exhaustion of the self-renewing population within a week of gene deletion, confirming the central importance of GILZ to germline maintenance. In contrast, populations of differentiating spermatogonia appeared unaffected. In order to define cellular pathways regulated by GILZ in spermatogonia we have also established cultures of undifferentiated cells from our mouse model allowing

inducible Gilz deletion in vitro. On-going work seeks to define the role of GILZ and other TSC22DF members in the undifferentiated spermatogonial population and identify underlying mechanisms contributing to GILZ-dependent germline maintenance.

T-2126

CHAPERONIN RELATED PHOSDUCIN-LIKE PROTEIN 2 (PDCL2) IS ESSENTIAL FOR SPERMIOGENESIS AND MALE FERTILITY IN MICE

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Infertility affects about 15% couples worldwide, and male factor infertility is responsible for 50% of infertile couples. Investigation into spermatogenesis defects will deepen our understanding of spermatogenesis regulation and improve clinical therapy results. By comparing the transcriptome of germ cells in mouse testis, mouse embryonic stem cells, mouse embryonic fibroblasts and other somatic cells, we found that Pdcl2 was specifically expressed in mouse testis. And from the online database, we found that Pdcl2 was also highly expressed in human testis and sperm. Pdcl2 is a highly conserved member of the phosducin family in yeast, mouse and human, and it is reported that Pdcl2 can regulate meiosis and cytoskeleton rearrangement in yeast. Overexpression of mouse PDCL2 in PLP2, yeast orthologue of PDCL2, deficient yeast can completely rescue the meiotic defect phenotype, showing conserved function from yeast to mammalian. However, the function of PDCL2 in higher eukaryotes remains obscure. Based on these data, we speculate that Pdcl2 may be involved in spermatogenesis regulation. We confirmed that Pdcl2 was highly expressed in mouse testis by western blot and immunofluorescence assay. Besides, we have already generated Pdcl2 knock-out founder mice by CRISPR/Cas9. Pdcl2-null male mice were infertile. Testis weight, epididymis weight and sperm number of Pdcl2-null male mice were less than those of wild-type littermates. Sperm of Pdcl2 knock-out mice were malformed and immotile. In addition, more apoptotic cells were found in Pdcl2 knock-out testis and epididymis. To elucidate the mechanism underlying spermatogenesis defects, we identified protein-protein interaction between PDCL2 and the protein folding-associated CCT complex. Collectively, PDCL2 may function as a chaperon to promote protein folding during spermatogenesis. Our study may provide important references for the diagnosis and therapy of clinical male infertility.

T-2128

THE DEVELOPMENTAL POTENTIAL OF THE GERM LINE IS RESTRICTED AFTER PGC COLONIZATION OF THE NASCENT GONAD IN MAMMALS

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In mammals, primordial germ cells (PGCs), the precursors of gametes, emerge from the epiblast and migrate to the gonads, where they later undertake gametogenesis for reproduction. Evidence from diverse vertebrates indicates that migratory PGCs are not irreversibly committed to gametogenesis, instead maintaining the potential to give rise to pluripotent cell lines and to teratomas. To identify when the germ line undertakes irreversible commitment to gametogenesis, we performed genome-wide transcriptional analyses of the embryonic human and mouse germ line through the migratory and gonadal phases. We discover that a set of deeply conserved germ cell determinants are first expressed in both human and mouse germ line only after PGCs colonize the nascent gonad. Through genetic studies, we show that one such factor, the RNA-binding protein DAZL, is necessary for the restriction of developmental potency in mammals. In Dazl-deficient mice, PGCs colonize the gonads, but continue to express a network of 'naïve' pluripotency factors, and retain the capacity for pluripotent cell derivation until at least E15.5. In DAZL-deficient mice and pigs, we find the failure to restrict developmental potency of the germ line results in spontaneous gonadal teratomas in both sexes. These observations, concordant across humans, mice and pigs, lead to a new understanding of germ line potential, the establishment of gametogenesis, and for the origin of germ line neoplasms in humans.

TECHNOLOGIES FOR STEM CELL RESEARCH

T-2130

SELECTION-FREE, HIGH FREQUENCY GENOME EDITING BY HOMOLOGOUS RECOMBINATION OF HUMAN PLURIPOTENT STEM CELLS USING CAS9 RNP AND AAV6

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Combination of genome editing and human pluripotent stem cells (hPSCs) offers a platform for in vitro disease modeling, drug discovery and personalized stem cell therapeutics. However, incorporation of large modifications using CRISPR/Cas9-based genome editing in hPSCs typically requires the use of selection markers due to low editing efficiencies. Here we report a novel editing technology in hPSCs using Cas9 protein complexed with chemically modified single guide RNA (sgRNA) and recombinant AAV6 (rAAV6) vectors for donor delivery without marker selection. With these components, we demonstrate targeted integration of a 2.2 kb DNA expression cassette in hPSCs at frequencies up to 94% and 67% at the HBB and MYD88 loci,respectively. We used this protocol to correct the homozygous sickle cell disease (SCD) mutation in an iPSC line derived from a SCD patient with a frequency of 63%. This Cas9/AAV6 system allows for both the integration of large gene cassettes and the creation of single nucleotide changes in hPSCs at high frequencies, eliminating the need for multiple editing steps and marker selection, thus increasing the potential of editing human pluripotent cells for both research and translational applications.

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T-2132

SORTING OF MSCS WITH CD271 AND MSCA-1 ANTIBODIES IN A CLOSED CARTRIDGE SYSTEM USING THE MACSQUANT® TYTO® SORTER

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Mesenchymal stem cells (MSCs) have raised great expectations in a number of clinical trials studying the regeneration of bone, cartilage, and heart or autoimmune diseases including GvHD, Crohn's disease, and diabetes. MSCs are an extremely rare cell type in cord blood, adipose tissue, or bone marrow (0.01%). Several groups worked on the identification of MSCs, using markers such as CD271, Anti-MSCA-1 (TNAP), CD73, and STRO-1. The MACSQuant® Tyto®, a benchtop microfluidic flow sorter, enables purification of cells in a fully closed, sterile single use cartridge, thus completely eliminating the risk of external contaminations and sample-to-sample carryover. Using this technology, we demonstrate the capacity to sort CD271+MSCA-1 (TNAP)+ MSCs. The gentle cell sorting process resulted in a highly pure population of viable CD271+MSCA-1+ cells. Subsequently the clonogenic potential as wells as the expansion capacity was compared between the CD271+MSCA-1+ MSC fraction, CD271- cells, and unsorted bone marrow MNCs (BM-MNCs). Furthermore, freshly sorted cells were compared with sorted and expanded cells regarding their potential to differentiate into osteoblasts and chondrocytes as well as their T cell-suppressive potential. In summary, the MACSQuant Tyto enabled isolation of CD271+MSCA-1+ MSCs from human BM-MNCs. The gentle sorting conditions and the sterile, fully closed system of the MACSQuant Tyto Cartridge provides an optimal basis for subsequent expansion of the isolated cells.

T-2134

TOXICOLOGICAL EVALUATION OF CONVULSANT AND ANTICONVULSANT DRUGS IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL NEURONAL NETWORKS USING AN MEA SYSTEM

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Functional evaluation assays using human induced pluripotent stem cell (hiPSC)-derived neurons can assess the convulsion toxicity of new drugs and the neurological effects of antiepileptic drugs. However, differences in the responsiveness of convulsant types and antiepileptic drugs as well as an evaluation index able to compare in vitro responses with in vivo responses are little known. Using multi-electrode arrays (MEAs), we observed differences in synchronized burst patterns in epileptiform activities induced by pentylenetetrazole (PTZ) and 4-aminophylisine (4-AP) with their different action mechanisms. We also found that 100 μM phenytoin suppressed epileptiform activities induced by PTZ, but increased those induced by 4-AP. To compare in vitro results with in vivo convulsive responses, we performed a frequency analysis below 250 Hz and excluded the spike component. In vivo convulsive firing enhancement of the high γ wave and β wave components were remarkably observed in in vitro hiPSC-derived neurons in co-culture with astrocytes. MEA measurements of hiPSC-derived neurons in co-culture with astrocytes as well as frequency analysis can effectively predict convulsion toxicity, side effects, and action mechanisms of drugs. These methods also enable the comparison of convulsions induced in vivo with those induced in vitro.

T-2136

ODONTOBLASTIC CYTODIFFERENTIATION OF HUMAN DENTAL PULP CELLS AND IMMUNOMIC SCREENING OF ODONTOBLAST-SPECIFIC CELL SURFACE MARKERS

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Odontoblasts, which are derived from cells of neural crest origin that is part of the outer surface of the dental pulp, play a role in dentin formation and regeneration. Odontoblastic cytodifferentiation of human dental pulp cells (hDPCs) was stimulated by activation of Smad signaling. The combinatorial treatment with BMP2 and BMP4 increased the expression of dentin-specific genes expression and the mineralization efficiency of hDPCs. By immunization of odontobasts differentiated from hDPCs, we constructed monoclonal antibodies (mAbs) against the odontoblast-specific cell surface antigens. As a result of this decoy immunization, we finally retained 12 of IgG-type and 15 of IgM-type mAbs, which contain the specific binding affinity on odontoblasts. According to immunohistochemistry, cell-rich zone in between dentin and pulp and perivascular region in pulp were strongly stained with these IgG-type mAbs. By immunoprecipitation and mass spectrometry, one of IgG-type mAbs, OD46 was interacted to a known cell surface antigen, Annexin-A6 (ANXA6), which is a calcium-dependent phospholipid-binding protein. Depletion of ANXA6/OD46 suppressed cell proliferation of hDPCs and induced cell death during odontoblastic cytodifferentiation, suggested that it might regulate a checkpoint between differentiation and proliferation. ANXA6/OD46 mAb and other mAbs will be useful for detection and separation of odontoblasts from the primary human pulp cells.

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T-2138

DEVELOPMENT OF A NEEDLE FOR A CELL TRANSPLANTATION THERAPY FOR PARKINSON'S DISEASE

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We plan to start 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, stereotactic systems for deep brain stimulation and brain biopsy are approved for clinical use by Ministry of Health, Labor and Welfare. However, these systems are not approved for cell transplantation, and moreover, these system is not suitable for our cell transplantation therapy, considering a small amount of transplanted cell suspension (about 5 uL). We decided to use the approved stereotactic system (Leksell Stereotactic System®) and 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 3 2 mm. In conclusion, we developped 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.

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T-2140

THE NOVEL EMBRYONIC STEM CELL LINES ESTABLISHED FROM COMMON MARMOSET

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Embryonic stem (ES) cells are useful tools for studying early embryonic development. Although, studies using mouse ES cells have been extensively conducted to understand embryonic development, it has been known that mouse ES cells and primate ES cells including human are greatly different characteristics. One of the major differences is chimeric competencies. When mouse ES cells are injected into early embryos such as blastocysts, ES cell-derived germline chimera individuals can be produced. On the other hand, although primate ES cells can differentiate into three germ layers, the primate ES cells do not have chimeric competency. In order to investigate and elucidate this different between mouse and primate ES cells, it is necessary to establish and use primate ES cells, but if done on a human sample, we take advantages of nonhuman primates ES cells that are less ethical issues compare to human ES cells. The common marmoset (Callithrix jacchus) is a useful experimental animal in biomedical research because of its similarity to humans, high reproductive efficiency, and easy handling. Given its prolificacy, the marmoset is suitable for studying primate reproductive and developmental biology. To date, several marmoset ES cells have been established and reported. However, in order to investigate and analyze the embryogenesis, new ES cell clines with young passage number are required. In this study, we established new ES cell lines. Establishment of ES cells was carried out by isolating Inner Cell Mass (ICM) from blastocyst stage marmoset embryos by immunosurgery. These ICMs were cultured on iMatrix that is recombinant protein of the laminin 511 extracellular matrix coated dishes. As the results, new feeder free 7 ES cell lines have been established. Furthermore, 2 out of 7 lines were derived from in vitro fertilized (IVF) embryos. This is the first case of establishment of ES cells without feeder cells and from IVF embryos. These ES cell lines would be nice tools to understand cytokine signaling to maintain undifferentiated status of ES cells and differentiation of ICM to cultured ES cells.

T-2142

STANDARDIZED QC ASSAYS AND (AUTOMATED) EXPANSION OF PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) hold great promise for disease modeling, drug discovery and clinical applications. Therefore, working with highly pluripotent, well characterized and quality controlled cell stocks during development is crucial to ensure reproducible experimental conditions. Here, we established a workflow encompassing stable expansion of hPSCs using a xeno-free cultivation medium, assessment of pluripotency using a defined marker combination and assessment of differentiation potential based on lineage-specific, complete media both combined with quantitative, multicolor flow cytometry analysis to characterize and quality control the cultivated hPSCs, as well as cryopreservation of hPSCs using an animal component-free, chemically defined cryopreservation media. Following this workflow, hPSCs could be stably expanded over 20 passages with persistent, high expression of pluripotency markers (>92%) TRA-1-60, SSEA-4, SSEA-5, Oct-4, Sox2 and almost no expression of differentiation maker SSEA-1, showed a homogenous morphology and also retained a stable karyotype. Quantitative, flow cytometry-based analysis reproducibly proved their differentiation potential into ectoderm, mesoderm, and endoderm. Cells cultivated under the same conditions showed a reproducible recovery after cryopreservation and thawing resulting in a 8-12 fold expansion in passage 1, high pluripotency marker expression as well as genomic stability confirmed 5 passages post thaw. Thus, the workflow shown here assures a standardized, robust expansion of hPSCs, includes characterization and quality control of the expanded cells, as well as efficient cryopreservation. The flow based QC strategy was also successfully applied for characterization of cells cultivated under standardized, automated, closed system conditions using the CliniMACS Prodigy Instrument which is of major relevance for generation of master cell banks (MCB) and working cell banks (WCB) for future clinical cell manufacturing.

T-2144

BIOREACTOR-EXPANDED HUMAN INDUCED PLURIPOTENT STEM CELLS AND PRINTABLE HYDROGELS FOR BIOFABRICATION

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Biomedical applications of human stem cells will require standardized procedures for scale-up and flexible bioprocessing in terms of cell type specification and customizable topographical layout. Here we aim at developing an integrated workflow to generate and scale-up of stem cells for bioprinting desired stem cell composites. To overcome the limited scalability of standard adherent culture, we have established a suspension culture-based strategy to expand human induced pluripotent stem cells (hiPSCs) in quantities exceeding 1 × 10^9 per reactor (1 liter working volume, 2.4 liter maximum volume), without using coated microcarriers. This 14-day, two-phase process generates hiPSC aggregates of 198 3 58 µm diameter on the day of final harvest, representing 2 × 10^9 cells. hiPSCs can be cultured and passaged in these bioreactors for at least 7 weeks in suspension while expressing pluripotency-associated markers TRA-1-60, TRA-1-81, SSEA-4, OCT4, and SOX2. These hiPSCs retain their potency to differentiate into cells of all three germ layers, maintain a stable karyotype, and respond to specific differentiation cues, exemplified here by differentiation into cardiomyocytes. For bioprinting, we used a 3D Discovery RegenHU bioprinter coupled with a water bath conjugated to a syringe heating apparatus at 30 °C and equipped with a custom cooling plate at 4 °C for the printing stage. Bioreactor-expanded hiPSCs and gelatin-alginate hydrogels were combined in a bioink, using an external mechanism for gelation by cross-linking with calcium. We present our findings on cell viability and maintenance of pluripotency post-printing. Our study provides a robust basis for bioprinting customized stem cell composites for toxicity screens, disease modeling and cell replacement.

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T-2146

VITRONECTIN-MODIFIED RECOMBINANT SPIDER SILK PROTEIN FOR HUMAN IPS-DERIVED NEURAL PROGENITOR CULTURE

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Tissue engineering by neural stem cells holds great promise for regenerative medicine and drug discovery. To increase the reproducibility and relevance of the approach, neural stem cells optimally should be grown in defined conditions free of animal-derived components. Here we explore the biocompatibility of recombinant spider silk protein functionalized with vitronectin (VNN-NT2RepCT) using human iPS-derived neuroepithelial-like stem (NES) cells. The material was used as a coating providing the extracellular matrix component for the cells and compared to the current golden standard for NES cells in vitro culture, namely poly-l-ornithine/laminin coating, as well as unmodified NTRepCT protein and pure vitronectin. Viability, proliferation, spontaneous neuronal differentiation, cytoskeleton arrangement, and adhesion of the cells were assessed. Our results demonstrate that the vitronectin modification of NT2RepCT was essential for proper adhesion and proliferation of the NES cells. Culturing the NES cells on VNN-NT2RepCT was as efficient as the current standard coatings for NES culture. Viability and proliferation rates remained similar when comparing VNN-NT2RepCT, poly-l-ornithine/laminin and vitronectin coatings. Moreover, TuJ1-positive cells with neuronal morphologies were generated in differentiating conditions when using the modified spider silk. Preliminary analysis of vinculin appearance by immunocytochemistry showed that NES cells grown on VNN-NT2RepCT displayed an increased number of focal adhesion points that potentially may influence various cell characteristics, such as migration. Our findings suggest that VNN-NT2RepCT is a promising substrate for xeno-free NES cell culture opening for the possibility to engineer more complex and biologically relevant constructs by creating 3D scaffolds using biomimetic silk spinning and/or bioprinting.

T-2148

P53 GENE REGULATES THE CELLULAR SENESCENCE OF MESENCHYMAL STEM/ STROMAL CELLS

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Mesenchymal stem/stromal cells (MSCs) are defined as self-renewing cells with the ability to differentiate into osteoblasts, adipocytes, and chondrocytes. Due to their multipotency, MSCs are useful resources to regenerate tissues. Extensive cell expansion is required to obtain the substantial number of cells needed for clinical applications. However, prolonged culture of MSCs reduces their proliferation ability and induces them to acquire mature phenotypes. Cellular senescence is caused by various stresses, including DNA damage, expression of oncogenes, and oxidative stress. The tumor suppressor, p53, responds to DNA damage and oxidative stress. p53 mutations are common in human tumors and linked to poor patient prognosis. Therefore, expression of p53 gene is essential for the safety of therapeutic cells, but it could become an obstacle for cell expansion. In this study, we isolated MSCs from BM of p53 knock out mice using the FACS-based approach and carried out to investigate the stem cell profiling in vitro and in vivo. We evaluated colony formation after sorting single cells into 96-well culture plate (PDGFRa+Sca-1+ cells), the colony forming ability was not different between wild type- and p53 knock out- MSCs. Next, we cultured MSCs and examined their proliferation capacity. When wild type-MSCs are cultured several times, the proliferative capacity gradually decreased. By contrast, p53 knock out-MSCs retained their proliferation ability even few passages. We performed to analyze the gene expression profile and to stain the senescence-associated beta-galactosidase (SA-β-gal) for detecting senescent cells. The result demonstrated that p53 knock out-MSCs do not become senescent and continue to proliferate after long-term culture. Furthermore, the differentiation capacity of p53 knock out-MSCs was maintained differentiation ability for mesenchymal lineage. Tumor formation was not observed following subcutaneous transplantation of p53 heterozygous MSCs. It is suggested that MSCs have anti-tumorgenic ability in correlation with stem cell capacity. Partial or transient inhibition of p53 gene expression may facilitate stem cell expansion and cell transplantation for regenerative medicine.

T-2150

OPTIMISED SURFACE-COATED MATERIALS FOR LONG-TERM MAINTENANCE OF HUMAN PLURIPOTENT STEM CELLS

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From scalable manufacturing, consistency and regulatory perspectives, culturing human pluripotent stem cells (hPSCs) on synthetic surfaces with well-defined chemical and physical properties is of great importance for future applications. Whilst many approaches are possible, a scalable approach to surface preparation which can be readily manufactured is crucial. We have developed a surface coating approach which can be readily applied to multiwell plates, tissue culture flasks and microcarrier particles. We have recently published a study where we showed that hPSCs could be cultured on a synthetic surface, presenting a cyclic RGD peptide (cRGDfK) optimised for highly specific avb3 and avb5 integrin binding. Culture was carried out for at least 10 passages in E8 media and the cells from three hPSC lines remained pluripotent. Pluripotency was assessed via colony morphology, proliferation rate, maintenance of OCT4 expression, cell viability at harvest, teratoma formation potential, and global gene expression as assessed by the PluriTest™ assay. Cells maintained on the cRGDfK presenting synthetic surfaces and control cultures maintained on Geltrex™ produced comparable results in most assays. In order to achieve these results we needed to optimise the surface coated materials for a variety of properties including chemistry, peptide conjugation methodology, peptide surface concentration, coating thickness, coating mechanical properties and coating structure as these are critical factors in the maintenance of pluripotency. The surface coatings were prepared in a one-step synthetic polymer coating approach which is readily scalable from a manufacturing point of view and was completely chemically defined. The process involved the chemical grafting of polymer molecules from the surface of tissue culture treated polystyrene, sterilisation using gamma-irradiation, followed by a simple peptide coupling step which yielded the desired peptide surface concentration. We observed that the chemistry of the coating used was significant; coatings with different thickness and mechanical properties were obtained, and control over these properties was observed to be crucial in PSC attachment, proliferation and maintenance of pluripotency.

T-2152

THE CULTURE SYSTEM OF MURINE SALIVARY GLAND-DERIVED STEM-LIKE CELLS USING SMALL MOLECULES

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The Sjögren's syndrome and radiotherapy to the tumor in the head and neck lead to damage on the salivary gland. Mechanism of the salivary gland stem cell culture and salivary gland disease has rarely been studied. The aim of this study was to treat small molecules to salivary gland-derived stem-like cells (SCs) and to identify the mechanism which had a significant effect to enhance the propagation potency in vitro. Primary SCs were characterized by FACS. Mesenchymal marker and salivary gland stem cell markers (CD24, CD44, and CD117) were highly expressed while the expression level of hematopoietic cell marker (CD45) was low. When SCs were long-term cultured, the proliferation rate rapidly decreased in passage 2. Small molecules which were known to have the effect to enhance pluripotency, proliferation, and viability were treated. In passage 2, the proliferation rates of MEK inhibitor-treated groups (PD184352: PD1 and PD0325901: PD3) were decreased compared to the control group, while the proliferation rates of PD98059-, SU5402-, CHIR99021-, and Y27632-treated groups were significantly increased. The proliferation rate of the Y27632-treated group increased more than twofold of other groups, which indicated the rock signaling pathway was significant for in vitro culture of the SCs. Additionally, cytotoxicity assay confirmed that small molecules had no effect on cytotoxicity. The gene expression patterns of CHIR99021- and Y27632-treated groups were similar to those of the normal salivary gland group. As a result, our study suggests ROCK and GSK signaling pathway could play a significant role in the maintenance of salivary gland stem cells. Therefore, additional studies about PD1- and PD3-treated groups will be required to identify the specific mechanism of in vitro culture and proliferation of SCs.

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T-2154

ADHESION AND ECM GENE EXPRESSION OF MOUSE NEURAL STEM/PROGENITOR CELLS ATTACHED ON POLY(VINYLALCHOL) HYDROGELS CROSS-LINKED WITH GAMMA IRRADIATION

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Poly(vinylalcohol) (PVA) hydrogels cross-linked with gamma irradiation can be used for the attachment culture of neural stem/progenitor cells (NSPCs), even though fibroblasts and osteoblasts can not adhere to them. In order to understand the mechanism that NSPCs adhere to PVA hydrogels, we investigated adhesion and extracellular matrix (ECM) expression of NSPCs. PVA hydrogels were respectively prepared from 3.75, 7.5, and 15% PVA solution with 10, 20, and 40 kGy radiation from Co-60 γ-irradiation source. The mechanical properties of these hydrogels were characterized by the Young's modulus based on the data of compression test. The Young's modulus of respective PVA hydrogels were 1.130.2 kPa for 3.75%-10kGy PVA, 9.931.5 kPa for 7.5%-20kGy PVA, and 22.134.9 kPa for 15%-40kGy PVA. The structure of PVA hydrogels was investigated by Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectra of PVA hydrogels showed the similar patterns of the relative intensity of the band related to hydroxyl groups. Neural stem/progenitor cells isolated from a forebrain tissue of E14 ICR mouse were used for the experiments. NSPCs were cultured in DMEM/F-12 medium supplemented with B-27 solution and growth factors (EGF, b-FGF) at 37°C, 5%CO2 on PVA hydrogels (3.75%-10kGy PVA, 7.5%-20kGy PVA, and 15%-40kGy PVA). The gene expression of integrins and ECM (fibronectin and tenascin) of NSPCs incubated on each PVA hydrogel for 7days were analyzed using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Additionally, the protein expression of tenascin C in NSPCs attached on PVA hydrogels was examined using immunocytochemistry and western blotting analysis. The gene expression levels of tenascin C and fibronectin were downregulated in NSPCs in response to the increasing stiffness of PVA hydrogels. NSPCs attached on a soft hydrogel (3.75%-10kGy PVA) showed the highest level of tenascin C gene and protein expression. Immunocytochemical analysis using fluorescence microscopy revealed the tenascin C localization around the cells directly attached on PVA hydrogels in cell clusters and the boundary surface. NSPCs may self-organize the substrate to attach on PVA hydrogels in response to the stiffness.

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T-2156

DEVELOPMENT OF EFFICIENT CELL CULTURE METHOD USING PREVELEX®, A NOVEL COATING MATERIAL THAT PREVENT PROTEIN ADSORPTION TO THE SURFACE OF CULTURE VESSELS

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Proteins nonspecifically adhere to the surface of cell culture vessels by hydrophobic interaction. For examples, growth factors or cytokines in medium adhere to the surface of vessels during cell cultures and are likely to lose their capacity to regulate cell growth and differentiation. To address this issue, we have developed a novel coating material prevelex® that prevents protein adhesion to the surface of culture vessels. prevelex® is a solution of polymers which are composed of amine and phosphoric acid ester monomers, and form a thin film when coated to vessels. In this study, we examined basic characteristics of prevelex® against protein adsorption and apply it to cell culture. prevelex® was coated to culture plates made of polypropylene (PP), polystyrene (PS) and glass, and adhesion of HRP-labeled IgG protein to the plates were evaluated. As a result, prevelex® decreased the adhesion amount to the plates at the level of 99.5(3 0.247) %. In addition, this adhesion-suppressing effect did not depend on the molecular weight or isoelectric point of tested proteins. The effect of prevelex® was kept even after γ-ray sterilization. Next, we investigated whether prevelex® inhibit the adhesion of growth factors such as basic fibroblast growth factor (bFGF) to culture vessels. We coated prevelex® onto culture plate, and cultured cell spheroids of human tumor cell line from uterus leiomyoma (SKN cells) in the presence of 0.01-100 ng / ml of bFGF for 4 days. Cells cultured in the prevelex®-treated plates proliferated 1.5 times faster compared to those in normal plates. The EC50 of bFGF was reduced to 1/6 or less by prevelex® coating. Correspondingly, we observed by ELISA test that prevelex® decreased the adhesion amount of bFGF to the surface of plates. Taken together, our findings suggest that prevelex® coating will lead to reduction of the amount of growth factor for cell culture. We are currently examining hematopoietic stem cell culture system using prevelex®.

T-2158

SCALABLE PRODUCTION OF HIPSC-DERIVED CARDIOMYOCYTES IN STIRRED-TANK BIOREACTORS

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Stem cell-based technologies lay the basis for pioneering approaches in drug discovery, drug safety testing, and regenerative medicine. The routine application of human induced pluripotent stem cells (hiPSCs) and functional cells derived from hiPSCs in high throughput applications for drug discovery will require the constant supply of high cell numbers at a consistent, high quality. Two-dimensional systems like T-flasks are widely used for the cultivation of stem cells; however, they are limited in control and scalability. Stirred-tank bioreactors have emerged as promising culture system for large-scale expansion of stem cells and their differentiation into the desired cell type. Ncardia is a stem cell drug discovery and development company developing highly predictive human cellular (disease) assay systems for safety and efficacy testing. The company's goal is to accelerate and improve drug candidate selection, reduce drug development costs and ultimately increase drug discovery and development efficiency. Large-scale manufacturing of hiPSC-based cardiomyocytes at high quality is integral for its cardiovascular services. Here we demonstrate the suitability of the Eppendorf DASbox® Mini Bioreactor system for the expansion of hiPSCs and subsequent production of Ncardia's Pluricyte® Cardiomyocytes. We show that using the DASbox system, hiPSCs were successfully expanded as cell aggregates in a highly reproducible manner. During the production of three different batches, aggregates were of homogenous size and the cells retained key iPSC markers including Sox2, Oct3/4 and Nanog during the expansion phase. More importantly, using the DASbox, a fully controlled production process scalable for large-scale manufacturing of hiPSC-derived cardiomyocytes could be established. Quantification of cardiomyocyte markers (cTNT and MLC2v) revealed the robust and efficient generation of multiple and pure ventricular-like cardiomyocyte batches. Analysis of the electrophysiological properties by multielectrode arrays (MEA) demonstrated the mature phenotype of the cells. The expected response to cardioactive reference compounds further confirmed the functionality of bioreactor-derived cardiomyocytes.

T-2160

LACTATE ACCUMULATION PROFILE OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN THE DEFINED AND XENO-FREE CULTURE MEDIUM StemFit®

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Human induced pluripotent stem cells (hiPSCs) represent a promising tool for regenerative medicine given their infinite proliferative capacity and potential for differentiation into various cell types. Lactate is a major metabolite of glycolysis, and its excessive accumulation in culture media could cause some deleterious effects on hiPSCs, such as growth inhibition, loss of pluripotency, and increased DNA damage. To obtain high-quality hiPSCs, the lactate level in the culture medium should be tightly controlled. We have developed a defined and xeno-free culture medium, StemFit®, that can be used to robustly maintain hiPSCs in feeder-free systems. StemFit® enables the growth of hiPSCs at a very low seeding density in a single-cell expansion protocol. Repeated measurement of metabolite levels over time demonstrated that the cell number is a major determinant for lactate levels. In addition, cultures initiated with a lower seeding density tend to prevent excess accumulation of lactate than those that require a higher initial seeding density. In fact, lactate levels can be well controlled in cultures with low initial seeding densities using StemFit®. These results would help to establish robust and stable culture systems for good-quality hiPSCs.

PLURIPOTENCY

T-2162

UTILIZING RNA SEQUENCING TO IDENTIFY CANCER-RELATED MUTATIONS IN HUMAN PLURIPOTENT STEM 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. Recently, point-mutations in the gene coding for the p53 tumor suppressor (TP53) have been found in hPSCs using whole exome sequencing (WES). These mutations were shown to gradually take over the culture, suggesting they provide a growth advantage in vitro. In order to analyze a large collection of hPSC samples, we developed a pipeline using publicly available RNA-sequencing data sets to identify mutations within the TP53 gene. Our analysis discovered that 5% of hPSC lines, and up to 19% of all analyzed samples carry TP53 loss-of-function mutations. As cancer-related mutations in other genes can also affect cells' tumorigenicity, we have sought out to discover such mutations in tumor suppressor genes and oncogenes using a similar approach. We first eliminated SNPs originally found in the commonly used, 20-years old WA01 and WA09 cell lines using WES. Screening over 160 different RNA sequencing samples from these lines allowed us to identify acquired mutations listed in the Catalogue of Somatic Mutations in Cancer (COSMIC), predicted to severely impair proteins' function. Furthermore, following the identification of genes impaired in both cell lines, we screened over 400 induced pluripotent stem cell samples for mutations within these genes, aiming at identifying recurrent aberrations acquired in culture. Our results highlight the need for mutation identification and validation in hPSCs prior to their use in both basic and clinical research.

T-2164

TRANSCRIPTIONAL ASSOCIATION OF MYRF WITH SOX2 IN MOUSE EMBRYONIC STEM CELLS

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Transcription factors are critical regulators of pluripotency in embryonic stem (ES) cells, control lineage commitment and determine cellular phenotypes. Myelin Regulatory Factor (MYRF) is a transcription factor critical for the terminal differentiation of oligodendrocytes in the central nervous system as it is required for the expression of genes involved in myelination. In oligodendrocytes MYRF functions together with SOX10 at regulatory elements that control transcription of the myelination genes. We determined that MYRF is expressed in mouse ES cells and up-regulated as cells differentiate. Using Proximity Ligation Amplification (PLA), we show interactions between MYRF and RNA polymerase II at several nuclear foci demonstrating that MYRF interacts with the transcriptional machinery even at this early stage. We explored potential partners for MYRF by testing the interaction between MYRF and several pluripotency-related transcription factors (OCT4, SOX2, NANOG and YY1). Quantitative analysis of interacting foci show a higher association between MYRF and SOX2 compared to the other transcription factors. Knockdown of SOX2 disrupted the interaction of RNA polymerase II with MYRF indicating a critical role of SOX2 in MYRF function in ES cells. Given that MYRF works cooperatively with SOX10 in oligodendrocytes to activate target gene transcription, these results suggest a regulatory role for MYRF in association with SOX2 during early lineage commitment.

T-2166

WNT3A ACTIVATE WNT-YAP/TAZ SIGNALING PATHWAY TO SUSTAIN CDX2 EXPRESSION IN BOVINE TROPHOBLAST STEM-LIKE CELLS

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As the precursor cell of placental cells, trophoblast stem cell (TSC) has become an effective tool to study the formation of placenta by the differentiation in vitro. Using 2i and L-Wnt3A/MEF mixed feeder cells, we have previously established bovine trophoblast cell line BTS-1. In this study, we replaced the L-Wnt3A/MEF mixed feeder cells with bovine fetal fibroblasts and added WNT3A to the 2i medium, and established another bovine trophoblast stem-like cell line, known as BTSW. The RT-PCR and immunofluorescence staining results showed that the BTSW cells expressed pluripotency genes including NANOG, SOX2, OCT4, TRA-1-60, TRA-1-81, SSEA4, E-CADHERIN and KRT18, and trophoblastic stem cell marker gene CDX2, TEAD4, ERR2 and so on. Methylation sequencing of the promoter regions of NANOG, OCT4 and CDX2 revealed that there was no significant difference between BTS-1 and BTSW. After removal of the WNT3A from the culture medium, NANOG, OCT4 and SOX2 were all down-regulated ($P<0.05$) and multiple trophoblast stem cell marker genes were down-regulated as well, while some TSC differentiation marker genes were up-regulated, which included MASH2, GCM1, and PAG. Western blot results showed that Wnt-YAP/TAZ signaling pathway was all activated in three trophoblastic stem-like cells, which activated the transcription of TEAD4, whereas this signaling pathway was not activated in the established bovine ES-like cells that expressed OCT4, SOX2 and NANOG, but not CDX2, named as BCFF cells. In conclusion, WNT3A activated and regulated the expression of CDX2 through the WNT-YAP/TAZ signaling pathway, thereby played an important role in maintaining properties of the bovine trophoblast stem cells.

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T-2168

GSK INHIBITION MEDIATES EFFICIENT DERIVATION OF GERM-LINE EMBRYONIC STEM CELLS FROM NON-OBESE DIABETIC MICE

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The non-obese diabetic (NOD) mouse strain is a predominant model of type 1 diabetes and a valuable tool for studying immune tolerance mechanisms. Hence embryonic stem (ES) cell lines derived from this mouse strain would prove useful for basic medical research. However, this mouse strain is considered to be a non-permissive strain for ES cell derivation using conventional methods. Instead we have used small molecule inhibition of MAP kinase and glycogen synthase kinase 3 (GSK3) to block spontaneous cell differentiation and therefore promote pluripotency persistence. Here we show a single pharmacological GSK3 inhibitor, 6-bromoindirubin-3'-oxime (BIO), in combination with leukemia inhibition factor (LIF; 1i/LIF) sufficiently promoted generation of stable NOD ES cell lines. Once established, expansion of the NOD ES cell lines on longer required treatment with BIO. One of these NOD ES cell lines contributed to chimeric mice; and transmitted to germline progeny that spontaneously developed diabetes. By contrast, we show that 5-aza-cytidine, a small molecule inhibitor of DNA methylation, and/or trichostatin A; and valproic acid, small molecule inhibitors of DNA methyltransferase, could not promote generation of NOD ES cells by epigenetic remodeling. These findings provide new insights into imposing pluripotency in cells isolated from a previously considered non-permissive strain.

T-2170

THE INHIBITORY MECHANISM OF THE FGF4 SIGNALING BY O-GLCNAC IN MOUSE EMBRYONIC STEM CELLS TO MAINTAIN THE UNDIFFERENTIATED STATE

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The undifferentiated state of mouse embryonic stem cells (mESCs) is maintained via several signal pathways such as leukemia-inhibitory factor (LIF), bone morphogenetic protein (BMP4) and Wnt signal pathways. Fibroblast growth factor 4 (FGF4) signal pathway functions as a trigger for the differentiation of mouse ES cells. After LIF, BMP4, Wnt and FGF4 bind to their respective receptors, many intracellular downstream molecules are activated. For example, MEK and ERK1/2 are key downstream molecules of FGF4 signal pathway and they are activated (phosphorylated)

by FGF4 stimulation. ERK1/2 is phosphorylated by phosphorylated MEK and phosphorylated ERK1/2 induces mESC differentiation. Therefore, in mESCs, the activation (phosphorylation) of FGF4-MEK-ERK1/2 pathway is inhibited to maintain the undifferentiated state. Here, we showed that a novel inhibitory mechanism of the FGF4-MEK-ERK1/2 pathway by the intracellular glycosylation in mESCs. We focused on O-linked β-N-acetylglucosaminylation (O-GlcNAcylation), which is an intracellular glycosylation, as a novel regulatory factor of FGF4 signal pathway in mESCs. O-GlcNAcylation is one of the posttranslational modification in the cytoplasm and nucleus. O-GlcNAc transferase (OGT) transfers a single N-acetylglucosamine (GlcNAc) to serine and threonine residues of nuclear and cytoplasmic proteins. It has been reported that proteins of many types such as signal components, epigenetic factors, cytoskeletal proteins, and transcription factors are O-GlcNAcylated. O-GlcNAcylation is competitive with phosphorylation of serine and threonine residues. Therefore, we hypothesized that O-GlcNAc inhibits FGF4-MEK-ERK1/2 pathway via the inhibition of the phosphorylation of FGF4 signaling components. We showed that the decrease of O-GlcNAc by Ogt knockdown inhibited marker genes of the undifferentiated state (OCT3/4, SOX2 and NANOG) and induced the differentiation from mESCs. This induction of the differentiation of mESCs was caused by the activation of FGF4-MEK-ERK1/2 pathway. Here, we showed that O-GlcNAc inhibits the activation of FGF4-MEK-ERK1/2 pathway to maintain the undifferentiated state. We clarified the signaling mechanisms by O-GlcNAc in mESC.

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T-2172

MEASLES VIRUS PERSISTENT INFECTION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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In this study, we found that the measles virus (MV) can infect human induced pluripotent stem cells (hiPSCs). Wild type measles virus strains generally use human signaling lymphocyte activation molecule (SLAM; CD150) as a cellular receptor, while vaccine strains such as the Edmonston strain can use both CD150 and CD46 as receptors. It is not yet known how early in the embryonal differentiation stages these receptors are expressed. We established two hiPSCs (BGU-iPSCs and EMF-iPSCs) which express CD46 and CD150. Both cells types can be infected by MV to form persistent, non-cytopathic cell lines that release infectious MV particles. Following MV persistent infection, BGU-iPSCs and EMF-iPSCs remain pluripotent and can differentiate in vitro into the three germ layers. This includes cells expressing the neuronal differentiation markers: NF68 and miRNA-124. Since the MV does not integrate into the cell's genome, it can be utilized as a vehicle to systematically introduce genes into hiPSCs in order to dissect and to define factors regulating lineage differentiation.

T-2174

IDENTIFICATION OF THE O-GLCNAC MODIFICATION SITE OF PKC ZETA AND FUNCTIONAL ANALYSIS OF O-GLCNAC ON PKC ZETA IN MOUSE EMBRYONIC STEM CELLS

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Differentiation of mouse embryonic stem cells (mESCs) is triggered by fibroblast growth factor 4 (FGF4)/ Mitogen-activated Protein Kinase (MAPK) signaling. Activation of MAPK is regulated by phosphorylation. We have reported that glycan on cell surface regulates MAPK activation in mESCs. O-linked β-N-acetylglucosaminylation (O-GlcNAcylation) is a sole glycosylation in nucleus and cytoplasm. O-GlcNAc is transferred by O-GlcNAc transferase (OGT) to serine and threonine residues of proteins, and removed by O-GlcNAcase (OGA). We have reported that OGT and OGA are necessary for survival of epiblast stem cells (EpiSCs) and reversion from EpiSCs to mESCs. It is also known that O-GlcNAc modification competes with phosphorylation of proteins. However, the molecular mechanism regulating intracellular signaling via O-GlcNAc was not uncovered in mESCs. In this study, we knock downed (KD) Ogt in mESCs. Phosphorylation of Protein Kinase C (PKC) ζ, an intracellular signaling molecule, was promoted in Ogt KD mESCs. Therefore, we hypothesized that O-GlcNAc inhibits phosphorylation of PKCζ. To investigate whether PKCζ is modified by O-GlcNAc, we precipitated mESC lysate using anti-O-GlcNAc antibody. Western blot analysis showed that PKCζ was co-precipitated, demonstrating that PKCζ is

O-GlcNAcylated. T410 of PKCζ is the key phosphorylation site for PKCζ activation. To identify the O-GlcNAcylated site of PKCζ, we expressed T410A-mutant PKCζ-FLAG in mESCs. Then, we performed immunoprecipitation assay with anti-FLAG antibody and western blot analysis using anti-O-GlcNAc antibody. Compared with control cells, O-GlcNAc significantly decreased in T410A-mutant PKCζ-FLAG expressed mESCs, demonstrating that T410 of PKCζ is O-GlcNAcylated. These results showed that O-GlcNAc competes with phosphorylation on T410 of PKCζ. In addition, PKCζ was a downstream component of FGF4 signaling. We identified the kinase which phosphorylates PKCζ in the downstream of FGF4. Furthermore, MAPK was activated in the downstream of PKCζ, and promoted differentiation in Ogt KD mESCs. We showed a novel molecular mechanism regulating intracellular signaling that O-GlcNAc on T410 of PKCζ inhibits PKCζ phosphorylation and mESC differentiation.

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T-2176

IDENTIFYING ESSENTIAL GENES FOR HUMAN PLURIPOTENCY: A COMPARISON BETWEEN HAPLOID EMBRYONIC STEM CELL AND CANCER CELL LINES

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The survival and pluripotency of stem cells require the coordinated expression of a set of essential genes. Identification of these genes is vital for a better understanding of the pluripotent state. Recently, we have isolated and characterized haploid human pluripotent stem cells (hPSCs) that allow high-throughput genetic screening for the identification of pluripotency-essential genes. Here, we generated a genome-wide loss-of-function library in haploid hPSCs using the CRISPR/Cas9 technology, with nearly 180,000 single guide RNAs (sgRNAs) that targets 18,166 protein coding genes. As the first screen for essentiality in hPSCs, we were interested in the differences and similarities with prior screens performed in human cancer cell lines, in order to define the core essential genes across cell types and also highlight essential genes specific to hPSCs. About 80% of the 1,661 genes we defined as essential were also identified as essential by the other screens. In addition, our hPSCs as well as a near haploid leukemic cell line showed a similar pattern of essentiality across various cellular compartments. Nevertheless, essentiality among developmental growth retardation-related genes and pluripotency transcription factors was specific only to hPSCs. Moreover, the aberrant genetic backgrounds of the cancer cell lines affected the essentiality landscape to a considerable extent. Various tumor-suppressor genes failed to appear as essential or growth-restricting in various cell lines, presumably due to loss of dependence in their function, whereas gain-of-function oncogenes became essential. Overall, we could demonstrate in this comparison the unique characteristic of hPSC screening for the identification of human essential genes.

PLURIPOTENT STEM CELL DIFFERENTIATION

T-3002

IDENTIFICATION OF THE MIRNAOME OF EARLY MESODERM AND CARDIAC PROGENITOR CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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MicroRNAs are small non-coding RNAs involved in post-transcriptional regulation of gene expression related to many cellular functions. We performed a small-RNAseq analysis of cardiac differentiation from pluripotent stem cells. Our analyses identified some new aspects about

microRNA expression in this differentiation process. We described a dynamic expression profile of microRNAs where some of them are clustered according to their expression level and we explored the extensive network of isomiRs and ADAR modifications in three cell populations: human pluripotent stem cells, early mesoderm progenitor cells and cardiac progenitor cells. We identified the microRNAs families and clusters involved in the cardiac differentiation and defined the mirRNAome based on these groups. Finally, we were able to determine a more accurate miRNAome associated with cardiomyocytes by comparing the expressed microRNAs with other mature cells. MicroRNAs exert their effect in a complex and interconnected way, making necessary a global analysis to better understand their role. Our data expands the knowledge of microRNAs and their implications in cardiomyogenesis.

T-3004

IN-VIVO IMAGING OF PLURIPOTENT STEM CELL DIFFERENTIATION USING THE ANTERIOR CHAMBER OF THE EYE

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Since the discovery of iPS cells, stem cell research has advanced rapidly and stem cell products are now seeing therapeutic applications. iPSC (induced pluripotent stem cells) derived cells should be examined using a suitable animal transplantation model prior to clinical application. However, such transplantation models require long-term observation. Herein, by taking advantage of the unique characteristic that the cornea is transparent, and that the anterior chamber is an immune-privileged site, we examined the anterior chamber of the eye as a potential transplantation site for iPS derived cells, and also evaluated whether this system could be used to evaluate differentiation status. First, immunodeficient nude rats were used to assess teratoma forming capacity of iPSC directly transplanted into anterior chamber. Next, iPS-derived neural crest cells were transplanted into the anterior chamber and observed for chondrocyte differentiation capacity. This method enabled us to detect differentiation rapidly and accurately. Similar differentiation potential was observed in two iPSC lines. This transplantation model should prove useful for investigating the differentiation capacity of iPSC-derived cells non-invasively in vivo.

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T-3006

SFRP2 ENHANCES THE OSTEOGENIC DIFFERENTIATION OF APICAL PAPILLA STEM CELLS BY ANTAGONIZING THE CANONICAL WNT PATHWAY

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Exploring the molecular mechanisms underlying directed differentiation is helpful in the development of clinical applications of mesenchymal stem cells (MSCs). Our previous study on dental tissue-derived MSCs demonstrated that secreted frizzled-related protein 2 (SFRP2), a Wnt inhibitor, could enhance osteogenic differentiation in stem cells from the apical papilla (SCAPs). However, how SFRP2 promotes osteogenic differentiation of dental tissue-derived MSCs remains unclear. In this study, we used SCAPs to investigate the underlying mechanisms. SCAPs were isolated from the apical papilla of immature third molars. Western blot and real-time RT-PCR were applied to detect the expression of β-catenin and Wnt target genes. Alizarin Red staining, quantitative calcium analysis, transwell cultures and in vivo transplantation experiments were used to study the osteogenic differentiation potential of SCAPs. SFRP2 inhibited canonical Wnt signaling by enhancing phosphorylation and decreasing the expression of nuclear β-catenin in vitro and in vivo. In addition, the target genes of the Wnt signaling pathway, AXIN2 (axin-related protein 2) and MMP7 (matrix metalloproteinase-7), were downregulated by SFRP2. WNT1 inhibited the osteogenic differentiation potential of SCAPs. SFRP2 could rescue this WNT1-impaired osteogenic differentiation potential. The results suggest that SFRP2 could bind to locally present Wnt ligands and alter the balance of intracellular Wnt signaling to antagonize the canonical Wnt pathway in SCAPs. This elucidates the molecular mechanism underlying the SFRP2-mediated directed differentiation of SCAPs and indicates potential target genes for improving dental tissue regeneration.

T-3008

THE DIFFERENTIATION POTENTIAL OF PLURIPOTENT STEM CELL IS DETERMINED BY THE LEVEL OF CHROMATIN HELICASE BINDING DOMAIN 7

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Pluripotent Stem Cells (PSCs) have two distinct potentials: self-renewal and differentiation. In a range of experiments we found PSCs lost and regained the differentiation potential reversibly by changing culture media when seeded in single cells. To explore the molecular mechanisms that regulate the differentiation potential of PSCs, a methylation status comparison study of several PSC clones that retained (or lost) differentiation potential was conducted. We found the expression level of Chromatin Helicase DNA binding Domain 7 (CHD7) determines the differentiation potential of PSCs. Indeed, copy numbers of CHD7 in a range of PSCs (ESC:H9, KhES-1, iPSC: PFX#9, 201B7, SHh#2) cultured with Repro FF2 on Vitronectin-N coated in single cell seeding were below 800 copies/5ng total RNA and lost the potential to differentiate. However, by changing culture medium to Essential 8 (Es8) or Stem Partners, the copy number of CHD7 in these cells elevated to 3200 copies/5ng total RNA or above and cells resumed the differentiation potential manifested by three germ layer differentiation in an Embryoid body formation assay. PSCs underwent spontaneous differentiation when the level of CHD7 was upregulated following the introduction of mCHD7. In the same context, the differentiation potential of PSC cultured with Es8 was perturbed when siCHD7 was introduced into the cells. These results indicate there is both an upper limit of expression for CHD7 to maintain PSCs in an undifferentiated state and a lower limit to retain differentiation potential. PSC clones represent a gathering of heterogenous cells having a distinct CHD7 level determined by distinct epigenetic status of the CHD7 promoter in each cell and thereby a distinct differentiation potential. This is especially the case with iPC clones being generated by reprogramming somatic cells that themselves have differing epigenetic statuses. The heterogeneity of PSC clones can be maintained when PSCs are cultured in cell clumps, and the differentiated colonies needed to be removed daily to maintain the undifferentiated state of PSCs in the culture. Our results suggest we can easily establish “differentiation resistance-free” iPSC clones from heterogenous iPSC clones by single cell seeding, using copy number of CHD7 as an index for differentiation potential.

T-3010

DEVELOPMENT OF CUSTOMIZED HUMAN INSULIN PRODUCING CELLS OF TYPE 2 DIABETES DERIVED BY HUMAN INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells were generated by transducing specific transcription factors, which are Oct3/4, Sox2, c-Myc and Klf4, in various cells. The objective of study is comparison of induced pluripotent stem cells generation and inducing differentiation into insulin-producing cells between normal and type 2 diabetic patients. We generated induced pluripotent stem cells from fibroblast of normal and that of type 2 diabetic patients. To differentiate generated induced pluripotent stem cell into functional insulin-producing cells, We designed protocol of 4 stage. We infected adenoviral Pdx-1 in pancreatic progenitor stage. Pancreatic progenitor cells were aggregated like pancreatic islets. Differentiated cells were examined specific transcription markers in differentiation stage by qRT-PCR and identified by immunofluorescence. We investigated function of differentiated insulin producing cells by glucose-stimulated insulin secretion. Induced pluripotent stem cells of type 2 diabetes are required longer for generating induced pluripotent stem cells than normal. Survival of induced pluripotent stem cells from type 2 diabetic patients was significantly decreased. But, generated induced pluripotent stem cell lines were identical quality as induced pluripotent stem cells of normal. We successfully induced definitive endoderm, pancreatic endoderm, pancreatic progenitor and insulin-producing cells stage from induced pluripotent stem cells of type 2 diabetes as well as normal. We confirmed the secretion of insulin and C-peptide in differentiated insulin-producing cells. We identified a possibility of insulin-producing cells differentiation and induced pluripotent stem cells generation regardless of disease. We are confident that it would be the source for allo-transplantation and auto-transplantation to treat diabetes, once high quality of insulin-producing cells are developed.

T-3012

MIR-4662A ENHANCES GENERATION OF CYP2B6-EXPRESSING HEPATOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

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Pharmaceuticals and chemicals are primarily metabolized in the liver. Drug-induced liver injury (DILI) remains a leading cause of new drug withdrawal from human clinical trials. Hepatocytes differentiated from human embryonic stem cells or human induced pluripotent stem cells (ESCs/iPSCs) are expected to be useful for the prediction of DILI in drug development. However, the actual applicability is hampered by the limited availability of metabolically functioning hepatocytes derived from human pluripotent stem cells (hPSCs). Cytochrome P450 2B6 (CYP2B6) which belongs to the set of important hepatic drug-metabolizing CYPs, accounts for 3-6% of total hepatic CYP content and metabolizes several pharmaceuticals including efavirenz and cyclophosphamide. Therefore, CYP2B6 has been linked to mortality during cyclophosphamide therapy and to hepatotoxicity during efavirenz therapy. Therefore, a hepatic platform expressing substantial levels of CYP2B6 is perceived for drug development and hepatotoxicity screening. In order to generate hepatocytes expressing drug metabolic enzymes especially CYP2B6, the current work tried to enhance the metabolic function of hepatocytes from ESCs/iPSCs via employing microRNAs differentially expressed in primary human hepatocytes (PHH). We initially setup protocols to differentiate hPSCs to hepatocyte-like cells (HLCs). MicroRNA expression profiling and real time quantitative PCR analysis were then conducted in PSC-HLCs and PHH. There are 23 miRNAs that were found to be expressed at high levels in primary hepatocytes compared to PSC-HLCs. We validated the expression of seven miRNAs including hsa-miR-664a, hsa-miR-194, hsa-miR-29c, hsa-miR-4662a, hsa-miR-885, hsa-miR-126, hsa-miR-122. Among these miRNAs, we revealed transfection of hsa-miR-4662a can enhance metabolic gene expression and/or enzyme activity in both HLCs derived from ESCs and iPSCs including CYP2B6, CYP2C9, and CYP3A4. Moreover, combination of hsa-miR-4662a together with hsa-miR-126, hsa-miR-122 further upregulated enzyme activities of CYP2B6 in HLCs derived from ESCs and iPSCs. Hopefully, the findings from the current work can lead to develop a strategy that could enhance metabolic functions of PSC-derived HLCs to be used for the prediction of DILI in drug development in the near future.

T-3014

ASSESSING THE DIFFERENTIATION POTENTIAL OF HUMAN PODOCYTES USING NPHS2-GFP AND NPHS1-GFP-IPSCS

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Adult cell reprogramming to generate induced pluripotent stem cells (iPSCs) provides an unprecedented opportunity to elucidate disease mechanisms, and promote the development of replacement therapy. We have developed a differentiation protocol for kidney podocytes derived from iPSC (iPSC-PODs). However, purification of iPSC-PODs remains challenging. Additionally, there are limited studies showing the integration of iPSC-PODs into normal and damaged kidneys in vivo. The aim of the study was to generate a iPSC reporter cell line targeted to the NPHS2 locus encoding the podocyte-specific gene podocin tagged with enhanced green fluorescent protein (eGFP+) using clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9. These NPHS2eGFP modified human iPSCs (NPHS2-GFP-iPSCs) will enable selective isolation of iPSC-PODs for cell transplantation in vivo. Two plasmids: one containing the eGFP gene (within two podocin homology arms) and podocin-specific PX335 containing the pair of sgRNAs were designed. The positive clones were then selected with neomycin resistance and clonally expanded. NPHS2-iPSC-PODs were generated by the addition of iPSC-PODs medium containing the differentiation factors retinoic acid, BMP7 and action A for 10 days. Another well established cell line nephrin-GFP iPSC was subjected to the same differentiation protocol. GFP expression was confirmed by genotyping and fluorescent microscopy. iPSC-PODs were stained with CFSE dye and differentiated. Ten days after differentiation, iPSC-PODs were injected into newborn kidneys to assess integration efficiency. Using fluorescent microscopy, cells were detected in renal cortex of postnatal day 3 newborn mouse kidneys injected intrarenally with D10 iPSC-POD. This provides proof-of-principle for iPSC-POD integration in postnatal kidneys, ideal recipients due to the ongoing developmental environment and without the need for immunosuppression. This study has generated the first NPHS2-GFP-iPSCs reporter cell lines with initial findings confirming cell integration in postnatal kidneys established. iPSC-PODs can be potentially used as a valuable tool to develop a cellular replacement therapy or a test bed to study the mechanism of human glomerular disease.

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T-3016

LYSOPHOSPHATIDIC ACID RECEPTOR 4 IS A PIVOTAL REGULATOR OF CARDIAC DIFFERENTIATION FROM EMBRYONIC STEM CELLS AND ADULT CARDIAC STEM CELLS

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Discovering a lineage-specific marker based on a comprehensive understanding of development is a key process in which allows an efficient induction of differentiation. We here report a new cardiac progenitor stage-specific marker, lysophosphatidic acid receptor 4 (LPAR4), and show its functional significance of cardiac differentiation and therapeutic implication. To screen cell-surface expressing molecules on cardiac progenitor cells compared to undifferentiated cells, we isolated Flk1+/PDGFRα+ cells at differentiation day 3 and performed microarray analysis as compared with undifferentiated cells. Among candidates, we identified a new G protein-coupled receptor (GPCR), LPAR4. Consistent with LPAR4 expression in mouse iPSCs where it peaks at day 3 after differentiation and shuts down immediately, a heart-specific expression of LPAR4 in mouse embryo disappears completely in adults, suggesting the role of LPAR4 in stimulating differentiation at an early stage and must be shut off for further progression of differentiation. In mouse iPSCs, LPA (LPAR4 agonist) followed by antagonist combination (AM966 and BrP-LPA) treatment significantly increased cardiac differentiation efficiency. And we demonstrated the key signaling molecule of LPAR4 down-stream signals related to cardiomyocyte differentiation. To confirmed LPAR4 down-stream key signaling molecule, we checked typical cardiomyocyte differentiation signaling molecules p38MAPK, ERK1/2, and AKT compared mouse embryonic stem cells with LPAR4 knockdown embryonic stem cell line and p38MAPK signaling pathway was the LPAR4 key down-stream molecule. Furthermore, there was a substantial increase in LPAR4 (+) cells in the adult mouse after myocardial infarction (MI). In vivo, sequential stimulation and inhibition of LPAR4 resulted in the reduction of infarct size and improvement of heart dysfunction after MI. We demonstrate that LPAR4 is a new cardiac progenitor stage-specific marker, as expressed on the cell surface. The manipulation of LPAR4 signaling enhances in vitro differentiation of iPSCs and ESCs into cardiac cells and shows in vivo therapeutic potential in adult mice after MI. Our findings provide a new insight into embryonic cardiac development and regeneration after injury.

T-3018

ENHANCED DIFFERENTIATION OF HUMAN PANCREATIC ISLET-DERIVED INDUCED PLURIPOTENT STEM CELLS INTO INSULIN-PRODUCING CELLS USING A SMALL MOLECULE

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In this study, we established human pancreas islet-derived induced pluripotent stem cells (hpiPSCs) to treat diabetes. These cells are advantageous in that they may : 1) be sourced from redundant partial pancreas tissue obtained from a pancreatectomized patient can be used to treat patients in whom postoperative diabetes is induced, 2) be more likely to differentiate into islet cells owing to the specific genetic properties, such as the epigenetic memory and preferential lineage-specific differentiation. We isolated the pancreatic islets and dermal fibroblast cells from the pancreas and skin, respectively, and induced their differentiation into iPSCs using sendai reprogramming kit. Specific mRNA expression of these pancreatic cells, including islet-specific transcription factors, were compared and analyzed in the established hpiPSCs and iPSCs. There were also analyzed expressions of specific genes for each differentiation stage during differentiation into insulin-producing cells steps. Finally, mature differentiated IPCs were transplanted into STZ-induced diabetic mice to determine whether they could regulate glucose in vivo. The iPSCs specific expression markers such as Oct3, SSEA4, Sox2, and TRA-1 were stained in established both hpiPSCs and iPSCs. The identification markers of each differentiation step were expressed and mRNA expression of insulin, glucagon, and somatostatin were increased significantly in the differentiated IPCs. Furthermore, the amount of c-peptide secreted in the medium was also detected. Over 40% of insulin-producing cells were identified using FACS. hpiPSCs and iPSCs were maintained under normal blood glucose levels for 30 days or more post transplantation into diabetic mice. Although further studies supporting the analysis of afferent epigenomic memory should be supported, this study demonstrated that hpiPSCs differentiated into IPCs and functioned in vivo as mature islet cells capable of in vivo glucose regulation. Therefore, hpiPSCs will be capable of inducing higher IPC differentiation efficiency through study of the underlying mechanisms, which can serve as a good alternative to islet cell transplantation.

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T-3020

MITOCHONDRIAL AND GLYCOLYTIC METABOLISM DECREASE DURING NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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A main goal for human embryonic stem cell (hESC) research is to differentiate hESC into specialised cell types. Significantly, the metabolism and nutritional requirements of a cell change with cell state. Yet, no comprehensive study exists on the daily changes in metabolism throughout hESC differentiation, which is necessary for optimising targeted differentiation and reprogramming protocols. This study charted, every 24 h, the changes in mitochondrial and glycolytic metabolism as hESC were differentiated into neural precursor cells (NPC). Neural differentiation over 11 days to NPC was performed on two hESC lines (MEL1; MEL2) at 5% and 20% oxygen. Mitochondrial metabolism was assessed by mitochondrial mass, activity, mtDNA copy number and superoxide levels. Metabolism was determined by glucose and pyruvate consumption, lactate production and glycolytic flux. To confirm neural induction of hESC, neural precursor cell populations, neurospheres, and BIII tubulin positive neural networks were generated at 5% and 20% oxygen. Throughout hESC differentiation to NPC, mtDNA copy number ($p < 0.0001$) and mitochondrial mass decreased ($p < 0.01$) by ~40% and 20% respectively. Furthermore, NPC consumed approximately half the glucose ($p < 0.05$) and pyruvate ($p < 0.05$) of hESC and had ~50% lower mitochondrial membrane potential ($p < 0.05$), all describing a more quiescent metabolic state. hESC cultured at 5% oxygen displayed greater mitochondrial mass and greater carbohydrate fluxes than those at 20% oxygen, and hence displayed a more profound shift towards the quiescent NPC state during differentiation. The dogma that ESC are glycolytic and that mitochondrial metabolism becomes the primary ATP source during differentiation is disputed by these findings. Upon neural induction, both glycolytic flux and mitochondrial mass and activity decrease, reflecting a slowing metabolism. These findings suggest that glycolytic and mitochondrial carbon sources, glucose and glutamine, may need to be limited to support the most physiological and efficient neural differentiation.

T-3022

HOW DOES AR DEFICIENCY AFFECT FORMATION OF SPERMATOGONIUM-LIKE CELLS FROM PSCS?

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Infertility affects about 15% of couples world-widely. Among which, mutations of androgen receptor (AR) have been found in 2-3% of infertile males. Androgen is secreted by Leydig cells, whereas AR is mainly expressed in the testicular Sertoli and peri-tubular myoid (PTM) populations. Together these cells establish a microenvironment for proper germ cell development. AR deficiency leads to androgen insensitivity syndrome (AIS), an X-linked recessive disorder, which causes phenotypes ranging from genital ambiguity to male infertility. We recently developed a feeder- and xeno-free culture, and successfully induced high percentage of spermatogonium-like cells (SLCs) from human pluripotent stem cells (PSCs). In addition, a small minority of SLCs has the potential to go through meiosis to become haploid cells (Stem Cell Reports, in press). We thus speculate that the existence of androgen-secreting Leydig cells and AR-expressing Sertoli or PTM cells in our derivation culture to support the germ cell development from PSCs. Indeed, FSHR+/GATA1+ Sertoli cells and DHH+ Leydig cells were detected during SLC derivation. Upon treatment with an AR inhibitor, significantly fewer PLZF+ SLCs developed from PSCs, compared with controls. Consistently, expression of key genes in spermatogonial formation, including PLZF, NANOS3 and GFRα1, were dramatically reduced in AR inhibitor treated samples. In addition, the transcript levels of key genes in three germ layer development and BLIMP1, an important regulator in formation of primordial germ cells (PGCs) were not significantly altered, suggesting normal three germ layer development and PGC derivation in the presence of AR inhibitor. By contrast, expression of GATA1, FSHR, and DHH was obviously decreased, thus suggesting that the niche to support SLC formation is disrupted by AR inhibition, and this in turn leads to reduced germ cell development from PSCs. Currently, multiple PSC lines with AR mutations were created from infertile patients or by CAS9/CRISPR techniques. Those lines will allow us to further determine whether our differentiation system provides a suitable niche for

SLC derivation from PSCs. The knowledge gleaned from this study will also contribute to our understanding of pathology and clinical therapy for male infertility caused by androgen or AR deficiency.

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T-3024

ESTABLISHMENT OF THE INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATED TO CHONDROCYTES STRATEGY BY 3D SCAFFOLD COMBINE WITH FACTORS

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Articular cartilage (AC) is composed of chondrocytes, responsible for abundant matrix synthesis and maintenance. When pathological or traumatic damaged happened, AC does not heal spontaneously and resulted in Osteoarthritis (OA). OA is the most common musculoskeletal disease in the elderly. However, cartilage defect and OA currently lack effective therapeutic methods. Stem cells provide hope for cell therapeutic methods for cartilage defect and OA. In all type of stem cells, embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are the most powerful cells that could differentiate into all three-layer cells, including chondrocyte. Previously, we generated a novel iPS-OSH cells that under hypoxic conditions in the absence of viral infection and oncogenic factors. In this study, we differentiated the iPS-OSH cells into chondrocyte and cultured on the 3D bioresorbable scaffold (polycaprolactone) to compare the proliferation and differentiation ability between different factors treatment, including transforming growth factor-β1 (TGF-β1) and/or Granulocyte Colony-Stimulating Factor (G-CSF). We evaluated the cell proliferation rates and chondrogenic markers with several approach including trypan blue exclusion assay, alcian blue staining, immunofluorescent staining and real-time RT-PCR. The results showed that the differentiation and proliferation rates of chondrocytes were accelerated in 3D culture environment compared to the conventional 2D culture system. Additional supplements with TGF-β1 and G-CSF also promoted the differentiation and proliferation rates of chondrocytes both in both culture systems. Overall, iPS-OSH cells exhibited the highest chondrogenic activities in the 3D culture environment supplied with TGF-β1 and G-CSF. The present study assessed the clinical application of iPS cells and suggested a novel therapeutic strategy to improve the cartilage defect which lacking effective therapeutic methods nowadays.

T-3026

RNA-SEQUENCING OF HUMAN PLURIPOTENT STEM CELL-DERIVED KIDNEY CELLS

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Chronic kidney disease can lead to end stage renal disease, at which point there are only two life-saving options for the patient: kidney transplantation or life-long dialysis. Novel treatment options aim to regenerate the kidney by using the patient's own cells to derive new kidney cells. Using patient material to produce Induced pluripotent stem cells (iPS), it is possible generate renal cell types and kidney organoids, which could be used for disease modeling or cell therapy. We are using a custom protocol that differentiates human iPS cells into specific renal cell types, which could be used to generate organoids for disease modeling. Pluripotent stem cells (d0) were differentiated in a first step to primitive streak (d2), intermediate mesoderm-like cells (d4), and then separate conditions were used to derive podocytes and tubular epithelial cells (d14) from renal vesicle (d8). Bulk RNA-sequencing (1 x 50 bp) was completed on the differentiation time series. Additionally, renal progenitor cells (d6 stage) were allow to self-organize and spontaneously form organoids (d10). Hand-picked organoids were enzymatically dissociated into single cells. cDNA from single cells were produced using the Fluidigm C1 system and subjected to sequencing (2 x 100 bp). All raw reads were aligned to the hg38 reference genome with Tophat and relative transcript abundances (fpkm) were generated by Cufflinks software. The transcriptome of the bulk differentiated cells at day 8 shows that many markers of the renal vesicle are expressed. In mammalian kidney development, the renal vesicle gives rise to podocytes, mesangial cells, and epithelial cells (proximal/distal tubule cells). Specific markers for these cell types are detected in the day 14, which points to a mixture of these cell types. Single-cell sequencing of the d10 organoids shows that individual cells do express some kidney markers but due to the high dropout of genes in single-cell analysis, it is clear that a deeper bioinformatic analysis is required to identify these cells from their single cell profiles. By classifying the potency of single cells to become specific renal types we hope to better understand the specification of renal cell types during nephron morphogenesis and provide a baseline for a cell-matrix which allows the assessment of disease-associated deviations.

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T-3030

AN INACTIVATING MUTATION IN THE HISTONE DEACETYLASE SIRT6 CAUSES A HUMAN PERINATAL LETHALITY SYNDROME

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It has been well established that histone and DNA modifications are critical to maintaining the equilibrium between pluripotency and differentiation during early embryogenesis. Mutations in key regulators of DNA methylation have shown that the balance between gene regulation and function is critical during neural development in early years of life. However, there have been no identified cases linking epigenetic regulators to aberrant human development and fetal demise. Here, we demonstrate that a homozygous inactivating mutation in the histone deacetylase SIRT6 results in severe congenital anomalies and perinatal lethality in four affected fetuses. *In vitro*, the amino acid change at aspartate 63 (Asp63) to a histidine results in virtually complete loss of H3K9 deacetylase and demyristoylase functions. Functionally, SIRT6 D63H mouse embryonic stem cells (mESCs) fail to repress pluripotent gene expression, direct targets of SIRT6, and exhibit an even more severe phenotype than Sirt6 deficient ESCs when differentiated into Embryoid Bodies (EBs). When terminally differentiated towards cardiomyocyte lineage, D63H mutant mESCs maintain expression of pluripotent genes and fail to form functional cardiomyocyte foci. Lastly, human induced Pluripotent Stem Cells (iPSCs) derived from D63H homozygous fetuses fail to differentiate into embryoid bodies, functional cardiomyocytes and neural progenitor cells, due to a failure to repress pluripotent genes. All together, our study described a germline mutation in SIRT6 as a cause for fetal demise, defining SIRT6 as a key factor in human development, and identifying the first mutation in a chromatin factor behind a human syndrome of perinatal lethality.

T-3032

SCALABLE PROCESS FOR STANDARDIZED GENERATION OF HUMAN PSC-DERIVED CARDIOMYOCYTES

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Fast and highly efficient generation of pure pluripotent stem cell-derived cardiomyocytes is a prerequisite for therapeutic applications, drug development and heart disease modeling. All these applications require controlled and standardized processes to allow for cost effective, large scale production of cardiomyocytes (CMs). Nevertheless, cardiovascular differentiations of human pluripotent stem cell cultures (PSC) do not generate homogeneous cell populations but a rather heterogeneous composition of CM subpopulations and non-CMs. The final cell composition and differentiation efficiency is currently depending e.g. on the stem cell clone, its passage and the differentiation protocol used. To circumvent these experimental variations and prepare for standardized processes suitable for automation and clinical scale up, we have now established a complete workflow from controlled cardiac differentiation to CM harvesting, purification, storage and analysis. Our workflow protocol allows for robust, highly efficient and scalable generation of CMs within less than 10 days of differentiation, thereby solving several technical issues related to generation of PSC-derived CMs. Previously, we have developed a cultivation and expansion workflow for PSCs using the CliniMACS Prodigy. In a next step, our workflow protocol for CM generation will be transferred to this functionally closed system enabling for scale up of CM manufacturing. First data indicate that 9x10e7 CMs could be generated in a single production run of the CliniMACS Prodigy connected to a one layer cell stack system. The newly generated CMs show a similar expression pattern as compared to small scale differentiations. Transfer of the complete workflow and reagents to the integrated cell processing platform will pave the way for standardized, large scale manufacturing of PSC-derived cardiomyocytes using the CliniMACS Prodigy.

T-3034

THE INFLUENCE OF STAT5 ACTIVATION BY CELECOXIB ON DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO HEPATOCYTES

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The liver is abundantly expressing various drug-metabolizing enzymes, and it plays a central role in drug metabolism. Especially, cytochrome P450 (CYP) 3A4 is one of the most important enzyme in the liver. Therefore, in drug development, the metabolism by CYP3A4 in the liver is important for predicting pharmacokinetics. Currently, human induced pluripotent stem (iPS) cell-derived hepatocytes (hiHep) have become a major focus as a source for drug development study. However, hiHep have lower expression of drug metabolism-related genes and lower drug metabolism activity such as CYP3A4 than human primary hepatocytes. Then, hiHep have insufficient ability to use drug metabolism studies. Recently, it was revealed that celecoxib restores expression of various CYPs to normal levels through the activation of signal transducer and transcriptional activation factor 5 (STAT5) in rat hepatocarcinoma. Therefore, in this study, we investigated whether celecoxib contributes the maturation of hiHep. The hiHep were expressed hepatocytes markers such as albumin, α-fetoprotein and hepatocyte nuclear factor 4α. The gene expression levels of hepatic marker genes (asialoglycoprotein receptor 1 and tyrosine aminotransferase), metabolic enzyme genes (UDP-glucuronosyltransferase 1A1 and CYP3A4), nuclear receptor genes (pregnane X receptor and constitutive androstane receptor) significantly increased by celecoxib compared to the control. Furthermore, the CYP3A4 activity was also increased significantly by celecoxib, and the activity was decreased by the addition of ketoconazole as a selective inhibitor of CYP3A4. Similarly, in other iPS cell lines, the gene expression level of CYP3A4 was increased by celecoxib. To investigate how celecoxib increased CYP3A4 gene expression, we added pimozide as an inhibitor of STAT5 simultaneously with celecoxib. Consequentially, the expression of CYP3A4 was almost the same as that of control by pimozide. These results suggested that celecoxib contributes to the maturation of hepatocytes by activation of STAT5, and celecoxib is useful for the generation of more functional hiHep.

PLURIPOTENT STEM CELL: DISEASE MODELING

T-3038

DIAGNOSTIC AND THERAPEUTIC SIMULATION OF OSTEOGENESIS IMPERFECTA USING INDUCED PLURIPOTENT STEM CELL AND CRISPR/CAS9 GENE EDITION

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Osteogenesis imperfect is a genetic disease characterized by repeated bone fragility fractures. The cause of bone fragility of osteogenesis imperfect is due to defective collagen formation from mutation of COL1A1 or COL1A2. Osteogenesis imperfect is impossible to cure with current therapeutic strategies. Induced pluripotent stem cell (iPSC) from patients with specific diseases is now utilized for disease simulation and drug screening platform. Here, we generated iPSCs from a family of osteogenesis imperfect (OI). Single gene mutation of COL1A1 gene was identified in disease family. OI-iPSCs revealed defected mineralization *in vitro* osteogenesis system. CRISPR/Cas9 vector was cloned for correcting mutated genes in OI-iPSCs. CRISPR/Cas9 system was successful in editing COL1A1 mutated OI-iPSCs. Gene corrected OI-iPSC showed the recovery of their osteogenic potential. Recovered osteogensis was also reproduced *in vivo* at calvarial bone defect model of SCID mice. OI-iPSC could help to study the core pathophysiology of this rare disease. Furthermore, CRISPR/Cas9-based gene correction may give the chance to therapeutic application on osteogenesis imperfect.

T-3040

BANKING OF CLINICAL GRADE HPSC: PAVING THE WAY FOR FUTURE CLINICAL APPLICATIONS

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The UK Stem Cell Bank (UKSCB) is a key partner of the UK regenerative medicine infrastructure, charged with procuring, processing (banking and testing) and distributing seed stocks of human embryonic stem cell (hESC) lines for research and clinical application. One of the key objectives of the UKSCB is to bank and release stem cell lines that meet the EU Tissue and Cell Directives (EUTCD) criteria as set out in Human Tissue Authority (HTA) regulations. These directives set a

benchmark for the standards that must be met when carrying out any activity involving tissues and cells for human clinical application. All cell lines are subject to ethical approval by a national Steering Committee, following which the UKSCB performs a due diligence protocol and determines if each cell line meets the requirements set and is therefore suitable as a starting material for clinical trials. Currently, 38 hESC lines have been approved for deposit as EUTCD-Grade by the UK Steering Committee. At present, over 10 lines have been banked on feeders and fully characterised to the EUTCD grade standard. The bank is now focusing its activities on supplying feeder-free deeply characterised EUTCD-grade hESC lines. Here, we describe the key elements of the UKSCB banking process for these lines including review, processing, characterisation, storage and distribution; all of which meet the requirements of the UKSCB's regulatory licence. This programme of work represents the start of a pipeline of EUTCD-grade hESC lines to support the regenerative medicine community in the development of quality and safety-assured cell therapies.

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T-3042

MODELING SENSORY NEURON PLASTICITY IN PULMONARY DISEASE USING HUMAN EMBRYONIC STEM CELL DERIVED SENSORY NEURONS AND PRIMARY MOUSE VAGAL NEURONS WITH HUMAN EPITHELIAL CELLS

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Hypersensitivity of the sensory circuitry innervating the respiratory tract accompanies mucosal dysfunction in a variety of pulmonary diseases, and contributes significantly to patient morbidity. The interplay between respiratory epithelial cells and sensory neurons is poorly defined and difficult to study in vivo. We set out to develop novel in vitro preparations consisting of stem cell-derived or primary vagal sensory neurons and human airway epithelial cells. Differentiation of H9 human embryonic stem cells (hESC) into sensory neurons was optimised using small inhibitors and growth factors. hESC-derived neurons stain positive for B-tubulin III, Neurofilament, Peripherin, TRPV1, Piezo2, vGluts1/2, and Calbindin neuronal markers and express key sensory genes (TAC1, SCN9A, P2RX3, TRPV1, ASIC2), and are electrically active (membrane potential -44.67310mV, action potential threshold -36.0234.88mV). Murine primary vagal sensory neurons were enzymatically dissociated and cultured. Media conditions were optimised to allow human epithelial cells grown at air-liquid interface (ALI) to be co-cultured with primary vagal or hESC-derived sensory neurons. Neurons co-cultured with differentiated epithelial cells at ALI possess significantly longer neurites than those grown alone (neurons only = 6403212cm; co-culture = 9163328cm; $P<0.05$, paired T-test). In addition, co-cultured neurons displayed molecular expression profiles closely resembling acutely isolated cells compared to neurons cultured for the same time alone. Altered growth and gene expression profiles of neurons in co-culture conditions suggests the existence of epithelial paracrine mediators in the co-culture system that support the maintenance of neurons. Future experiments will utilise hESC-derived neurons and epithelial cells derived from patients with respiratory disease to better define mechanisms of sensory nerve plasticity.

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T-3044

X CHROMOSOME INACTIVATION STATUS OF KLINEFELTER SYNDROME DERIVED HIPSCS

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Klinefelter syndrome (KS; 47, XXY karyotype), is associated with a wide phenotypic variability, with the primary features being sterility and small testicles. The improper testicular environment, as well as failures in the meiotic progression, may contribute to the progressive germ cell depletion after the onset of puberty, however, the link between germ cell degeneration and the additional X chromosome remains still unclear. Although X chromosome inactivation (XCI), a silencing mechanism of one X chromosome in female cells, also occurs in KS patients, the known XCI escaping genes can contribute to the clinical picture of KS patients. In addition, early embryonic development occurring before XCI, could be affected and lead to the widespread defects observed in KS patients. Thus, human induced pluripotent stem cell (hiPSC) -based model for KS could help gathering more information about possible developmental defects. In this study, we derived hiPSC lines from skin fibroblasts biopsied from two KS patients, and analyzed the whole transcriptome by RNA-sequencing and XCI status by RNA fluorescence in situ hybridization (FISH) for XIST. We found 332 genes differentially expressed between KS and normal male fibroblasts, and gene

ontology (GO) enrichment analysis for up-regulated genes showed enrichment for regulation of urogenital system development including reproductive system development and epithelial cell proliferation. When comparing KS hiPSCs and normal male hiPSCs 1061 genes were found differentially expressed, and GO terms for down-regulated genes included endoderm cell differentiation, regulation of metanephros development and blood vessel remodeling. For KS fibroblasts, only 8 genes out of 220 up-regulated genes were X-linked, with 3 known XCI escapes (including XIST). For KS hiPSCs, 124 genes out of 357 up-regulated genes were X-linked, with just 26 known XCI escapes, indicating incomplete XCI for KS hiPSCs. RNA FISH confirmed XCI for all cells in KS fibroblast cultures, whereas KS hiPSC cultures had two cell populations; with either one or no XIST cloud. Further investigation on XCI and in vitro germ cell differentiation of KS hiPSCs will contribute to a better understanding of the germ cell development failures in KS patients.

T-3046

THE SPONTANEOUS DIFFERENTIATION AND CHROMOSOME LOSS IN IPSCS DERIVED FROM HUMAN AMNIOTIC FLUID CELLS OF TRISOMY 18 SYNDROME

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Trisomy 18 syndrome is the second most common aneuploidy after trisomy 21 syndrome. Patients are characterized by multiple malformations and extremely short lifespan. However, the molecular mechanisms remain unclear. Here, we successfully generated several iPSC lines derived from human amniotic fluid cells of two patients with trisomy 18. We found that trisomy 18 iPSCs (18T-iPSCs) were prone to differentiate spontaneously. Intriguingly, 18T-iPSCs lost their extra 18 chromosomes and converted to diploid cells after 10 generations. To obtain a high-resolution genome-wide DNA copy number data, we performed a DNA copy number variants (CNVs) analysis using the Affymetrix CytoScan Arrays. The results confirmed that 18T-iPSCs carried an extra chromosome 18, while no other obvious microdeletions or microduplications happened in the genome. Fluorescence in situ hybridization analysis showed chromosome loss was a random event that might happen in any trisomic cells. Selection undifferentiated cells for passage accelerated the recovery of euploid cells. To investigate the mechanisms underlying the phenotypes observed in 18T-iPSCs, we compared the global gene expression profile of 18T-iPSCs with that of isogenic diploid iPSCs. Changes in gene expression between the isogenic cells were caused by the extra copy of chromosome 18 but not individual variation. RNA seq analysis indicated a relative small change between these six samples among the 20049 to 25 334 identified genes in 18TiPSCs compared to diploid iPSCs. GO analysis of the upregulated genes showed that genes associated with neural differentiation, ectoderm development and cell motility were significantly enriched in the18T-iPSCs, which was consistent with our observation that these cells were prone to spontaneous differentiation, especially towards neural differentiation. Further we found these genes also was involved in the epithelial-mesenchymal transfer (EMT) process. Among these genes, the Cdh2 gene is located in chromosome 18. Overexpression of Cdh2 in human ES cell line H9 suggested that it contributes to the neural differentiation of 18T-iPSCs. Moreover, Cdh2 regulated the expression of NPTX1 and promoted a neural lineage fate of the 18T-iPSCs. Overall, our findings indicate the genomic instability of 18T-iPSCs bearing an extra chromosome 18.

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T-3048

AN IN VITRO HUMAN SENSORY NEURON MODEL OF PIEZO2 DEFICIENCY

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PIEZO2 is a mechanically activated ion channel responsible for the mechano-senses of light touch and proprioception, the perception of body position and movement. Individuals with rare inactivating mutations in PIEZO2 have profound loss of gentle touch discrimination and display strikingly uncoordinated motor movements. PIEZO2 patients thereby exhibit delays in achieving developmental motor skills, heavily relying on visual compensation. While the clinical phenotype of PIEZO2 loss of function is unique and consistently discernible, our understanding of how PIEZO2 deficiency impacts human peripheral sensory neuron development and physiology is incomplete. We have established a method to produce sensory neurons in vitro by forced overexpression of developmental transcription factors. Here, we applied this technique to rapidly generate sensory neurons directly from PIEZO2 patient induced pluripotent stem cells. These induced neurons displayed morphological and biochemical hallmarks of human peripheral sensory neurons through expression of specific neurotrophin receptors, neurofilaments, and transcription factors. Importantly, we did not observe any differences in the efficiency of

differentiation or expression of sensory neuron markers between control and patient neurons. Because the differentiation proved robust across multiple cell lines, we considered this model system well-suited to assess sensory neuron disease phenotypes in vitro. When presented with a mechanical stimulus, high amplitude PIEZO2-mediated currents were reliably evoked in 50% of neurons from an unaffected control. However, no responses were detected in patient-derived neurons. Consistent with previous findings, our model system confirms that PIEZO2 is an essential component of human mechanosensation, and may provide a unique opportunity to elucidate the physiological consequences of PIEZO2 loss of function in human sensory neurons.

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T-3050

PATIENT-DERIVED IPS CELLS AS A NEW MODEL SYSTEM FOR STUDYING NEUROBLASTOMA INITIATION AND PROGRESSION

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Neuroblastoma (NB) is an embryonic cancer originated from the sympathoadrenal lineage of neural crest. Children diagnosed with NB over 1 year of age often have extensive or metastatic disease resulting in a poor prognosis. NB develops in the peripheral nervous system, specifically in the adrenal ganglia. Neural crest cells (NCC) is a transient multipotent cell population present only in the developing embryo. The emergence of cellular reprogramming techniques has made it possible to create models of otherwise scarce disease-relevant human cells, such as NCC. Anaplastic lymphoma tyrosine kinase (ALK) is thought to play a role in the development of the nervous system during embryogenesis. Mutations in the ALK gene are common events in NB. To study the contribution of ALK mutations in NB initiation, we have successfully reprogrammed non-cancerous fibroblasts from NB patients, carrying germline mutations in ALK (R1275Q) into induced pluripotent stem (iPS) cells. The patient-specific iPS cells and iPS cells from healthy individuals have been characterized by morphology, expression of pluripotency markers, as well as differentiation to all three germ layers. More importantly, we have established a fully defined, xeno-free NCC differentiation protocol using iPS cells grown on Laminin-521 and are able to generate NCC that express relevant markers such as p75, SOX10, TFAP2A and PAX3. To examine the tumorigenic ability of these cells, we have orthotopically transplanted luciferase-labeled healthy (Ctrl) or patient NCC into the adrenal gland of immunocompromised mice and monitored tumor growth over time using the IVIS in vivo system. Luciferase activity could be detected in mice injected with patient NCC eight weeks after injection the signal increased over time, while no luciferase activity was observed in mice injected with Ctrl NCC. By using somatic cells carrying germ line mutations as the cell source, instead of using cancer cells derived from an established tumor that carry many more mutations, we will be able to follow the stepwise tumor development resulting from the original mutation. We believe our model will help to uncover new networks important for tumor initiation, discover potential early-stage biomarkers, and give us better models system in which therapeutics can be evaluated.

T-3052

IDENTIFICATION OF DISEASE CAUSING PATHWAYS AND DRUG SCREENING USING AN IPSC-DERIVED MODEL OF MARFAN SYNDROME

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Marfan syndrome (MFS) is a connective tissue disorder caused by mutations in FBN1, with pleiotropic manifestations including severe cardiovascular complications, such as aortic aneurysms and dissection. Current treatments focus on minimising aortic wall stress by controlling haemodynamics but do not tackle the underlying pathology. Although angiotensin II receptor (AngIIR) blockade prevented aortic dilatation and disease in a MFS mouse model, results in clinical trials have been disappointing. We speculate that there are multiple disease perturbations downstream of the FBN1 mutations and that AngIIR antagonism is only interfering with a subset of signalling abnormalities in MFS. Our recent work using our human induced pluripotent stem cell (iPSC) model for MFS identified p38 and KLF4 as novel disease drivers; we hypothesise that there are additional disease-related abnormalities in MFS. Therefore, we used both RNA sequencing and a small-molecule drug library to identify novel pathways responsible for the disease phenotypes. Here, patient iPSC-derived smooth muscle cells (SMCs) are treated with an annotated phenotypic compound library (AstraZeneca UK). We used proteolysis inhibition as an initial measure to screen for positive hits with the intention of further validating putative compounds

for their potential to rescue other MFS abnormalities. Our phenotypic assay reliably identified a number of compounds inhibiting MMP activity, that are under further investigation. In addition, RNA sequencing on MFS and CRISPR-corrected isogenic SMCs identified novel genes and pathways involved in MFS. These complementary techniques will enable the identification of novel disease-causing pathways and drugs to treat MFS, and offer strategies for clinical intervention using our in vitro disease model as a screening platform.

T-3054

DEVELOPMENT OF AN EBOLA VIRUS PATHOGENESIS MODEL USING IPSC-DERIVED HEPATOCYTES

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Hemorrhagic fever viruses are prime examples of zoonotic viruses that accidentally infect humans, causing severe disease. One of the most severe examples of these viruses is Ebola virus (EBOV), which infection causes acute immune-dysregulation and severe liver damage in humans. The recent EBOV outbreak in West Africa with over 28,000 reported cases and more than 11,000 deaths demonstrates the devastating consequences of highly pathogenic emerging viruses hitting an unprepared population in resource-poor, densely populated areas. Studies aimed to dissect mechanisms of pathogenicity are mainly limited to conventional, immortalized cell culture systems, bearing the risk that the results obtained with these systems do not recapitulate the response to viral infection in primary human cells. Our goal is to use induced pluripotent stem cell (iPSC)-derived hepatocytes to develop a platform for modeling EBOV pathogenesis. iPSC-derived hepatocytes were characterized using intracellular staining, qRT-PCR, and functional assays. In a pilot study, iPSC-derived hepatocytes, primary hepatocytes, and hepatic carcinoma cells (Huh7) were infected with a vesicular stomatitis virus expressing the EBOV glycoprotein (VSV-Z76-GFP). Upon VSV-Z76-eGFP infection, iPSC-derived hepatocytes expressed significantly higher levels of IFNβ than Huh7 cells. Infection with VSV-Z76-eGFP at a high MOI did not lead to cell death whereas Huh7 cells died within 24 hours, indicating that iPSC-derived hepatocytes have a competent antiviral response, more closely related to what has been reported in human infection. We then tested infectivity using wild-type EBOV under BSL4 conditions. iPSC-derived hepatocytes and primary hepatocytes supported EBOV infection as indicated by the presence of cytoplasmic viral inclusions and showed significant transcriptomic differences when compared to Huh7 cells. Our data suggest that iPSC-derived hepatocytes represent a powerful platform for the study of human host responses to EBOV infection and pathogenesis and offer a novel strategy for testing new potential antiviral treatments.

T-3056

SPONTANEOUS NETWORK ACTIVITY DEVELOPMENT IN CULTURED CORTICAL NEURONS: COMPARISON BETWEEN RAT EMBRYONIC AND HUMAN PLURIPOTENT STEM CELL -DERIVED SYSTEMS

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Electrical activity development of embryonic rat cortical neurons has been typically measured using microelectrode array (MEA) technology, which enables efficient study of neuronal populations and allows repeated measurements of the same network. Although rodent MEA studies form the basis for the technology, human specific characteristics need to be better evaluated for applications such as in vitro modelling and drug discovery. In the present study, human pluripotent stem cells (hPSC) were differentiated to cortical neurons and their functional development was compared with primary rat cortical cultures (E17-E18). The hPSCs differentiated into mixed neural culture where development of neurons was followed by emergence of astrocytes after extended culture time. Neurons expressed markers for both upper and lower cortical layers and consisted mainly of glutamatergic and GABAergic subtypes. Our MEA data demonstrates that with current differentiation method for hPSC-derived cortical neurons, we can achieve spike rates close to that seen in rat cortical networks in vitro. Also, these hPSC-derived neurons form synchronous networks that reflect those seen in rat cortical cultures. However, there are distinct characteristics in hPSC-derived networks that need to be realized in data analysis and interpretation. Thus, these results suggest that hPSC-derived networks offer promising platform for functional in vitro studies together with rat counterparts.

T-3058

SIALIDOSIS NEURONS FROM PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELL EXHIBIT SYNAPTIC DYSFUNCTION

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Sialidosis is a lysosomal storage disease with the defect of enzyme activity of lysosomal neuraminidase due to the gene mutation of NEU1 encoding the enzyme. Because NEU1 is responsible for the degradation of N-terminal acetylneuraminic acid (major form of sialic acid) residue in sugar chain, sialidosis patients show systemic accumulation of sialyl-glycoconjugates. Although sialidosis patients suffer from progressive neurological symptom such as myoclonic seizures, visual impairment, epilepsy and ataxia, cellular and molecular basis of sialidosis pathology is poorly understood. Patient-derived induced pluripotent stem cell (iPSC) is a promising tool for the investigation of genetic disorders because it holds genetic information of patient and can differentiate into a variety of somatic cells which are difficult to be collected directly from patients such as neurons. In this study, we established sialidosis patient-derived iPSC and investigated the cellular phenotype of iPSC-derived neural cells. These cells show reduced enzymatic activity of neuraminidase, accumulation of sialyl-glycoconjugates and lysosomal abnormality, which are common cellular phenotypes in sialidosis. Moreover, we newly found that disturbance of synaptic neurotransmission in sialidosis neurons. Our new cellular model of sialidosis would be useful for further investigation of sialidosis pathology and drug screening.

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T-3060

A PRECISE CORRECTION OF THE F508DEL CFTR IN CYSTIC FIBROSIS HIPSCS VIA CRISPR/CAS9 RIBONUCLEOPROTEINS

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CRISPR/Cas9 is a potent tool for editing detrimental mutations in disease-specific hiPSCs. Here, we have designed Cas9/gRNA ribonucleoprotein(RNP) for targeting a F508del mutation in cystic fibrosis(CF)-hiPSCs. A F508del mutation was efficiently targeted and corrected, when Cas9/gRNA RNP complex and ssODN were electroporated in CF-hiPCS. In addition, Gene-corrected CF-hiPSC clones were established and showed stemness and pluripotency in vitro. We also tested genome-wide specificity of sgRNA for correcting a F508del mutation in hiPSCs. We investigated indel mutations in 6 potential off-target sites with ~ 3bp mismatches in the sgRNA target sequence from the Cas-OFFinder web tool. No mutational indels were found in those genomic sites. To analyze genome-wide off-target effect, Digenome-seq have also performed in CRISPR/Cas9 treated CF-hiPSCs. From Digenome-seq analysis, we found that 74 genomic loci were cleaved by CRISPR/Cas9 and CF-hiPSC-derived genomic DNA in vitro (DNA cleavage score >0.1). However, top 10 DNA cleavage scoring sites in 74 potential off-target sites were intact in CRISPR/Cas9 treated CF-hiPSCs, in vivo. There results indicate that a F508del mutation in CF-hiPSCs could be precisely targeted and corrected by Cas9/gRNA ribonucleoprotein and ssODN.

T-3062

INVESTIGATING LONG QT SYNDROME TYPE 1 BY IN VITRO GENERATION OF HUMAN PACIENT-SPECIFIC CARDIOMYOCYTES

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Long QT syndrome type 1 (LQT1) is a severe cardiac arrhythmogenic syndrome associated with a high risk of sudden death. It is characterized by an abnormal prolonged QT interval on electrocardiogram caused by the dysfunction of a potassium channel coded by the KCNQ1 gene. The use of patient specific iPSC-derived cardiomyocytes (CM-iPSC) for modelling LQT1 can provide important mechanistic insights, which may help us better understand the pathophysiology of the disease. In this study, we developed an in vitro cell model of LQT1 using CM-iPSC. Blood samples from patients with clinical suspicion of LQT1 were genotyped for mutations at the KCNQ1 gene. A mutation c.1763T>C was identified in: (1) a proband with clinical history of aborted sudden death during physical exercise (QTc of 516 ms while resting), confirming LQT1; and (2) an asymptomatic relative. A healthy donor was also used as control. iPSC were generated from erythroblasts by viral transduction (CytoTune-iPS 2.0 Sendai virus) and presented normal karyotype, as verified by G-banding assay. Its pluripotentiality was confirmed by the presence of transcripts (Oct3/4, Sox2, Nanog, Klf4, Rex1, Dnmt3B, Lin28, Dppa4) and proteins (OCT3/4, SOX2, NANOG, TRA1-60, TRA1-81), as well as its ability to spontaneously differentiate in vitro into the three embryonic germ layers, as verified by RT-PCR (Bmp4, Nestin, Tubulin β3, Gata6, Afp, Sox17) and immunofluorescence (Nestin, Brachyury, Afp). iPSC were differentiated into CM by Wnt-pathway modulation, presenting spontaneous beating and expression of cardiac Troponin T (43,96 3 20,47%). LQT1 modelling was demonstrated by electrophysiological analysis, which showed a significant increase in the action potential duration at 90% of repolarization (APD90) of the CM-LQT1 (312.1 3 79.64; n = 51) when compared to the CM-carrier (177.2 3 68.27; n = 8) and CM-control (271.6 3 71.99; n = 48). In conclusion, the use of CM-iPSC proved to be a reliable tool for the customized studies of LQT1, replicating in vitro the clinical phenotype presented by the patients.

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T-3064

A HIPSC MODEL TO STUDY HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS

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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 due to the fact that there is no in vitro model to study the direct interactions between HIV-infected macrophages/microglia and neurons. 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. Our novel protocol rapidly produces microglia that express the classical markers TMEM119, P2RY12, and TREM2, among others in mono-culture and are productively infected with HIV and respond to antiretroviral treatment. In addition, we have developed a rapid differentiation protocol for astrocyte-like cells that express hallmark proteins and promote overall neuronal health. This all human cell-based, novel, reductive system allows us to study the direct interactions and mechanisms by which microglia cause synaptodendritic damage during HIV infection

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REPROGRAMMING

T-3066

NEURAL CREST CELLS ARE THE CELLS OF ORIGIN IN REPROGRAMMING

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The discoveries made by the Yamanaka and other groups in reprogramming somatic cells either to pluripotent stem cells (iPSCs) or directly to other differentiated cells has opened a new field in stem cell research. However, these reprogramming techniques are plagued by problems relating to the efficiency of the process and the poor understanding of their cellular mechanisms. There are still no clear studies which show that all differentiated cells have the potential of being reprogrammed or whether there exists an elite population of cells that are selectively being reprogrammed. We hypothesise that the primary cell of origin of iPSCs and directly reprogrammed cells are neural crest stem cells (NCSCs) found in most tissues. It is well established that multipotent NCSCs migrate to many parts of the developing embryo where they can produce a vast array of cell types, some of them remain as undifferentiated stem cells throughout adulthood. We traced the lineage of neural crest cells in mice embryos using a Wnt1-Cre and ROSA-YFP reporter system. We found that YFP positive cells (NC derived) were present in the primary cell culture of embryonic skin and they increased in proportion with passage number. We reprogrammed skin samples of mouse embryonic

fibroblasts (MEFs) and found that 100% of the iPSCs produced were YFP positive (from NC origin). Following these findings, we sought to test whether the same is true in direct reprogramming experiments that attempt to convert MEFs to neurons directly using Brn2, Ascl1, Myt1l. We hypothesized that these "induced" neurons (iN) are descendants of NC progenitors and stem cells that already have the potential to differentiate into neurons. And indeed, all the iNs were YFP+ (NC derived). Even when the NC and non-NC cells were sorted prior to reprogramming, only the NC derived cells produced neurons while the non-NC were unable to do so. We now are attempting to test another stem cell population in the skin, epidermal stem cells, for their potential to reprogram to iNs to assess whether this property is unique to NSCSs only or to other stem cells. Finally we will genetically delete the NCSCs and test the skin for its reprogramming potential. These studies can help better understand the cellular mechanism of reprogramming which will help contribute to new therapies for treating degenerative diseases.

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T-3068

DISSECTING SOMATIC CELL REPROGRAMMING USING SINGLE-CELL ANALYSIS

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Induced pluripotent stem cells (iPSCs) is a delicate model for the study of cell fate transition. However, although plenty of great work have been published, the mechanism hidden under the reprogramming process is still unclearly. One of the difficulties for mechanism study is the heterogeneous of the reprogramming cells. To this end, we use single-cell RNA sequencing to solve this problem. In this study, we use OCT4/SOX2/KLF4 to reprogram MEF cells. we found that the reprogramming cells initial with a homogeneous mesenchyme-epithelial transition (MET), and then bifurcated into a keratinocyte-like non-reprogramming (NR) and a reprogramming (R) fates. The R cells continue to acquire pluripotency and then bifurcate again into Dppa5a+ chimera competent and Dppa5a- chimera incompetent cells. We further found IFN-gamma induced immune response is one of the barriers for the Dppa5a rate-limiting step. These new discoveries of somatic cell reprogramming could help deep understanding of cell fate transition.

T-3070

GENERATION OF INDUCED PLURIPOTENT STEM CELLS USING HUMAN CIRCULATING STEM CELLS ISOLATED FROM PERIPHERAL BLOOD

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The discovery of induced pluripotent stem cells (iPSCs) has opened up new possibility of molecular understandings for development and therapeutic applications for patient-specific disease. One of the important issues for clinical applications is cell source. Human peripheral blood is one of the easy accessible cell sources. However, isolated peripheral blood cells have shown low gene transfection efficiency and inconveniences requiring specific methods to isolate. Here, we report a novel population of peripheral blood-derived stem cells, which can be easily reprogrammed to iPSCs. We freshly isolated peripheral mononuclear cells (PBMC) from human peripheral blood and seeded on the fibronectin-coated plate. We observed adherent cell population from as early as 5 days after the start of culture and these cells had NFATc1+CD31+CD45- markers. Inhibition of contact with CD3+ cells was a way to increase the isolation efficiency of these cells. When we examined the peripheral blood of various organ transplant patients, we found that the origin of these cells was the endocardium of the heart. We were able to produce iPSCs more successfully than human dermal fibroblasts because of the increased gene transduction efficiency and enhanced stemness. Furthermore, we have also confirmed that these endocardial cells can be differentiated to osteogenic, adipogenic, and myogenic-lineage cells. Therefore, we named these cells circulating multipotent stem cell. We obtained reprogrammed colonies in 8 days after 4 factor virus transduction without feeder cells. The characteristics of endocardial-iPSCs were similar to embryonic stem cells. In addition, these endocardial-iPSCs were particularly effective in

differentiating into cardiovascular cells. We obtained more than 50 iPSC lines from PBMC of patients with cardiovascular disease and normal volunteers. Our study showed new methods to isolate stem cells from peripheral blood and to generate iPSCs with high efficacy. This suggests that our new approach could be one of ideal methods for clinical application of iPSCs in future.

T-3072

PREDICTING CELLULAR REPROGRAMMING FACTORS FOR TRANSDIFFERENTIATION BY MODELLING THE CELL'S EPIGENETIC LANDSCAPE

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Transdifferentiation is the process of directly reprogramming cells from one somatic lineage to another, which can be achieved by inducing changes in gene expression. Current computational methods which predict transcription factors (TFs) that can induce these changes, are based on analysing differences in gene expression profiles between cell/tissue types and do not consider the epigenetic state of the cell. Here, we developed a new computational method (EpiMogrify) to predict reprogramming factors for transdifferentiation, by systematically analysing and modelling informative aspects of the cell epigenetic landscape. In particular, broad H3K4me3 domains have been associated with cellular identity genes, while developmental genes are known to be marked by poised chromatin (both activation (H3K4me3) and repressive (H3K27me3) marks. We constructed cell/tissue-specific epigenetic landscapes by integrating H3K4me3 and H3K27me3 ChIP-Seq data with protein-protein interaction networks in order to prioritise reprogramming factors for transdifferentiation. We integrated data from 111 human cell/tissue types made available by the ENCODE and Roadmap consortia, producing a set of predicted reprogramming factors for each of the possible conversions (i.e., 12,210). In contrast with previous methods, our predictive framework has a unique ability to not only identify TFs but also lncRNAs, receptors or growth factors for reprogramming. The accuracy of these predictions has been demonstrated by comparisons with the factors used and experimentally validated in existing transdifferentiations, and with TFs predicted by existing computational methods (CellNet and Mogrify). We demonstrate that EpiMogrify provides a significant improvement over the existing methods, by broadening the type of possible reprogramming factors and therefore providing novel candidate sites for CRISPR-targeted epigenetic remodelling. The predicted reprogramming factors for all available human cell conversions will be made available as a resource for the community, and experimental validations for selected predictions are ongoing.

T-3074

OOCYTE-SPECIFIC HOMEOBOX 1, OBOX1, FACILITATES REPROGRAMMING BY PROMOTING MESENCHYMAL-TO-EPITHELIAL TRANSITION AND MITIGATING CELL HYPERPROLIFERATION

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Mammalian oocytes possess fascinating unknown factors, which can reprogram terminally differentiated germ cells or somatic cells into totipotent embryos. Here, we demonstrate that oocyte-specific homeobox 1 (Obox1), an oocyte-specific factor, can markedly enhance the generation of induced pluripotent stem cells (iPSCs) from mouse fibroblasts in a proliferation-independent manner and can replace Sox2 to achieve pluripotency. Overexpression of Obox1 can greatly promote mesenchymal-to-epithelial transition (MET) at early stage of OSKM-induced reprogramming, and meanwhile, the hyperproliferation of THY1-positive cells can be significantly mitigated. Subsequently, the proportion of THY1-negative cells and Oct4-GFP-positive cells increased dramatically. Further analysis of gene expression and targets of Obox1 during reprogramming indicates that the expression of Obox1 can promote epithelial gene expression and modulate cell-cycle-related gene expression. Taken together, we conclude that the oocyte-specific factor Obox1 serves as a strong activator for somatic cell reprogramming through promoting the MET and mitigating cell hyperproliferation.

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T-3076

ELECTROMAGNETIZED GOLD NANOPARTICLES MEDIATE DIRECT LINEAGE REPROGRAMMING INTO INDUCED DOPAMINE NEURONS IN VIVO FOR PARKINSON'S DISEASE THERAPY

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Electromagnetic fields (EMF) are physical energy fields generated by electrically charged objects, and specific ranges of EMF can influence numerous biological processes, which include the control of cell fate and plasticity. In this study, we show that electromagnetized gold nanoparticles (AuNPs) in the presence of

specific EMF conditions facilitate an efficient direct lineage reprogramming to induced dopamine neurons in vitro and in vivo. Remarkably, electromagnetic stimulation leads to a specific activation of the histone acetyltransferase Brd2, which results in histone H3K27 acetylation and a robust activation of neuron-specific genes. In vivo dopaminergic neuron reprogramming by EMF stimulation of AuNPs efficiently and non-invasively alleviated symptoms in mouse Parkinson's disease models. This study provides a proof of principle for EMF-based in vivo lineage conversion as a potentially viable and safe therapeutic strategy for the treatment of neurodegenerative disorders.

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T-3080

REPROGRAMMING OF HUMAN FIBROBLAST TO SKIN PRECURSOR CELLS BY PLANT STEM CELL EXTRACTS

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We have previously established a new method to produce induced pluripotent stem cells by delivering embryonic stem cell-derived proteins into adult mouse fibroblast. However, the protocol was not optimized in human because of its difficulty to prepare sufficient yield of human ES cell extracts. To overcome this problem, we hypothesized whether plant stem cell (callus)-derived proteins could reprogram human fibroblasts. The plant stem cell or callus, a dedifferentiated plant cell mass, can regenerate itself and differentiate into many tissues of a whole plant body. In this study, based on the dedifferentiation characteristic of plant callus, we observed reprogramming activities of plant callus extract on human dermal fibroblast. Here, we demonstrate molecule 'S', major component of Sequoiadendron Giganteum (SG) callus extract, reprogrammed somatic fibroblast to Mesodermal and Ectodermal precursor cells. These cells expressed neural precursor specific protein Nestin as well as Fibronectin and Vimentin and could differentiate into ectodermal and mesodermal lineage but not into endodermal lineage. These gene expression might be regulated by epigenetic modification including promoter methylation and H3K4me3. These results indicated that the molecule 'S' could be an effective agent for direct conversion of fibroblast to Mesodermal and Ectodermal precursor cells and for tissue regeneration.

Funding Source: This study was supported by the Korea Health Technology R&D Project (HI-17 C-2085 & HI-14 C-1277) through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare (MHW).

T-3082

TOOLS THAT SUPPORT CONSISTENT GENERATION OF HIGH QUALITY IPSC USING SENDAI VIRUS REPROGRAMMING FOR TRANSLATIONAL RESEARCH

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Induced pluripotent stem cell (iPSC) research is rapidly moving towards translational and clinical applications. A prerequisite for these applications is consistent production of high quality iPSC lines from different donor cells. In addition, the initial stages of iPSC generation need to be further streamlined to minimize extra effort and costs associated with clones that fail to expand, or do not meet quality standards for downstream use. Methods that enhance consistency between different donors and tools that facilitate identification, elimination, and quantification of residual reprogramming factors enable faster generation of high quality iPSC clones, suitable for use in downstream applications. Previously, we reported the first off-the-shelf reprogramming kit designed for clinical and translational research. Here, we describe xeno-free workflows and associated tools that can be used in conjunction with this kit to further streamline iPSC generation and help ensure the consistent creation of high quality iPSCs. Optimization of a combination of conditions including hypoxia, matrix, and seeding density was shown to offer consistent iPSC generation from difficult to reprogram donor cells. In addition, a panel of antibodies specific for unreprogrammed and partially reprogrammed cells along with an assay that detects residual Sendai virus enabled visualization and elimination of unwanted cells, thereby enriching for high quality, footprint-free iPSC. Lastly, a qPCR-based absolute quantification assay was developed to determine the copy number of Sendai virus present in iPSC clones for further confirmation of foot-print free iPSC. Together, these tools support a complete workflow that will allow researchers to more easily progress their investigations toward translational and clinical research.

T-3084

STIMULATION OF CXCR2 ENHANCES REPROGRAMMING EFFICIENCY OF THE HUMAN INDUCED PLURIPOTENT STEM CELLS

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In our previous study, we demonstrated that the chemokine (C-X-C motif) receptor 2 (CXCR2) and its related ligands support the pluripotency and proliferation of the human pluripotent stem cells (hPSCs) via mTOR-β-catenin-hTERT axis. Our data indicated that CXCR2 is associated with the cell lineage determination during differentiation. The role of CXCR2 in the generation of hPSCs, however, has not been investigated. Here, we demonstrated that the CXCR2 signaling is critical for the reprogramming of somatic cells using human placenta-derived conditioned medium (hPCCM), which contains CXCR2-specific ligands, such as IL-8 and GROα. We evaluated the efficiency of reprogramming in various cell types; human umbilical vein endothelial cells (HUVEC), human placenta cells (HPC) and human dermal fibroblast (HDF). We found a high expression of CXCR2, mTOR, and β-catenin in the generated iPSC colonies (AP+) compared to the non-colonized cells. Interestingly, the efficiency of reprogramming of HUVEC was significantly higher (7-10 times higher, $p<0.01$) than other cell lines in this system. In addition, the efficiency of reprogramming was significantly decreased in a shRNA lentiviral-suppressed CXCR2 expression. Together, our data indicate that the CXCR2 signaling plays an essential role in reprogramming and this suggests a novel and humanized (xeno-free) system for generation iPSCs.

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T-3086

REPROGRAMMING NON-LIMB SOMATIC CELLS TO THE MAMALLIAN LIMB PROGENITOR

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In birds and mice, we know the origin of the limb - the limb progenitor. This cell contains the full capacity to generate an entire limb. Demonstrating this, these progenitors can be isolated, dissociated and transplanted to a non-limb site such as the hindbrain and still produce a fully patterned limb. Despite their dramatic self-organizing capacity and potential use in regenerative medicine, deriving these cells is tedious as they are only present transiently during early development. To circumvent this problem, we have developed a protocol to reprogram non-limb somatic cells to the limb progenitor by transcription factor overexpression. Four transcription factors were identified in a systematic screen to induce the limb specific Prx1-EGFP reporter. Transcriptional analyses of our Prx1-EGFP(+) putative limb progenitors demonstrated reactivation of the limb progenitor program as shown by upregulation of limb specific genes including Sall4, Lhx2 and Msx2 and the forelimb specific gene Tbx5. Differentiation assays in vitro showed that they are have functional properties similar to the mammalian limb progenitor including the ability to generate Alcian Blue(+) cartilage nodules. Transplantation of these putative progenitors have shown they are able to engraft in vivo and we are now assessing their differentiation capacity in the developing embryo. With these findings, we are building a tool to not only interrogate the limb progenitor in vitro but we are also building the foundation for generating a limb de novo.

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T-3088

THE CIS-REGULATORY LOGIC OF REPROGRAMMING COMPETENT AND INCOMPETENT POU TRANSCRIPTION FACTORS

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Direct DNA dependent interactions between Sox2 and Oct4 are intimately linked to the maintenance and induction of pluripotency. To decode the relevance of this partnership for chromatin engagement, remodelling and activation of the pluripotency network, we profiled genomic occupancy and transcriptional outcome of Oct4, Sox2 and reprogramming incompetent Pit-Oct-Unc (POU) factors. At the onset of reprogramming, an Oct4 mutant with destroyed Sox2 dimerization interface is able to effectively target chromatin closed in somatic cells that are marked by composite SoxOct DNA elements. The dimerization defective Oct4 supports somatic silencing reminiscent to the wild-type protein. However, it gets cleared from SoxOct elements as reprogramming progresses and the activation of pluripotency genes drastically fails. This suggests that the target search of Oct4 is not depending on a Sox2 co-selection mechanism. Yet, the formation of long-lived enhanceosomes requires effectively formed Sox2/Oct4 dimers. Moreover, the promiscuous but lineage biased binding to the closed chromatin of the donor cells is inherently shared by POU factors and independent of the interaction with Sox2. This indicates that Oct4 functions as a pioneering factor at early and lineage safeguard at late reprogramming stages and these molecularly independent functions can be separated by protein engineering.

LATE BREAKING ABSTRACTS

T-4002

EPIGENETICALLY REGULATED HEDGEHOG SIGNALING TO STROMA DETERMINES MOLECULAR SUBTYPE OF HUMAN BLADDER TUMOR INITIATING CELLS

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Hedgehog (Hh) pathway inhibition fails therapeutically or even accelerates progression in treatment of endoderm-derived colon and pancreatic cancers. In bladder, another organ of endodermal origin, despite its initial presence in the cancer cell of origin, Sonic hedgehog (Shh) expression is lost during progression to invasive urothelial carcinoma and blockade of stromal response to Shh dramatically accelerates progression. This cancer-restraining effect of Hh pathway activity is associated with stromal expression of BMP signals, which stimulate urothelial differentiation. Here, we show that the loss of Shh expression during bladder cancer progression results from the hypermethylation of CpG shore of Shh gene, regions that flank promoter CpG islands with less CG density. The expression of Shh is dramatically increased by pharmacological inhibition of DNA methyltransferase activity, thus restraining cancer progression and growth. Importantly, urothelial carcinoma with the increased expression of Shh develops into less aggressive, more differentiated luminal subtype whereas the loss of Shh expression induces basal-like invasive urothelial carcinoma with molecular profiles of tumor initiating cells associated with aggressive behaviors. A favorable effect of BMP pathway activation and the inhibition of DNA methylation with FK506 and Azacitidine, respectively, suggests the potential combinatorial therapy to control the proliferation of tumor initiating cells in invasive urothelial carcinoma.

T-4004

GENERATION AND GENETIC CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM PRIMARY LEUKEMIC PATIENT SAMPLES

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Leukemias are malignancies that arise from hematopoietic stem and progenitor cells (HSPCs) and are often the result of genetic and epigenetic changes that alter major cellular processes including self-renewal, proliferation, and differentiation. New drugs to treat these diseases will require large numbers of leukemic cells for drug discovery, screening, and target validation efforts. While immortalized cell lines and primary malignant cells are invaluable tools for drug development and understanding cancer development, cell lines can fail to retain key attributes of the original leukemic cells and primary malignant cells can be difficult to obtain in large numbers. Human pluripotent stem cells (hiPSCs) derived from leukemic cells have been proposed as a potential unlimited source of leukemic cells but reprogramming of cancer cells has proven to be challenging as only a few hiPSC lines from leukemic patient samples have been generated. In this study, we demonstrate the generation of hiPSCs from a primary acute promyelocytic leukemia (APML) patient sample utilizing reprogramming methods optimized in healthy patient samples. Numerous hiPSC clones were generated from the primary APML sample and initial analysis by array comparative genomic hybridization showed that several of the hiPSC clones share a majority of aberrations with the parent sample, including a loss of heterozygosity event (LOH) in chromosome X. Interestingly, the clones analyzed do not possess a PML/RARA rearrangement or a LOH event in chromosome 11 present in the parent sample. We believe these aberrations may be unfavorable for reprogramming, explaining their absence in the analyzed hiPSC clones.

This study is one of the first to report the reprogramming of an APML sample to hiPSCs carrying numerous aberrations of the parent sample. While the initial results of the study are promising, further analysis is required to determine if the shared aberrations play a role in disease development and how the missing aberrations in the hiPSCs affect reprogramming.

T-4006

MECHANICALLY TUNABLE HYDROGELS PROMOTE THE SURVIVAL OF INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS IN TISSUE ISCHEMIA

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A key feature of peripheral arterial disease (PAD) is damage to endothelial cells (ECs), resulting in restricted blood flow, foot ulcers, lower limb pain, and lower limb amputation. Recent preclinical studies suggest that transplantation of ECs via direct injection into the affected limb can result in significantly improved blood circulation. However, the clinical adoption of this therapy has been limited by low cell viability and poor transplanted cell function. To address these limitations, we have developed an injectable hydrogel termed SHIELD (Shear-thinning Hydrogel for Injectable Encapsulation and Long-term Delivery). In this study, we demonstrate improved acute viability of encapsulated induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) following syringe injection in an in vitro model of transplantation. Additionally, SHIELD improves iPSC-EC proliferation and angiogenetic factor secretion in in vitro hypoxic culture conditions, which mimic the cellular environment of PAD. Using a murine hind limb ischemia model for PAD, we demonstrate enhanced iPSC-EC retention in vivo following transplantation in SHIELD. Further, histological explants indicate improved re-vascularization of the ischemic limb. Together these results suggest that SHIELD provides mechanical cues to protect iPSC-ECs from mechanical forces during injection and may influence iPSC-EC paracrine angiogenic signaling to promote long-term cell survival and potency within the ischemic tissue.

T-4008

HYPOXIC CHANGES IN MULTI-DIMENSIONAL HISTONE METHYLATIONS IN HUMAN ADIPOCYTE DERIVE STEM CELLS

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Hypoxia increases both active and repressive histone methylation levels via decreased activity of histone demethylases. However, how such increases coordinately regulate induction or repression of hypoxia-responsive genes is largely unknown. Here, we profiled active and repressive histone tri-methylations (H3K4me3, H3K9me3, and H3K27me3) and analyzed gene expression profiles in human adipocyte-derived stem cells under hypoxia. We identified differentially expressed genes (DEGs) and differentially methylated genes (DMGs) by hypoxia and clustered the DEGs and DMGs into four major groups. We found that each group of DEGs was predominantly associated with alterations in only one type among the three histone tri-methylations. Moreover, the four groups of DEGs were associated with different TFs and localization patterns of their predominant types of H3K4me3, H3K9me3 and H3K27me3. Our results suggest that the association of altered gene expression with prominent single type histone tri-methylations characterized by different localization patterns and with different sets of TFs contributes to regulation of particular sets of genes, which can serve as a model for coordinated epigenetic regulation of gene expression under hypoxia.

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T-4010

YAP/TAZ-DEPENDENT DEPLETION OF HUMAN EPIDERMAL STEM CELLS IN JUNCTIONAL EPIDERMOLYSIS BULLOSA (JEB)

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Laminin 5-dependent Junctional Epidermolysis Bullosa (JEB) is a severe genetic skin adhesion disorder. At variance with other forms of EB, as Dystrophic or Simplex EB, JEB is characterized by epidermal stem cell depletion, the origin of which is unknown. Here we show that nuclear YAP is expressed in epidermal stem cells, detected as holoclones, and decreased or

absent in transient amplyfing progenitors. Ablation of YAP cause a selective depletion of holoclones, whilst ablation of both YAP and TAZ wipes off the entire clonogenic, proliferative epidermal compartment. In contrast, enforced YAP extends keratinocyte life-span. Epidermal stem cell depletion of primary JEB keratinocytes is due to perturbation of the YAP/TAZ pathway. YAP/TAZ expression is significantly decreased in JEB keratinocytes, which do not contain nuclear YAP but only phosphorylated, inactive YAP. The JEB phenotype is recapitulaled by Laminin 5 ablation and consequent YAP/TAZ down-regulation in normal cells. Restoration of adhesion properties by Laminin 5-gene therapy rescues normal nuclear levels of YAP/TAZ and clonogenic potential. Enforced YAP recapitulates Laminin 5-gene therapy in JEB cells, thus uncoupling adhesion from proliferation in epidermal stem cells. This work has important clinical implication for an efficient ex vivo gene therapy of JEB.

T-4012

PRELIMINARY ANALYSIS OF THE CURRENT STATUS OF STEM CELL THERAPY AND ITS EXPERIMENTAL USE IN JAPAN

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Continuing advances in stem cell research have added to the expectation that this could be a common form of clinical treatment. Stem cell therapy has been studied worldwide, and yet, in some cases has caused severe health hazards. In September 2010, a Korean patient, who received adipose-derived stem cell therapy in Japan, died of pulmonary embolism. The lack of rules for performing cell therapies in private clinics has become a grave issue in Japan. In November 2014, the Act on the Safety of Regenerative Medicine (ASRM) was enforced. Accordingly, all medical institutions providing cell therapy or clinical trials in Japan have to first submit their informed consent documents and clinical protocols to the Ministry of Health, Labor, and Welfare (MHLW). In addition, regenerative medicines are classified into three risk categories. Treatments involving the use of ES cells, iPS cells, or allogenic cells are classified as high-risk (Class I) regenerative medicines. Middle-risk (Class II) medicines use somatic stem cells or cell cultures. The low-risk (Class III) group comprises cancer immunotherapy and treatments involving the homologous use of somatic cells. In November 2017, MHLW revised the ordinance for the enforcement of ASRM. They disclosed the names of registered institutes, names of regenerative medicines, and informed consent documents for each provided medicine on their website, where the general public can search for a cell therapy or trial. However, stem cell therapy and research as a whole is not clearly understood. Our aim was to elucidate the present status of cell therapy and its experimental use in Japan. In February 2018, we investigated the MHLW website and exhaustively collected informed consent documents. In total, there were 2568 medical institutions providing cell therapy or trials. To particularly focus on stem cells, we searched for Class I and II regenerative medicines. These were provided by 138 institutions (79 provided treatment and 59 conducted clinical trials). Several institutions providing stem cell therapy were private clinics. Additionally, clinical trials are conducted by three private clinics. In our poster, we will present the details of the Class I and II regenerative medicines used, such as the target disease, the type of cells used, and the manner of transplantation.

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T-4014

ANALYSING THE ROLE OF INI1 ON THE EARLY DEVELOPMENT

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Global chromatin remodeling takes place during differentiation of pluripotent stem cells, which results in a progressive transition from an open chromatin configuration to a closed compact state. A previous study demonstrated that chromatin remodeling complex SWI/SNF is associated with the maintenance of pluripotency, suggesting that chromatin remodeling indeed plays a role in early differentiation of pluripotent stem cells. Consistently, mouse embryos deficient for Ini1, which encodes BAF47, a member of SWI/SNF, fail to hatch and result in peri-implantation lethality. Therefore, it is predicted that Ini1 functions in the early mouse development. However, the precise role of Ini1 in early mouse embryos remains unclear. In the present study, we aim to investigate the role of Ini1 in differentiation of pluripotent stem cells. For this purpose, we established Ini1 KO mouse ES cells by CRISPR/Cas9 technology. Then we examined differentiation propensity of Ini1 KO ES cells by teratoma assay and generating chimeric embryos. We found that Ini1 KO ES cells differentiate into three germ layers, which include ectoderm, endoderm and mesoderm, indicating that Ini1 is dispensable for the maintenance of pluripotency. Notably, Ini1 KO ES cells often differentiated into PL-1-positive giant cells in teratomas, which is hardly observed in the control ES cell-derived teratomas. Consistent with this observation, qRT-PCR and immunocytochemistry revealed that Cdx2, one of the trophectoderm lineage marker genes, is upregulated in Ini1 KO ES cells relative to wild type ES cells. Collectively, these results raised the possibility that Ini1 might play a role in the cell fate maintenance of pluripotent stem cells at preimplantation stage. In future experiments, we will try to uncover how Ini1 regulates the early mouse development, which includes the initial cell fate decision of early embryos, by utilizing combinatorial approaches in vivo and in vitro.

T-4016

GENERATION AND CHARACTERIZATION OF HPSC-DERIVED CD8 ALPHA/BETA POSITIVE T CELLS WITH TUMOR-SPECIFIC CYTOTOXICITY FOR ''OFF THE SHELF'' CANCER IMMUNOTHERAPY

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Emerging interests have been remarkably expanded to realize allogeneic T cell transfer for the next generation of cancer immunotherapy. Although a number of therapeutic concepts have been investigated as "off the shelf" approach, one of the promising strategies is the application of human iPSC (hPSC)-derived T cells with tumor-specific cytotoxicity. Here we report tumor-specific cytotoxic T cells could be efficiently differentiated from hPCSs without using any feeder cells. These cytotoxic T cells were derived from CD4 and CD8 double positive fraction, which reinforced the reminiscence of normal T cell development in human thymus. hPSCs-derived T cells expressed CD3 and CD8αβ, and they had monoclonal TCRα and β chains which we intended to express for antigen specific cytotoxicity. Interestingly, hPSCs-derived cytotoxic T cells were applicable to repeated expansion procedure, which reached the level required by large scale manufacturing. Expanded hPSCs-derived T cells showed tumor antigen-specific cytotoxicity in vitro and significantly reduced tumor growth in vivo. Furthermore, their cytokine and receptor profiles were similar to cytotoxic T cells in peripheral blood. On the other hand, surface expression of co-inhibitory receptors such as PD1 and TIGIT were negligible, suggesting potential clinical benefits better than current therapy of autologous T cell transfer. Taken together, we conclude that our hPSCs-derived T cells are potentially adaptive for clinical-grade manufacturing process along with superior tumor-specific cytotoxicity and functional properties, thereby contribute to the new era of allogeneic T cell cancer immunotherapy.

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T-4018

PERINEURIUM-LIKE SHEATH DERIVED FROM LONG-TERM SURVIVING MESENCHYMAL STEM CELLS CONFERS NERVE PROTECTION TO THE INJURED SPINAL CORD

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The functional multipotency enables mesenchymal stem cells (MSCs) promising translational potentials in treating spinal cord injury (SCI). Yet the fate of MSCs grafted into the injured spinal cord has not been fully elucidated even in preclinical studies, rendering concerns of their safety and genuine efficacy. Here we used a rat spinal cord transection model to evaluate the cell fate of allograft bone marrow derived MSCs. With the application of immunosuppressant, donor cells, delivered by biocompatible scaffold, survived up to 8 weeks post-grafting. Discernible tubes formed by MSCs were observed beginning 2 weeks after transplantation and they dominated the morphological features of implanted MSCs at 8 weeks post-grafting. The results of immunocytochemistry and transmission electron microscopy displayed the formation of perineurium-like sheath by donor cells, which, in a manner comparable to the perineurium in peripheral nerve, enwrapped

host myelins and axons. The MSC-derived perineurium-like sheath secreted a group of trophic factors and permissive extracellular matrix, and served as a physical and chemical barrier to insulate the inner nerve fibers from ambient oxidative insults by the secretion of soluble antioxidant, superoxide dismutase-3 (SOD3). As a result, many intact regenerating axons were preserved in the injury/graft site of the spinal cord following the forming of perineurium-like sheath. A parallel study utilizing a good manufacturing practice (GMP) grade human umbilical cord-derived MSCs or allogenic MSCs in an acute contusive/compressive SCI model exhibited a similar perineurium-like sheath formed by surviving donor cells in rat spinal cord at 3 weeks post-grafting. The present study for the first time provides an unambiguous morphological evidence of perineurium-like sheath formed by transplanted MSCs and a novel therapeutic mechanism of MSCs in treating SCI.

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T-4020

DNAJB6, A KEY FACTOR IN NEURAL STEM CELL RESISTANCE TO POLYGLUTAMINE PROTEIN AGGREGATION

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Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disorder caused by the expansion of polyglutamine (polyQ)-encoding CAG-repeats in the ATXN3 gene. The CAG-repeat length is proportionally related to the aggregation propensity of the ataxin-3polyQ protein. Although the protein is ubiquitously expressed, it only causes toxicity to neurons. To better understand this neuronal hypersensitivity, we generated iPSC-lines from three SCA3 patients. iPSC generation and neuronal differentiation is unaffected by the expression of the ataxin-3polyQ. No spontaneous aggregate formation is observed in the SCA3 neurons. However, upon glutamate treatment, aggregates form in SCA3 neurons but not in SCA3-derived iPSCs or iPSC-derived neural stem cells (NSCs). Analysis of chaperone proteins expression reveals a drastic reorganization of the chaperone network during differentiation, including an almost complete loss of expression of the anti-amyloidogenic chaperone DNAJB6 in neurons. Knockdown of DNAJB6 in NSC derived from SCA3 patients leads to spontaneous SCA3 aggregation. Moreover, DNAJB6-knockout cells are hypersensitive to polyQ aggregation, which is prevented by DNAJB6 re-expression. Our data show that downregulation of DNAJB6, which occurs upon neuronal differentiation, is directly linked to neuronal toxicity of polyQ aggregation.

T-4022

ANALYSIS OF CELLULAR PROCESSES WITHIN 3D CELL MODELS USING FLUORESCENCE MICROSCOPY

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High attrition rates in drug development and lack of translation relevance can be traced back to a lack of physiological relevance when performing target identification, lead identification and optimization. Whilst more physiologically relevant, animal studies are time consuming and expensive. Therefore a niche exists for experimental models that span the gap between in vitro cell-based assays and in vivo animal studies. These systems would mimic the in vivo setting while recapitulating the ease of manipulation of a cell based assay. Traditional two dimensional cell culture models lack physiologically relevant environmental conditions. Their whole physical and biochemical setting is drastically different. For this reason researchers have been turning to three dimensional organoid and tumor spheroid systems. In this systems, cells benefit from cell to cell and cell to ECM contacts. Moreover the cells exist in a more biochemically relevant state with gradients through the 3D system existing, primarily these are oxygen, nutrients and metabolites. Functionally different zones also exist within the three dimensional models with apoptotic or necrotic regions observed in one location (typically at the core) while zones of proliferative cells can be detected along the periphery. These conditions closely resemble the macro environment surrounding the cells in an intact organism. Noninvasive approaches such as fluorescence microscopy are highly advantageous as they allow for the study of these three dimensional systems. We describe the application of a suite of fluorescent biosensors in combination with automated fluorescence microscopy for the high throughput, quantitative analysis of 3D cell models. Data will be shown quantifying the induction of

apoptosis in spheroid models in combination with either mitochondrial membrane potential measurements or the sequestration of therapeutic antibodies labeled with environmentally sensitive fluorescent dyes. Assessment of other pertinent cellular parameters such as viability, proliferation, cell cycle and ROS production will be demonstrated within 3D cell models. Finally, live-cell analysis of the penetration of activated immune cells into a tumor spheroid model will be shown.

T-4024

HUMAN PLURIPOTENT STEM CELL-DERIVED ERYTHROPOIETIN-PRODUCING CELLS IMPROVE RENAL ANEMIA IN MICE

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The production of erythropoietin (EPO), a principal hormone for the hematopoietic system, by the kidneys is reduced in patients with chronic kidney disease (CKD), eventually resulting in severe anemia. Although recombinant human EPO treatment improves anemia in patients with CKD, returning to full red blood cell production without fluctuations does not always occur. In this study, we established a method to generate EPO-producing cells from human induced pluripotent stem cells (hiPSCs) by modifying previously reported hepatic differentiation protocols. These cells showed increased EPO expression and secretion in response to low oxygen conditions, prolyl hydroxylase domain-containing enzymes inhibitors and insulin-like growth factor-1. The EPO protein secreted from hiPSC-derived EPO-producing (hiPSC-EPO) cells induced the erythropoietic differentiation of human umbilical cord blood progenitor cells in vitro. Furthermore, transplantation of hiPSC-EPO cells into mice with CKD induced by adenine treatment improved renal anemia. Thus, hiPSC-EPO cells may be a useful tool for clarifying the mechanisms of EPO production and may be useful as a therapeutic strategy for treating renal anemia.

T-4026

MIS416 ENHANCES THERAPEUTIC FUNCTIONS OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS AGAINST EXPERIMENTAL COLITIS BY MODULATING SYSTEMIC IMMUNE MILIEU

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Human adult stem cells, including umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs), have recently been considered a promising alternative treatment for inflammatory bowel disease due to their unique properties, immunomodulation and tissue regeneration. Despite many years of research and pre-clinical studies, controversial therapeutic outcomes have been produced from actual cell application. The discrepancy is caused by several factors such as poor engraftment, low survival rate and donor-dependent variation of the cells. Enhancement of consistency and efficacy of MSCs remains a challenge for the feasibility of cell-based therapy. In the present study, we investigated whether administration of MIS416, NOD2/TLR9 agonists could enhance the therapeutic efficacy of hUCB-MSCs against Crohn's disease using DSS colitis model. Colitis was experimentally induced in mice by using 3% DSS, and mice were administered a retro-orbital injection of MIS416 and subsequent intraperitoneal injection of hUCB-MSCs. Mice were then examined grossly, and blood, spleen and colon tissues were subsequently collected for further ex vivo analyses. To explore the effect of MIS416 on other cells, hUCB-MSCs and primary isolated immune cells were cultured with MIS416, and in vitro assays were performed. Compared to the single administration of hUCB-MSCs, co-administration with MIS416 improved the therapeutic efficiency of the stem cells by significantly alleviating the symptoms of inflammatory bowel disease. Interestingly, MIS416 did not exert any direct effect on the immunomodulatory capacity of hUCB-MSCs. Instead, systemically injected MIS416 changed the immune milieu in the colon and it caused hUCB-MSCs to more efficiently suppress inflammation and mobilize them toward lesion sites. In addition, considerable numbers of regulatory-type immune cells were stimulated due to the synergism of MIS416 and hUCB-MSCs. These findings indicate that co-administration with MIS416 enhanced the therapeutic potential of hUCB-MSCs by systemically regulating the immune response, which might be a strategy for overcoming the current obstacles to stem cell therapy in clinical practice.

T-4028

EVALUATION OF HUMAN iPSC-DERIVED MESENCHYMAL STEM/STROMAL CELLS FOR USE IN CELL-BASED THERAPY

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Donor procurement makes the logistics of obtaining human bone marrow (BM) as a source of mesenchymal stem/ stromal cells (MSCs) complicated. Although BM MSCs from adult donors still have satisfactory abilities to be used for regenerative medicine purposes, from the manufacturing perspective, the best donors are in the pediatric age range. In addition, the procedure requires planning, it is painful and the quality of cells varies from among donors. Therefore, the MSCs therapy field is constantly looking for alternative sources. We hypothesized that human MSC derived from induced pluripotent stem cells (iPSCs) have biological qualities of native MSC and could be scaled up and used for therapeutic purposes. To test the hypothesis, we isolated native MSC (nMSC) from umbilical cord's Wharton's Jelly (WJ) of two donors (#012 and #013) in xeno-free conditions. Next, we reprogrammed them into iPSC (iPSC012 and iPSC013) and subsequently differentiated them back into MSC using two different protocols ARG and TEX (iMSC012ARG, iMSC012TEX, iMSC013ARG, and iMSC013TEX). Using the same protocols, we also differentiated the clinical grade human embryonic stem cell line (ESC) KCL034 into MSC (eMSC034ARG and eMSC034TEX). To assess which of the two differentiation protocols worked better, we compared differentiation capability, transcriptomics, metabolomics and immunomodulatory potential of the iMSCs (iMSC012ARG, iMSC012TEX, iMSC013ARG and iMSC013TEX) and eMSCs (eMSC034ARG and eMSC034TEX) with nMSCs (nMSC012, nMSC013 and BM MSC). Based on the expression of all expressed genes, the data demonstrated that both iMSCs and eMSCs differentiated following TEX protocol are closer to nMSC with higher differentiation potentials but lower immunomodulatory properties than ARG. Our data suggest that, following a careful selection and screening of donors, nMSCs from umbilical's cord WJ can be easily reprogrammed into iPSCs providing an unlimited source of material for differentiation into iMSCs of similar therapeutic potential as nMSC. iMSCs could be easily scaled up under cGMP conditions and serve as a replacement of BM and MSCs from other sources for therapeutic purposes.

T-4030

ZEB1 LEVERS PROGRESSION AND NEURONAL FATE DURING NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Human pluripotent stem cells hold great promise in regenerative medicine. However, risk of tumor formation and difficulties in generating large amount of subtype derivatives remain the major obstacles for practical application. Here we have discovered that ZEB1 is highly expressed upon differentiation of human embryonic stem cells (hESCs) into neuronal precursors. Depletion of ZEB1 did not impede neural fate commitment, but it prevented hESCs-derived neural precursors from differentiating into neurons, indicating the necessity of ZEB1 for neuronal differentiation. ZEB1 overexpression not only expedited neural differentiation and neuronal maturation which ensured safer neural cell transplantation, but also facilitated the acquisition of excitatory cortical neurons which were valuable for curing certain neurological disorders. We thus contribute novel information on how human neural cells are generated, which is also informative to develop cell replacement therapy for neurological diseases.

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T-4032

A SINGLE DIFFERENTIATION SYSTEM MAPS MULTIPLE HUMAN KIDNEY LINEAGES FROM PLURIPOTENT STEM CELLS

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The mammalian adult kidney, metanephros, develops by mutual interaction between two progenitor tissues, the ureteric bud (UB) and metanephric mesenchyme (MM). MM contains nephron progenitors (NPs), stromal progenitors (SPs) and endothelial cells (ECs). An embryonic germ layer, intermediate mesoderm (IM), gives rise to NPs and UB cells, and ECs are derived from lateral plate mesoderm (LPM). On the other hand, previous reports indicate that SPs are derived from several tissues, including IM or paraxial mesoderm (PAM). Recently, several studies have developed induction protocols of kidney lineage cells and reconstruction of kidney organoids from human pluripotent stem cells. However, there are no reports so far that separately

generate these progenitor cell types, which constitute embryonic kidneys, in one differentiation culture system in vitro. In this study, we aim to establish a novel differentiation culture system which solves the unachieved problem. We created the culture system in which three mesoderm subtypes, IM, LPM and PAM, and their derivatives, NPs, UB cells and ECs, were separately and robustly generated from hiPSCs. Moreover, our results also suggest that UB cells have different origins at the epiblast stage from NPs. We are currently examining the detailed mechanisms of cell fate decision and lineage commitment during kidney differentiation. Our selective and robust differentiation protocols may contribute to understanding the mechanisms underlying human kidney development and congenital renal disorders and supplying cell sources for regenerative therapies.

T-4034

HUMAN IPSC-DERIVED NEURAL CREST CELLS UNRAVELS PATHOGENIC MECHANISMS UNDERLYING RICHIERI-COSTA-PEREIRA SYNDROME

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Richieri-Costa-Pereira syndrome (RCPS) is a rare autosomal-recessive acrofacial dysostosis characterized by cleft mandible, laryngeal anomalies, Robin sequence and severe limb defects. RCPS is mainly caused by large number of the repeat motifs in the 5'UTR of EIF4A3, which in turn lead to decreased expression of EIF4A3. The eukaryotic initiation factor 4A3 (eIF4A3) is a core component of the Exon Junction Complex (EJC), being involved in various aspects of RNA processing including splicing, mRNA export, cytoplasmic localization, and nonsense-mediated decay. Recently, we have shown that eIF4A3 deficiency results in disturbances in neural crest cell development, involving decreased migratory capacity and disturbed osteochondrogenic differentiation, using complementary induced pluripotent stem cells (iPSCs) and mouse models. In order to investigate the molecular mechanisms leading to these altered cellular functions, we performed RNA sequencing of iPSC-derived neural crest cells (iNCCs) from RCPS patients and control individuals. We have identified 121 differentially expressed genes (DEGs) with enrichment for genes related to regulation of actin cytoskeleton, protein digestion, ECM-receptor interaction and focal adhesion biological processes. Most of these DEGs were validated by RT-qPCR and they support the previously observed disturbance in NCCs migration of RPCS patients. Preliminary analyses of alternative splicing revealed prevalence of skipped exon events and enrichment for splicing defects in ribosomal proteins (RPL37A, RPS9, RPS2, RPS28, RPL9, MRPS10 and MRPL18), which may parallel craniofacial disorders caused by defective ribosome biogenesis, such as Treacher Collins syndrome and Diamond Blackfan anemia. These data suggest a dysregulation of ribosomal biogenesis in the molecular pathogenic mechanism underlying Richieri-Costa-Pereira syndrome. A further understand on this mechanism can give insights on how mutations in genes with basic and ubiquitous function results in specific phenotypes. Investigating RCPS pathophysiology will also clarify mechanisms governing human craniofacial development, which remain largely unknown.

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T-4036

GENERATING MULTIPOTENT STEM CELLS FROM PRIMARY HUMAN ADIPOCYTES FOR TISSUE REPAIR

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We previously described a vector and transcription factor-free method to reprogram murine and human somatic cells into induced multipotent stem (iMS) cells combining 5'-azacitidine (AZA) with platelet derived growth factor (PDGF)-AB (Chandrakanthan et al. PNAS 2016). However, the use of bovine serum and sub-optimal efficiency of cell conversion (~ 1:500) limited the potential of this method for clinical application. We have now optimised xeno-free conditions that has yielded autologous iMS cells at high efficiency (~ 1:40) from human adipocytes harvested from subjects aged 18-80 years, and have characterised their in vitro and in vivo properties. Human iMS cells expressed CD73, CD90 and CD105 and lacked expression of CD14, CD20, CD34 and CD45. They displayed serial re-plating, multi-lineage differentiation capacity and maintained a stable karyotype. Human iMS cells can be expanded long-term in autologous or allogeneic human serum containing medium. These cells expressed pluripotency factors (OCT4, Nanog, SOX2 and SSEA4) but unlike pluripotent stem cells, lacked spontaneous teratogenicity. Human iMS cells were transplanted into NOD/SCID mice to assess their persistence, teratogenicity and in vivo plasticity at sites of injury in a postero-lateral inter-lumbar vertebral injury model. Transplanted iMS cells were retained at the site of injection for the duration of assessment (1 year) with no evidence of malignant transformation, and contributed to the formation of new blood vessels, bone, cartilage and smooth muscle at the site of injury. To assess the specificity of cell plasticity, human iMS cells were also injected into the tibialis anterior muscles of SCID/Beige mice following cardiotoxin induced skeletal muscle injury. Donor cells contributed to hCD56 expressing muscle satellite cells and hSPTBN1 expressing myofibres without heterotopic transformation. Taken together, these data provide the foundation to evaluate the tissue regenerative potential of iMS cells in controlled clinical trials.

T-4038

ACTIVATION OF THE MURINE CALVARIAL SUTURE NICHE ENHANCES REGENERATION OF CALVARIAL BONE DEFECTS VIA WNT SIGNALING

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We have previously characterized the requirement of skeletal progenitor cells expressing PRX1 for postnatal calvarial bone regeneration. These cells reside exclusively within the calvarial sutures of young and adult mice and decline with age. Since 7-day old mice are able to regenerate calvarial critical size defects (cCSD), we theorize that the inability of mature mice to regenerate cCSD is due to this decline. Therefore, here we aim at increasing their number within their suture niche to test whether such increment could foster regeneration of cCSD. Hence, we hypothesized that mechanical expansion of the calvarial sagittal suture can increase the number of PRX1-expressing cells (pnPRX1+) residing within the suture and, consequently, can foster regeneration of cCSD. We utilized PRX1-CreER-eGFP transgenic mice, which express GFP in Prx1 expressing cells, to evaluate PRX1-expressing cells and their progeny during suture expansion and regeneration of cCSD. The expansion group (n=5) received an expansion apparatus that was placed onto the sagittal suture, whereas the control group (n=6) underwent the same surgical procedure without the insertion of the expansion apparatus. In both groups, a cCSD of 2 mm in diameter was created in the left parietal bone. Results show that the expanded group presents with significantly higher number of pnPRX1+ cells (4-fold higher) and higher expression of proliferation markers within the sagittal suture (6-fold higher, on average). Further, we observed substantial healing of the cCSD when mechanical expansion was applied, with an average increment of the bone volume 20-fold higher in the group of mice that received the expansion devise. Post-natal ablation of pnPRX1+ cells by means of expression of Diphtheria toxin reduced healing of the cCSD. In addition, the ability to regenerate cCSD was significantly compromised in aged mice or after inactivation of Wnt signaling in pnPRX1+ cells (no quantifiable regeneration observed). In conclusion, this study, for the first time indicates that mechanical expansion can induce the proliferation of skeletal progenitor cells within their calvarial suture niche and can sustain the regeneration of cCSD remotely located in parietal bones. This regeneration potential is compromised in aging animals and requires activation of Wnt signaling.

T-4040

RELIABLE AND ROBUST ANIMAL- AND HUMAN-COMPONENT-FREE HUMAN MSC-BM EXPANSION ON A NEW SYNTHETIC READY-TO-USE FN1 MOTIFS SURFACE

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Human mesenchymal stem cells (hMSCs) consist in a heterogeneous population of multipotent cells, easily isolated from various tissues as adult bone marrow, adult adipose tissue, dental pulp, fetal or neonatal tissues. Combining multi-lineage differentiation potential, low immunogenicity, great immunomodulatory potential and tissue repair promotion capabilities, hMSCs represent a promising stem cell population in term of regenerative medicine, cell therapy and tissue engineering applications. Relatively in low abundance in their natural niches, hMSCs require a robust in vitro cell culture expansion process to obtain sufficient high-quality cell numbers for research and clinical applications. In order to reach efficient cell expansion with a high level of consistency and reproducibility, well-defined, serum-free, xenogenic-free (XF) or animal-component-free (ACF) hMSC culture systems are recommended. Therefore, a completely new human- and animal-component-free, ready-to-use surface had been developed. This proprietary coating technology is made up of synthetic fibronectin-derived motifs, specifically designed to mimic the cell attachment site of native extracellular matrix (ECM) proteins. This constitutes a completely defined xeno-free substrate to culture ECM protein-dependent cell types, especially in restrictive culture settings as serum-free conditions. The present experimental work demonstrates that this synthetic Eppendorf CCCadvanced™ FN1 motifs surface, used in combination with well-defined culture medium and dissociation solution, represents a highly effective and reliable alternative to biological coating-dependent hMSC culture systems.

T-4042

DEVELOPMENT OF A NOVEL CELL CULTURE IMAGING SYSTEM USING A DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

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Cell morphology and proliferation rate are important information to optimize cell culture processes. Although phase contrast microscopy is mainly used to get such information, there are some difficulties to use it for cell culture processes optimization, because it takes several tens of minutes to take whole culture plate images, and it is very time-consuming to check all the images. Moreover, it's difficult to acquire stable image quality because users have to adjust light intensity and its focus. Therefore, we developed a novel cell culture imaging system named CultureScanner CS-1. We adopted digital in-line holographic microscopy(D-IHM) for its optical system. Because this optical system enables having a wide field of view and high resolution, CS-1 can take 6-well plate images within 10 minutes. In addition, as this optical system is lens-free, CS-1 doesn't require optical adjustment by users. This feature enables users to acquire data of stable image quality over the entire plate. We also developed a viewer for easily checking a large amount of images. This viewer tiles all the captured images and enables users to change display magnification in seamless, so we can overlook the whole plate image and observe interested area in details very easily. Furthermore, we developed a function to analyze texture information of cells and detect characteristic parts. For example, this function can be used to detect partially differentiated areas from colonies of iPSCs. CS-1 is a novel imaging system that can evaluate large numbers of cells non-invasively and over time. The images and analysis data from CS-1 system can provide useful information for optimization of the cell culture processes.

T-4044

DEVELOPMENT OF A MASSIVE CARDIAC SPHEROIDS PRODUCTION AND TRANSPLANTATION SYSTEM IN HUMAN PLURIPOTENT STEM CELLS

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Cardiac regenerative therapy using human induced pluripotent stem cells (hiPSCs) is a potentially promising strategy for patients with heart disease; however, the inability to eliminate residual hiPSCs and generate a

massive amount of pure hiPSC-derived cardiomyocytes (hiPSC-CMs) has been a barrier to realizing this potential. Recently, we established an innovative method for purifying the bulk of hiPSC- CMs by focusing on glucose, glutamine and lactate metabolism in hiPSCs and differentiated cardiomyocytes (Tohyama S, Cell Metabolism 2016, Circ. Res. 2017). In addition, we also developed an advanced two-dimensional culture system using multilayer culture plates with active gas ventilation that yielded a large number of pure hiPSC- CMs (Tohyama S, Stem Cell Reports 2017). However, there are no efficient massive hiPSC-CM spheroids production and transplantation systems for heart failure patients. Thus, we developed a large-scale production system for pure hiPSC-CM spheroids using microwell culture plates on a clinically relevant scale, and an injection device for the direct intramyocardial hiPSC-CM spheroids transplantation. An injection device yielded an optimal three-dimensional distribution of transplanted hiPSC-CM spheroids in the myocardial layer. Old farm pigs were used for experiments to evaluate effectiveness of developed device. After the injection procedure, pigs were euthanized and the tissue was harvested to assess the retention of injected materials and histological analysis. Ex vivo device biocompatibility showed no detrimental effects on cell viability, spheroid shape, or size. Significantly, direct epicardial injection of hiPSC-CM spheroids mixed with gelatin hydrogel into beating porcine hearts with the injection device resulted in an optimal distribution and retention of transplanted hiPSC-CM spheroids in a layer within the myocardium, suggesting that the system yielded superior myocardial retention when compared to conventional needle injection procedures. These findings will provide a foundation for the clinical application of pure hiPSC-CM spheroids-mediated cardiac regenerative therapy for heart failure patients.

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T-4046

BONE REGENERATION USING A SYNTHETIC 3D MODEL WITH ADIPOSE MESENCHYMAL STEM CELL

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Adipose mesenchymal stem cell (AMSC) condensation is characterized by high concentration of cell masses, which contributes to differentiation in bone tissue. The advancement of tissue engineering associated with the regenerative medicine, produces new approaches to engage bone generation arises. We established a new method to create osseous tissue regeneration that was evaluated utilizing AMSC-spheroid, extracellular matrix (EM), BMPs, TGF-B, FGF, IGF and PDGF, applied on synthetic 3D printed vertebrae (n=17). We established two study groups; the first with AMSC-spheroid, EM, and growth factors; the second was the control group. Depending on the conditions, we used one-way repeated-measure ANOVAs. In the first group, bone generation was identified (n=17) in 35% of vertebral surfaces two weeks after; four weeks after management, vertebrae were covered with bone tissue in 65% of their surfaces. In the second group (n=17), 100% of synthetic models showed no bone tissue formation (p=0,001). This new approach could be used as a bone graft substitute.

T-4048

MATRIX PROPERTY-MEDIATED THERAPEUTIC COMPOUNDS IN EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS

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At present, various clinical trials using mesenchymal stem cells (MSCs) have taken place with increasing success rates. Given that their clinical efficacy is mainly attributed to paracrine effects, recent studies have drawn much attention to extracellular vesicles (EVs) secreted from MSCs. These bilipid-layered and nano-sized vesicles retaining medicinal potency equivalent to that of MSCs could reach a safe and effective cell-free treatment while circumventing current limitations of stem cell infusion therapies. However, conventional MSC culture methods only deliver an arbitrary cocktail of therapeutic molecules to collected EVs. Therefore, desired recruitment of the multifaceted therapeutic compounds in EVs should be addressed to customize their therapeutic capacity as primed for a targeted disease. In this study, we regulated cytokine inclusions packaging into EVs by 3D-organizing different physical interactions between MSCs and culture matrices. We used gelatin methacryloyl (GelMA) hydrogel that was amenable to highly cell-friendly microenvironments as well as tuneable mechanical properties. Therefore, MSCs could be encapsulated in different 3D-microenvironments with varying mechanical properties that were readily controlled by simple UV cross-linking procedures. EVs were collected from each condition and their therapeutic properties and efficacies were compared by various biological analyses and different culture models. Our study showed an efficient and scalable method to manipulate therapeutic compositions of MSC-derived EVs, which would practically contribute to translation of EVs to clinics.

T-4050

APPLICATION OF CATECHOLAMINES IN ADENO-ASSOCIATED VIRUS-MEDIATED GENE DELIVERY

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Investigating safe and valuable viral vectors is vital for pursuing the fundamentals of gene therapy for the treatment of many intractable diseases. Adeno-associated virus (AAV) is a functional gene vector that has been utilized in gene therapeutics by its lack of pathogenicity and effective gene delivery abilities. Although a variety of AAV serotypes can effectively transduce most cell types, their delivery efficiencies can vary widely depending on the target cell type. Engineering viral vectors has focused on the hypothesis that higher possibility of interaction between gene vehicles and receptors on the cell membrane will promote sufficient gene delivery. In this study, catecholamine moiety was applied to the AAV-mediated gene delivery to promote the increased endocytosis to target cells. Furthermore, diverse properties of the mixture, catecholamine polymer complexed AAV, will be applied to the fabrication of multiscale gene delivery platform, which can be used in dynamic in vivo-like conditions.

T-4054

REGULATION AND ROLE OF ARYL HYDROCARBON RECEPTOR IN HUMAN EMBRYONIC STEM CELLS AND THEIR DIFFERENTIATING COUNTERPARTS

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Toivo Maimets - *University of Tartu, Estonia*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor known for mediating the toxic effects of various environmental contaminants. Recent advances in the field, however, have broadened its role in normal physiologic processes. The significance of AHR in cells with high developmental potential (e.g. hematopoietic, neural, brest cancer stem cells/ progenitors) has been established. However the role and regulation of AHR has not been described yet in human embryonic stem (hES) cells and their differentiating counterparts. Thus we aimed to elucidate the role and regulation of AHR in pluripotent hES cells and during early differentiation into ecto-, endo- and mesodermal lineages. Our experiments show that AHR is expressed in hES cells and the most potent xenobiotic ligand and known carcinogen - TCDD - has no effect on the expression of pluripotency factors as well as cell cycle. We found that AHR is downregulated during differentiation into embryoid bodies, which contrasts previous findings in murine ES cells and published data about hES cell differentiation with retinoic acid (RA). Indeed, we also noticed upregulation of AHR expression during RA treatment. However directed differentiation with commercial kits towards three different lineages resulted in robust downregulation of AHR expression, being most prominent in early mesodermal cells at both mRNA and protein levels. By performing ChIP-Seq analysis we identified the binding of AHR, among others, on OCT4 promoter. Additionally our ongoing experiments aim to characterize a novel post-translational modification of AHR.

FRIDAY, 22 JUNE, 2018

POSTER SESSION III-ODD
18:00 – 19:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

F-1001

SIRT5-DEPENDENT CPT1/2-MEDIATED FATTY ACID OXIDATION PREVENTS TNF-α INDUCED HUMAN UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELL AGING AND LIPID ACCUMULATION

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Young Hyun Jung - *Seoul National University, Seoul, Korea*
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Umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) have therapeutic potential for the treatment of immune-mediated adverse reactions. Despite the therapeutic effects of UCB-MSCs on inflammatory diseases, it has not been fully elucidated the direct effect of proinflammatory cytokines on free fatty acid synthesis and lipid accumulation resulting in UCB-MSC senescence. In this study, we investigated the involvement of sirtuins in balancing of lipid metabolism and rescuing senescence of UCB-MSCs in inflammatory environment. TNF-α, a major proinflammatory cytokine, reduced the expression of Sirt5, a mitochondrial sirtuin having demalonylase and desuccinylase activity, and knockdown of Sirt5 expression promoted TNF-α-induced UCB-MSC senescence. Moreover, UCB-MSCs exposed to TNF-α have shown that increased lipid droplet formation and up-regulated SCD1, FAS and DGAT2 mRNA expression levels. Subsequently, TNF-α stimulated the nuclear translocation of SREBP1, a major transcriptional regulator of lipid metabolism. Inhibition of NF-κB signaling by Bay 11-7082 attenuated the TNF-α-mediated lipid droplet accumulation and UCB-MSC senescence, which was also prevented by fatostatin,

a SREBP inactivator. Whereas TNF-α-reduced Sirt5 expression hindered both demalonylation of CPT1 and desuccinylation of CPT2 which is crucial step for the entry of long-chain fatty acyl CoA into the mitochondria. Furthermore, overexpression of Sirt5 prevented the TNF-α-induced lipid droplet accumulation and UCB-MSC senescence with increase of fatty acid oxidation. In conclusion, TNF-α-mediated senescence of UCB-MSCs is aggravated by lipid accumulation with reduction of fatty acid oxidation through NF-κB signaling pathway dependent DGAT2 expression and loss of Sirt5-induced demalonylation of CPT1 and desuccinylation of CPT2. These results indicate that therapeutic effects of UCB-MSCs can be improved by maintaining Sirt5 expression level in the stem cell therapy of immune-mediated inflammatory diseases.

F-1003

THE PRECLINICAL STUDY FOR THERAPEUTIC EFFECTS OF PCMSCS IN MULTIPLE SCLEROSIS

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Cell therapy, including stem cell therapy, is not only a novel medical practice but also a medicinal product providing a promising strategy for the treatment of human diseases. Here, we first presented a new platform using albumin-conjugated fluorescent nanodiamonds (FNDs) as biocompatible and photostable labels for quantitative tracking of human placenta choriodecidual membrane-derived mesenchymal stem cells (pcMSCs) to provide a reliable methods for study the biodistribution and associated pharmacokinetics of transplanted cells in the animal models for preclinical evaluation. With this background-free detection technique and time-gated fluorescence imaging, we have been able to precisely determine the numbers as well as positions of the transplanted FND-labeled pcMSCs in organs and tissues of the animals after intravenous administration. The method is applicable to single-cell imaging and quantitative tracking of human stem/progenitor cells in rodents and other animal models as well. Furthermore, we demonstrated the application of pcMSCs for the treatment of the myelin oligodendrocyte glycoprotein $(MOG)^{35-55}$-induced experimental autoimmune encephalomyelitis (EAE) in C57BL/6J mice, which was used as the animal model of multiple sclerosis (MS). MS is an autoimmune disease of the central nervous system characterized by chronic inflammation, focal demyelination and widespread damage of axon. In this study, we found that pcMSCs could ameliorate the disease progress of $(MOG)^{35-55}$-induced EAE mice compared to control, and pcMSCs could not only repress T-cells proliferation but also inhibit the progress of differentiation and maturation of dendritic cells (DCs) in vitro. Our results also showed both the expression of typical DCs costimulatory molecules (CD80 & CD86) as well as antigen-presenting molecule MHC class-II peptides were strongly down regulated after monocyte, the progenitor cells of DCs, co-culture with pcMSCS. Taken together, this studies presented a preclinical evaluation model for a potential therapeutic option to treat of MS.

F-1005

KDM4 HISTONE DEMETHYLASE FAMILY IS REQUIRED TO MAINTAIN TROPHOBLAST STEM CELL FATE BY MODULATION OF H3K9ME3 STATUS

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Faithful maintenance of embryonic and trophoblast lineage resulting from the first differentiation event is crucial to the proper development of embryo and placenta. Epigenetic regulation involving DNA methylation and H3K9me3 has been shown to be important in stabilizing the embryonic fate through the repression of transcriptional program of the unrelated cell lineages. The histone H3K9 demethylase Kdm4 (Jmjd2) family was previously shown contributing to the murine embryonic stem (ES) cell state. However, their roles in trophoblast lineage determination and maintenance remain unclear. Here we show that Kdm4 activity is crucial to the maintenance of trophoblast stemness gene expression in murine trophoblast stem (TS) cells. Induction of TS cell differentiation leads to loss of Kdm4b binding to trophoblast stemness gene Cdx2, which is associated with increased H3K9me3 enrichment and gene repression. In contrast, loss of H3K9me3 at differentiation gene Ascl2 was independent of Kdm4b occupancy, suggesting the specific epigenetic regulation of trophoblast stemness genes by Kdm4 family. Although forced expression of Kdm4b in ES cells demonstrated no significant induction of trophoblastic transdifferentiation, the trophoblast lineage commitment can be further promoted together with 5-azacytidine treatment. Importantly, inhibition of Kdm4 activity in ES cells cultured in TS medium condition (primed ES cells) showed reduced expression of trophoblast lineage genes. We also observed stronger induction of trophoblast differentiation genes by continuous inhibition of Kdm4 activity in the TS-like

cells derived from the DNA hypomethylated Np95-/- ES cells, implying the crucial role of Kdm4 family in the maintenance of stem cell state. Our findings suggest that Kdm4 family sustains the transcriptional program associated with stemness in TS cells through epigenetic regulation of the H3K9me3 status.

Funding Source: This work was supported by the Research Grants Council of the Hong Kong Special Administrative Region (SAR), China (Grant no. HKU775510M).

F-1007

PROTECTIVE EFFECT OF 17β-ESTRADIOL ON HIGH GLUCOSE-INDUCED AUTOPHAGIC CELL DEATH THROUGH SIRT3 AND NRF2-DEPENDENT MITOCHONDRIAL ROS REDUCTION IN HUMAN MESENCHYMAL STEM CELLS

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Young Hyun Jung - *Seoul National University, Seoul, Korea*
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17β-estradiol (E2) has protective roles in mitochondrial dysfunction and damage during oxidative stress. Diabetes is closely related to oxidative stress and the risk of diabetes is high in postmenopausal women who are deficient in E2, but the exact mechanism is not clear. Therefore, the present study investigated protective role of E2 in high glucose-induced autophagic cell death and its related pathways. High glucose (D-glucose, 25 mM) increased the protein levels of Beclin1 and LC3 while p62 was decreased. In addition, mRNA expression levels of ATG14, BECLIN1 and LC3 were enhanced under high glucose conditions, along with decreased cell viability in human mesenchymal stem cells (hMSCs). High glucose elevated reactive oxygen species (ROS) levels in a time-dependent manner which was blocked by treatment with MitoTempo, a mitochondrial-targeted exogenous antioxidant. We also found that high glucose-induced mtROS production and cell viability reduction were reversed by E2 pretreated with high glucose treatment. E2 alone increased estrogen receptor alpha (ERα) expression in a time-dependent manner compared with high glucose alone in hMSCs. In addition, co-treatment of high glucose and E2 increased nuclear translocation of ERα, but not as much as E2 alone. In nucleus, translocated ERα bound to estrogen response elements and transcribed Sirtuin3 (Sirt3), NAD-dependent mitochondrial deacetylase, and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) expression. Sirt3 enhanced the activity of manganese superoxide dismutase (MnSOD), which then significantly prevented high glucose-induced increase of mtROS levels. Nrf2 also increased the expression of MnSOD. Nrf2-induced MnSOD reduced mtROS level which prevented autophagic cell death in hMSCs. In conclusion, our results imply that E2 leads to the protective effect on autophagic cell death under high glucose conditions through Sirt3 and Nrf2-dependent levels of mtROS reduction in hMSCs.

F-1009

SINGLE-STAGE CELL-BASED CARTILAGE REPAIR IN A RABBIT MODEL: CELL TRACKING AND IN VIVO CHONDROGENESIS OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS AND HYALURONIC ACID HYDROGEL COMPOSITE

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Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have gained popularity as a promising cell source for regenerative medicine, but limited in vivo studies have reported cartilage repair. In addition, the roles of MSCs in cartilage repair are not well-understood. The purpose of this study was to investigate the feasibility of transplanting hUCB-MSCs and hyaluronic acid (HA) hydrogel composite to repair articular cartilage defects in a rabbit model and determine whether the transplanted cells persisted or disappeared from the defect site. Osteochondral defects were created in the trochlear grooves of the knees. The hUCB-MSCs and HA composite was transplanted into the defect of experimental knees. Control knees were transplanted by HA or left untreated. Animals were sacrificed at 8 and 16 weeks post-transplantation and additionally at 2 and 4 weeks to evaluate the fate of transplanted cells. The repair tissues were evaluated by gross, histological and immunohistochemical analysis. Transplanting hUCB-MSCs and HA composite resulted in overall superior cartilage repair tissue with better quality than HA alone or no treatment. Cellular architecture and collagen arrangement at 16 weeks were similar to those of surrounding normal articular cartilage tissue. Histological scores also revealed that cartilage repair in experimental knees was better than that in control knees. Immunohistochemical analysis with anti-human nuclear antibody confirmed that the transplanted MSCs disappeared gradually over time. Transplanting hUCB-

MSCs and HA composite promote cartilage repair and interactions between hUCB-MSCs and host cells initiated by paracrine action may play an important role in cartilage repair.

F-1011

INVOLVEMENT OF EPIGENETIC REGULATION IN IMMUNOMODULATORY PROPERTIES OF HUMAN MULTIPOTENT MESENCHYMAL STROMAL CELLS (MSCS)

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Human mesenchymal stromal cells (MSCs) are multipotent somatic progenitors with strong immunomodulatory properties. One important molecule involved in these processes is HLA-G, a non-classical major histocompatibility complex (MHC) class I molecule. Human HLA-G is known to be expressed only by placental cytotrophoblasts and in the thymus, both transient organs. Thus, understanding of regulatory mechanisms involved in expression of this elusive molecule has proven difficult, relying almost exclusively on cancer cell line data and overexpression studies. We and others have recently shown that human embryonic stem cell-derived (hE-), bone marrow- (BM), placenta-derived MSCs (P-MSCs) express HLA-G that impart immunomodulatory actions. Using human BM- and P-MSCs, as well as human term placental tissue, we investigated the molecular mechanisms involved in HLA-G expression in these normal cells/tissue. We found that BM- and P-MSCs express several but not all HLA-G isoforms, with expression of unique glycosylated forms. Upon interferon-γ (IFN-γ) stimulation, HLA-G levels are upregulated in MSCs but not the JEG-3 choriocarcinoma cell line. Most interestingly, methylation patterns in the HLA-G proximal promoter of human BM- as well as P-MSCs are comparable to human placental tissue but not JEG-3. Our study implicates the importance of using normal cells and tissues for physiologic understanding of tissue-specific transcriptional regulation, and the utility of human MSCs in understanding the transcriptional regulation of HLA-G, an important but elusive immunomodulatory molecule.

ADIPOSE AND CONNECTIVE TISSUE

F-1013

THE ADULT MAMMALIAN DIGIT TIP BLASTEMA CONSISTS OF PRE-EXISTING MESENCHYMAL CELLS THAT ARE RECRUITED FOR REGENERATION

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Regeneration of appendages is relatively common among invertebrates. In mammals, however, multi-tissue regeneration is largely restricted to the distal portion of the digit tip and involves the formation of a blastema. While the source of regenerative blastema cells in amphibians has been widely studied, the cellular origin and phenotypic identity of mammalian blastema cells are not well understood. Here, using an adult murine digit tip regeneration model we show that the blastema is predominately comprised of mesenchymal precursor cells, and by utilizing several strains of transgenic mice including PdgfraCreERT2 and Msx1CreERT2, we demonstrate that the blastema originates from pre-existing mesenchymal tissue. To characterize these mesenchymal blastema cells, we performed single cell RNA sequencing (scRNA-seq) over the course of digit tip regeneration. This analysis confirmed that the blastema is largely comprised of mesenchymal precursors with a relatively homogeneous transcriptional signature that is similar to that of the developing embryonic limb bud and other embryonic mesenchymal tissues. Thus, Together, these data support a model where, during successful adult digit tip regeneration, pre-existing mesenchymal precursor cells are recruited into the blastema, where they adopt an embryonic transcriptional identity, and ultimately recapitulate a limb development program to regenerate the digit.

Funding Source: This work was funded by CIHR grants to DK and to FDM. MAS is funded by an OIRM Initiative fellowship.

F-1015

CHARACTERIZATION OF ISOLATED ADIPOSE-DERIVED STEM CELLS, ENDOTHELIAL PROGENITOR CELLS, AND PERICYTES FROM LIPOASPIRATE USED FOR BREAST RECONSTRUCTION IN BREAST CANCER PATIENTS

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Although fat autografting has been widely used in breast reconstructive surgery, the surgical outcomes have not yet satisfied and there is limited information of cell components in stromal vascular fraction (SVF) obtained from breast cancer patients for their breast reconstruction. This study thus aims to identify and quantify isolated cells including ADSCs, endothelial progenitor cells (EPCs) and pericytes in SVF from breast cancer patients as well as to confirm multipotency of cultured ADSCs. SVF was isolated from abdomen lipoaspirate obtained from twenty-two breast cancer patients aged 25 - 57 years. Freshly isolated SVF was analyzed for ADSCs, EPCs and pericytes by staining with fluorochrome-conjugated monoclonal antibodies against CD13, CD31, CD34, CD45, CD73, CD90, CD105, and CD146 prior to polychromatic flow cytometry analysis. SVF was then cultured to obtain only ADSCs. Expanded ADSCs were re-confirmed for their phenotypes and were detected for their differentiation ability towards adipogenic, osteogenic, and chondrogenic lineages by quantitative polymerase chain reaction (qPCR). Results show that ADSCs were a majority in SVF with 66% of CD45- population followed by equal numbers of EPCs and pericytes (~16%). The ADSC population was homogeneous with expression of CD13+CD31-CD34+CD45-CD73+CD90+CD105-CD146-, whereas the EPC and pericyte populations were heterogeneous with main expressions of CD13+CD31+CD34+CD45-CD73-CD90+CD105+CD146+ and CD13-CD31-CD34-CD45-CD73-CD90-CD105-CD146+, respectively. The characteristic of ADSCs was also changed over serial passages with downregulation of CD34 and upregulation of CD105. Multilineage differentiation of ADSCs was confirmed for both early and late passages. This study then suggests that ADSCs in breast cancer patients were found dominant and homogeneous which is suitable for autologous cell-assisted lipotransfer to use in breast reconstruction.

Funding Source: This project is supported by Siriraj Research Fund, Faculty of Medicine Siriraj Hospital, Mahidol University, Grant number: R016033006.

F-1017

SINGLE-CELL ANALYSIS REVEALS DISTINCT IMMUNOMODULATORY CAPACITY AND METABOLIC PATTERN OF MESENCHYMAL STEM CELLS FROM DIFFERENT TISSUES

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Adipose and bone marrow derived mesenchymal stem cells (ADSCs and BMSCs) have been widely applied in clinical trials. However, the differences of cellular heterogeneity and functional characteristics between ADSCs and BMSCs remain largely unknown. Here, we performed single-cell RNA-seq analysis of ADSCs and BMSCs obtained from the same donor. We found that BMSCs are more heterogeneous than ADSCs as measured by Kur value. In consistent with the gene expression signatures of these cells, functional analysis indicated that ADSCs exhibited lower immunogenicity and higher immunosuppression capacity when compared to BMSCs. Moreover, in metabolic pattern we found that ADSCs prefer glycolysis, while BMSCs prefer mitochondrial respiration, which may contribute to their distinct immunomodulatory characteristics. Our work provides key insights into the crucial features of human ADSCs and BMSCs at single-cell resolution, and may provide guidance for future MSCs clinical application.

Funding Source: This work was supported by the National Key R&D Program of China (grant no. 2017YFA0104902)

F-1019

EXPLORATION OF THE USE OF ADIPOSE TISSUE-DERIVED STEM CELLS FOR IN UTERO TRANSPLANTATION

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Since abnormal brain development in congenital neurological disorders has exerted the brain damage during development, it is still limited therapeutic benefit for treatments against these disorders. Adipose tissue-derived stem cells (ADSCs) are known to improve stroke symptoms by direct cell replacement and an increase of the viability of endogenous neurons. ADSCs may be a useful material of neural regeneration, however, there is less evidence that ADSCs are used as a cell source for in utero therapy. The purpose of this study is to establish the method for in utero transplantation of ADSCs. First, we characterized the ADSCs using the ratio of cell size, measured as forward scatter (FSC). Next, we compared cell proliferation, gene expression, and integration after in utero transplantation using these fraction between FSC-

high and FSC-low. From adhesive cells after collagenase-digested adipose tissue, the non-hematopoietic fraction was gated by FSC and analyzed with Sca-1 and PDGFR alfa. FSC-high and FSC-low have similar cell populations expressing Sca-1 and PDGFR alfa. FSC-high had higher proliferative ability than FSC-low. Only FSC-high ADSCs transplanted into the fetus ventricular lead to settle and migrated into the brain. We confirmed that neuron marker expressed in the engrafted ADSCs at two weeks after the delivery. Our findings characterize ADSCs into two cell populations, and FSC-high ADSC have high proliferation rate and differentiate into mature neuron after in utero transplantation. In conclusion, FSC-high ADSCs could be more proper cell source for in utero transplantation engraftment.

Funding Source: This work was supported by JSPS KAKENHI Grant Number JP17K16311

MUSCULOSKELETAL TISSUE

F-1021

INVESTIGATING THE ROLE OF TRANSCRIPTION FACTOR NKX2-5 IN TONGUE MYOGENESIS DURING EMBRYONIC DEVELOPMENT

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Orofacial muscles including tongue are believed to originate from the paraxial mesoderm progenitors of the occipital somites with principally skeletal muscle phenotypes. However, recent linage tracing experiments may dispute this notion, as multipotent splanchnic mesodermal cells that contribute to the myocardial cells and may also give rise to parts of tongue muscle. This study has investigated the cardinal cardiac transcription factor (TF) Nkx2-5 in the tongue development. Linage tracing of Nkx2.5Cre/Zeg mice, showed at least 65% of the adult tongue myocytes are derived from Nkx2.5+ progenitors. Adult tongue myocytes express Nkx2-5 mRNA and protein. Interestingly, adult tongue posses Nkx2.5+ satellite cells while the satellite cells are lost in the heart. Mouse embryonic tongue tissue persistently and strongly expressed Nkx2-5 when probed with immunohistochemical staining and qPCR. This is companied by co-expression of other cardiac markers such as Mef2C, Isl-1, SMA, α-actinin, Tbx1 and Tbx5. 5 from early lingual swell stage to full extent of tongue tissue formation. In the mean time, the embryonic and adult tongue also expresses skeletal myocyte markers Myf5, myogenin and MyoD starting from embryonic day 12.5. Demonstrating a hybrid phenotype of these muscles. These intriguing evidences that emerged from our work demonstrated that, contrary to the conventional belief, the tongue muscle is largely formed by a group of progenitors that posses the cardiac TF Nkx2.5, and in the maturation process, they adopt some of the skeletal muscle TFs to acquire structural properties of skeletal myocytes while maintaining cardiac TFs expressions in mature and satellite cells. The probing of the mechanistic pathways of the cardiac progenitor transition to hybrid muscle type switch is currently being investigated using an in vitro iPSC differentiation assay. This study may shed light to the understanding of role of cardiac progenitors in tongue and perhaps broader orofacial development and finding treatment modalities for regenerative repair of orofacial defects. As well, finding regulatory factor that medias the switch from cardiac to hybrid muscle differentiation may uncover the tongue satellite cell to cardiomyocyte differentiation cue which may in turn be useful for repairmen of damaged hearts.

Funding Source: Australian Dental Research Foundation (ADRF)

F-1023

IDENTIFICATION OF CANDIDATES MODULATING HUMAN SKELETAL MUSCLE PROGENITOR CELLS MIGRATION

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Michael Emami - *UCLA, Los Angeles, CA, U.S.*
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Skeletal muscle is one of few organs in the human body that contains endogenous stem cells capable of repopulating muscle fibers upon injury. In Duchenne Muscular Dystrophy (DMD), a devastating genetic disease characterized by muscle wasting, the muscle stem cells are rendered dysfunctional or exhaust following continuous muscle insult. We have described a CRISPR/Cas9 editing strategy using DMD- human induced pluripotent stem cells (hiPSCs), where exons 45-55 are deleted restoring a functional dystrophin protein. Muscle progenitor cells derived from CRISPR/Cas9 corrected hiPSC can be used as a personalized cell based therapy for DMD. However, current strategies do not result in large scale engraftment or ability to reside in the niche cross multiple muscles. Therefore, we are developing screening approaches to identify candidate factors that enhance migration and extravasation of skeletal muscle cell derived from hiPSCs. Although the mechanisms of migration and extravasation are well understood for leukocytes and hematopoietic stem cells, far less is known for muscle progenitor cell populations especially derived from hiPSCs. Understanding the regulation of muscle cell migration will provide insight into approaches to enhance muscle stem cell delivery to more regions of dystrophic muscle.

Funding Source: QRLP-Qatar Foundation, Doha, Qatar

F-1025

CELL THERAPY FOR TREATING ULLRICH CONGENITAL MUSCULAR DYSTROPHY (UCMD) MODEL MICE BY USING IPSC-DERIVED MESENCHYMAL STROMAL CELL (MSC)

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Mesenchymal stromal cell (MSC) is well known as a multipotent progenitor for osteogenic, chondrogenic and adipogenic lineage, and is also found in skeletal muscles. Recent studies reported that although they normally act as supportive cells on skeletal muscle homeostasis with producing collagen VI (COL6), they are also the responsive cells for fibrosis and fatty degeneration in diseased muscle. Moreover, mutations of Col6 genes, which are specifically expressed in MSC, cause a severe muscle disorder, Ullrich congenital muscular dystrophy (UCMD). UCMD patients' muscle cannot produce COL6 protein due to the mutations of Col6 genes, and show various phenotypes which are muscle atrophy, ectopic fat deposition and muscle weakness. But no effective cure was found for UCMD yet. Therefore, we assumed that COL6 positive MSC could be a cell source of cell therapy for UCMD. However MSC isolated from adult tissues are quite limited in expansion. To overcome such hurdle, we plan to use iPSC-derived MSC for future cell therapy of UCMD. When we transplanted human induced pluripotent stem cell (iPSC)-derived MSC (iMSC) in the Gastrocnemius / Tibialis Anterior muscles of UCMD model (COL6KO/NSG) mice, transplanted iMSC could be successfully engrafted, and COL6 protein was restored over a large region for at least 12 weeks. Moreover, we observed that the phenotypes of COL6KO mice was ameliorated in iMSC transplanted and COL6 restored legs : 1. impaired regenerating muscle fiber (eMYH3 +) could complete their muscle regeneration, 2. number of satellite cells (Pax7+) and myoblasts (Myod1+) increased, 3. muscle contraction force was improved. Then we started to elucidate the therapeutic mechanisms of iMSC transplantation in vivo and in vitro study by using COL6KO-iPSC (KO-iMSC) which was created by CRISPR-Cas9 system. Our research represent a first possible step towards establishment of effective cell therapy for UCMD.

Funding Source: Grant-in-Aid for JSPS Fellows

F-1027

TRANSPLANTING HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS AND HYALURONATE HYDROGEL REPAIRS CARTILAGE OF OSTEOARTHRITIS IN A MINI PIG MODEL

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Osteoarthritis (OA) is a chronic disease of the degenerative joint. Mesenchymal stem cells have been used for cartilage regeneration in OA. We investigated the therapeutic potential of transplantation of a combination of the human umbilical cord mesenchymal stem cells (HUCMSCs) with hyaluronic acid (HA) hydrogel in a porcine OA preclinical model. HUCMSCs were characterized with morphology, surface markers and differentiation capabilities. qRT-PCR was used for gene expressions of HUCMSCs with HA co-culture. In large animal study, two healthy female mini pigs, 4-month-old and weighing 30-40 kg, were used. A full-thickness chondral injury was created in the trochlear groove of each knee in two mini pigs. After three weeks, the second time of osteochondral defects was created. Then, a 1.5 ml of a mixture of HCUMSCs and HA composite (4%) was transplanted in the chondral injured area in the right knee of each pig. The same surgery created the osteochondral defect not treated in the left knee as the control. The pigs were sacrificed 12 weeks after transplantation. Macroscopic and microscopic histologies, qRT-PCR and immunostaining evaluated the degree of chondral degradation. HUCMSCs showed typical MSC characteristics, including spindle morphology, surface markers (CD29, CD4, CD73, CD90, HLA-ABC positive, CD34, CD45 and HLA-DR negative) and multipotent differentiation (adipogenesis, osteogenesis and chondrogenesis). More proliferations of HUCMSCs were noted in 4 and 25% of HA than without HA. The gene expressions of COL2A1 and aggrecan in HUCMSCs were increased in HA. The treated knees showed significant histological improvements in hyaline cartilage regeneration compared with control knees. ICRS histological score showed a higher scores of the treated knee than the control knee. Our findings suggest that cartilage regeneration using the mixture of HUCMSC and HA in a large animal model may be an effective treatment in OA and a stepping stone for future clinical trials.

CARDIAC TISSUE AND DISEASE

F-1029

HUMAN PLATELET LYSATE AS A XENO FREE ALTERNATIVE OF FETAL BOVINE SERUM FOR THE IN VITRO ISOLATION AND EXPANSION OF HUMAN CARDIAC PROGENITOR CELLS

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Multilineage differentiation and secretion of growth factors/cytokines introduce resident cardiac progenitor cells (CPC) highly attractive for clinical application. But, majority of published isolation and expansion methods for CPC presented challenges when considering good manufacturing practices (GMP) for clinical translation. Here, a new method for isolation and expansion of CPC using pooled human platelet lysate (PL) have been described as alternatives to fetal bovine serum (FBS) to achieve GMP-compliance. CPC were obtained from right atrial appendages by explant cultured in either IMDM (+PL) or IMDM (+FBS) and followed immunomagnetic selection with antibodies against c-kit and Lin. Immunophenotype, population doubling time, chromosomal stability, and differentiation capacities were compared in CPC cultivated with PL or FBS. Population analyzes of explant cultures showed that the proportion of CPC in IMDM (+PL) medium was significantly larger than after cultivation in IMDM (+FBS) (2.6% +/- 0.4% versus 1.2% +/- 0.3%). Isolated CPC cultivated in two types of medium conditions strongly expressed the stem cell surface marker c-kit, as well as mesenchymal markers D105 and CD73. CPC had similar proliferation properties after cultivation in IMDM (+PL) and IMDM (+FBS) mediums. Comparative genomic stability analyses revealed no chromosomal aberrations. Differentiation ability, tube formation on Matrigel were generally unaffected by the type of medium that was used. The present study demonstrates that the use of the pooled human platelet lysate as a growth supplement maintains growing potential, immunophenotype, chromosomal stability and differentiation capacity of human CPC similar to the cells cultivated in FBS, and could be reasonable as an alternative safe source of growth factors for isolation and expansion of human CPC for clinical purposes.

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F-1031

A RESIDENT POPULATION OF ADVENTITIAL MACROPHAGE PROGENTITOR CELLS PRESENTS IN POSTNATAL VASCULATURE

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Macrophages are fundamental regulators of vascular biology and disease. For a long time, it has been thought that all vascular macrophages come from monocytes, that originate in bone marrow, circulate in blood and are recruited to the artery wall to become macrophages. Recently, we have discovered a new population of stem/progenitor cells present in the outer layer of arteries (adventitia). These cells possess high proliferative capacity and potential to generate hematopoietic cell progeny. Functional studies showed that single-cell aortic disaggregates, prepared from adult C57BL/6 mice generated multipotent and lineage-specific hematopoietic colony-forming units, with a striking preponderance of macrophage colony-forming units (CFU-M). We named this cell population Adventitial Macrophage Progenitor Cells (AMPCs). Characterisation of these clonogenic vascular AMPCs indicated that they are Lin-CD11b-F4/80-Sca-1+c-Kit+CX3CR1+CD115+CD45+/Lo. Most importantly, the frequency of AMPCs and functional CFU-M colonies was significantly higher in apolipoprotein E-null (ApoE-/-) atherosclerotic mice than control mice, indicating that these AMPCs are more abundant in atherosclerotic blood vessels. When AMPCs were terminally differentiated along the myeloid pathway into macrophages with the presence of growth factors, we yielded sustained transgene expression during the

hematopoietic specification process and generated mature CD11b+F4/80+ macrophages. Parallel studies were performed using lineage-mapping by crossing Flt3-cre mice with Rosa26mT/mG reporter mice. Definitive hematopoietic stem cells in development and adult life transiently upregulate FLT3 (FLK2, CD135). In FLT3-mTmG mice, cells that pass through a FLT3+ stage of development, consistent with definitive hematopoiesis, express GFP (FLT3-Cre+), while all other cells are red fluorescent (TdTom+; FLT3-Cre-). These lineage-tracing studies demonstrated that AMPC-derived macrophages are maintained locally and do not come from BM or spleen via circulation. Altogether, these observations suggested that AMPCs constitute a source of tissue-resident macrophage precursors that are present in the outer layer of arteries and can generate macrophages in postnatal murine arteries independently of circulating blood monocytes.

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F-1033

ENHANCEMENT OF CARDIOMYOCYTE MATUARTION ON CARDIAC FIBROBLAST-DERIVED DECELLULARIZED EXTRACELLULAR MATRIX

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Based on the comprehensiveness reminiscent of a natural extracellular matrix(ECM), cell-derived ECMs(CDM) engineered de novo are attractive alternatives over conventional ECM platforms in directing cell fate. Although cell-ECM interactions are increasingly being recognized as key mediators of cell behavior, very few studies have investigated the impact of CDMs on cardiomyocyte maturation. Hence, we cultivated human pluripotent stem cell-derived cardiomyocytes(hPSC-CM) on decellularized-cardiac fibroblast ECM(dCFM) to investigate whether the inherent and various dCFM components influenced the characteristics of hPSC-CM. Our results revealed a four-fold acceleration of hPSC-CM maturation and enhanced electrophysiological function in comparison to the control, suggesting that dCFMs are propitious for hPSC-CM maturation. From our findings, we further propose an optimal dCFM substratum as a novel bioenvironmental culture platform for ameliorating cell maturation.

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F-1035

EXPANDABLE HUMAN CARDIOVASCULAR PROGENITORS AS A TOOL TO REGENERATE THE MURINE HEART AFTER MYOCARDIAL INFARCTION

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Myocardial infarction (MI) and other cardiovascular diseases are a major cause of mortality and morbidity worldwide, despite large progress in modern medicine. Since the adult human heart has low regenerative potential and adult cardiomyocytes (CMs) are very difficult to obtain or expand in vitro, human pluripotent stem cell (hPSC)-derived CMs provide a promising and innovative alternative cell source for cardiac repair. To promote the formation of larger vascularized grafts and a more mature cardiac cell phenotype, we investigated whether doxycycline-inducible (Tet-On-MYC) hPSC-derived cardiac progenitor cells (CPCs) have the potential to proliferate and differentiate into major cardiac lineages in a mouse model of myocardial infarction (MI). Additionally, we evaluated their impact on limiting cardiac remodeling and improving cardiac function. In conclusion, hPSC-derived CPCs actively proliferated and expanded into large grafts in vivo in response to doxycycline (Dox) and fibroblast-growth-factor (FGF2). Largest grafts (~0.3 mm2) were observed after 15-day treatment with Dox/FGF2 when compared to 30 days of treatment (~0.2 mm2) or no treatment (~0.04 mm2). Tumor formation was not observed during the 12 weeks observation. Upon withdrawal of Dox/FGF2, CPCs efficiently differentiated into major cardiac lineages including CMs, endothelial cells (ECs) and smooth muscle cells (SMCs) via WNT-inhibitor XAV939 driven in vivo differentiation. Analysis of magnetic resonance imaging (MRI) is currently in progress; ejection fraction and wall motion values will be the main parameters evaluated in order to understand if the formation of larger grafts significantly improves cardiac function. Importantly, CPCs were able to vascularize the graft area (~5-6% of cardiac graft area) and significantly reduced the amount of fibrosis in the infarcted area by 50%.

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F-1037

HUMAN IPSC-DERIVED CD82-POSITIVE CARDIOMYOCYTE-FATED PROGENITORS AS A PROMISING CELL SOURCE OF CARDIAC REGENERATION

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Recently, we successfully identified human cardiomyocyte-fated progenitors (CFPs), a hiPSC-derived cell population that overwhelmingly differentiates into CMs under diverse conditions in vitro and in vivo, are marked by the cell surface molecule CD82. Furthermore, we found CD82 contributes to CM differentiation by attenuating the Wnt/β-catenin signalling pathway through exosomal regulation (Cell Reports 2018). Cardiovascular progenitor cells (CPCs) have restricted differentiation potential to mesoderm cell lineages, which differentiate into CMs with high efficiency in optimized cell culture conditions in vitro, whereas they show deviated differentiation into non-CM populations in non-specific culture conditions and after transplantation in vivo. In contrast, CD82+ CFPs are gifted a privileged differentiation potential in which the direction of differentiation is limited to CMs almost exclusively even in non-specific and in vivo conditions. Consequently, CD82+ CFPs can differentiate into CMs specifically and stably in vivo after transplantation. Next, to examine the effect of CD82+ CFPs on improving cardiac function, we transplanted 2 million CD82+ CFPs into sub-acute myocardial infarction model of immune-deficient rats. One month after transplantation, we observed that CD82+ CFPs almost exclusively differentiated into cardiomyocytes and improved cardiac function, compared with sham-operated control. These results suggested that CD82+ CFPs would serve as a promising cell source of cardiac regeneration.

F-1039

EPIGENETIC REGULATION IN HEART CELL THERAPY

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Existing treatments for heart attack include Medication, intervention, and surgical bypass. These strategies are mainly used to prevent disease progression, but do not address the fundamental problem of CM loss. Therefore, developing new effective therapeutic strategies to preserve the pumping function after MI is highly desired. Cell plasticity or cell state changes, including dedifferentiation and maturation, are critical in disease pathogenesis and prevention, and are likely key to successful cell transplantation. To address the potentially critical role of epigenetic changes in cardiac cell therapy, we set out to examine the histone acetylation in transplanted CMs after infarction. Elevated histone acetylation is often accompanied by cell stage changes in response to various signals, particularly acetylation at the super enhancer regions. In this study, we showed that valproic acid (VPA), a pan HDAC inhibitor and a FDA approved drug for epilepsy and bipolar disorders, reduced ~50% of the infarct size and preserves the pumping function of heart after MI in rats. Moreover, even 60 min after infarction, VPA administration still significantly decreased infarct size in MI rats. Mechanistically, we identified that VPA treatment increased gene expression essential for cell survival and proliferation. Knock-down and over-expression experiments showed that VPA treatment triggered a Foxm1 pathway, which in turn activated genes essential for cell survival and proliferation and inflammation suppression. Importantly, overexpression of Foxm1 provided similar heart protective effect after MI as VPA treatment. In parallel, we have applied a novel biodegradable tri-block copolymer that can self-assemble into nanofibrous microspheres as cell carriers for cell transplantation. These microspheres dramatically improve engraftment of exogenously-delivered cells and functional recovery of the infarcted hearts. Intriguingly, the efficacy of cell engraftment appears to correlate with the cell carrier's ability to maintain the acetylation status of the cells. We expect that the combined HDACi treatment with cell carrier-mediated CM transplantation will work synergistically to regenerate infarcted heart and lead to effective clinical applications in heart cell therapy.

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F-1041

THE ESTABLISHMENT OF A NOVEL MODIFIED MRNA PARACRINE FACTOR LIBRARY TO COMPLIMENT CARDIAC REGENERATION AFTER INJURY

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Heart disease continues to be a major burden in the developed world, positioned as the leading cause of death. Heart diseases can progress towards heart failure, a condition to which there is no radical treatment except for heart transplantation. Stem cell technologies and paracrine treatments are both emerging biotherapies for conditioning the failing heart. One interesting approach

to repair the damaged heart would be to treat healthy segments of the heart with paracrine cues that could expand cardiac cells in situ. Alternatively, paracrine factors can have a positive effect on transplanted cardiac stem cells through pre-treatment strategies. Through understanding the molecular underpinnings that govern developmental cardiogenesis, these same molecules could positively augment reparative networks in the diseased heart. Here we employed single-cell RNA-seq analysis from human embryonic/fetal cardiac tissue and identified significantly up-regulated molecular targets, such as growth factors and chemokines, during the earliest stages of cardiac development. Some of those molecules were enriched in specified cardiac progenitor populations and may have profound properties responsible for driving cardiogenesis/myogenesis. In order to nominate paracrine factors for heart regeneration studies in the injured adult heart, interesting candidates from the embryonic/fetal cardiac tissue screen were compiled into a paracrine factor library consisting of generated modified mRNAs. Previously, synthetic, chemically modified mRNA (modRNA) has been touted as a novel technology platform for the expression of paracrine/autocrine cues for the treatment of heart failure. The use of modRNA has several favourable characteristics including high stability, pulse-like expression and limited activation of innate immune genes upon transfection. Using an in vitro human ES-derived cardiomyocyte proliferation assay, we further identified several candidates within our library that stimulate cardiomyocyte growth/proliferation. Lastly, modRNA candidates will be employed in an in vivo experimental approach of myocardial infarction, where we hope the pathological and functional evaluation will determine novel factors for the treatment of ischemic heart disease.

F-1043

ROLE OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 CHANNELS IN THE REGULATION OF CALCIUM TRANSIENTS AND ACTION POTENTIALS OF 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 and they are able to respond to a wide range of stimuli in the cellular environment. Among TRP channels, TRPV1 channel is activated by heat, pain inducers and the pungent component of hot chili peppers, capsaicin. Previous studies showed that upon stimulation, TRPV1 channel can generate intracellular Ca2+ concentration ([Ca2+]i) changes by Ca2+ entry via the plasma membrane or by Ca2+ release from intracellular organelles. As a fundamental property of cardiomyocytes (CMs), [Ca2+]i plays an important role in different cellular processes such as excitation-contraction coupling, cell proliferation and cell death. However, there is only limited knowledge on the function of TRPV1 channel in the Ca2+-handling properties and electrophysiological characteristics of CMs. In this study, we used embryonic stem cells (ESCs)-derived CMs as a model to study the role of TRPV1 channel in CMs. Our recent results revealed that TRPV1 is located on the sarcoplasmic reticulum (SR) of ESCs-derived CMs and acts as a Ca2+ release channel on the SR. In addition, activation of TRPV1 had no effect on Ca2+ sparks, which can reflect the opening of the ryanodine receptors. Functionally, TRPV1 agonist capsaicin decreased the rate and diastolic depolarization slope of action potential of ESC-derived CMs, as well as the amplitude and frequency of spontaneous Ca2+ transients of ESC-derived CMs. In the near future, to study the role of TRPV1 in ESC-derived CMs, TRPV1 will be knocked down by using adenovirus harboring shRNA against TRPV1. This study will not only give a better understanding of Ca2+ homeostasis of ESC-derived CMs, but also provide insights into the future cell replacement therapies.

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ENDOTHELIAL CELLS AND HEMANGIOBLASTS

F-1045

PLURIPOTENT STEM CELL MODEL OF PULMONARY ARTERIAL HYPERTENSION

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In order to understand the function of BMPR2 in human tissue development and disease such as pulmonary arterial hypertension, we utilised pluripotent stem cells as a human model system. The in vitro dissection of the role of BMPR2 in the development and function of the major cell lineages of the blood vessel were carried out using a suite of human pluripotent stem cell (PSC) lines containing an allelic series of BMPR2 mutations, including a patient-specific truncating mutation (2504delC). Using these reagents, we identified a key role for BMPR2 in the regulation of endothelial migration, cell fate decisions, angiogenesis and proliferation. Importantly, compensatory mechanisms, likely via alternate receptors such as ACVR2A/B, were identified that activated both SMAD-dependant and independent signalling when BMPR2 was perturbed. Thus, BMPR2 is

not essential for the differentiation of PSCs to mesoderm, cardiomyocytes, blood cells, smooth muscle cells (SMCs) and endothelial cells (ECs), in vitro. However, BMPR2 mutant cardiomyocytes displayed an altered transcriptional response to BMP4 exposure. Phenotypic characterization of BMPR2 mutant ECs revealed that a number of endothelial cellular functions relied upon BMPR2. Furthermore, all BMPR2 mutant genotypes displayed more rapid cell migration kinetics than wild type ECs. Metabolomic data and transcriptional data showed that BMPR2 mutant ECs have dysregulated metabolism and related-gene expression. Taken together, BMPR2 appears to have a protective function that maintains homeostasis of the vessel wall. These studies pave the way for future work to elucidate the mechanisms underlying BMPR2 mediated PAH.

F-1047

INHIBITION OF THE HYPER-FIBRINOLYTIC ACTIVITY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS IN AN IN VITRO CELL-BASED THERAPY WOUND HEALING MODEL

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Human induced pluripotent stem cell-derived endothelial cells (iPSC-EC) are a potential autologous cell source for cell-based therapy in wound healing applications. During the early stages of wound healing, microvascular endothelial cells invade the fibrin matrix of the wound clot to form capillaries that enable nutrient exchange for the healing process. We evaluated the efficacy of iPSC-EC as a potential cell source for cell-based therapy wound treatment by conducting an in vitro three-dimensional fibrin matrix sprouting assay, as a simulation of the early wound environment. Spheroids of 1000; 2000; 5000; 10000; or 20000 cell aggregates of iPSC-EC were embedded into a human fibrin matrix and cultured for three days to observe sprouting of capillary-like structures. As controls, primary human dermal microvascular endothelial cell (HDMEC) spheroids were cultured in identical conditions. The HDMEC groups sprouted capillary-like structures in the fibrin matrix within 24 hrs of culture, with extensions of the sprouts up to 72 hrs. In the iPSC-EC group, all spheroid configurations digested the fibrin matrices within 24 hrs. To address the hyper-fibrinolytic activity of iPSC-EC, we co-cultured iPSC-EC with human vascular smooth muscle cells (vSMC) or human adipose-derived mesenchymal stem cells (ASC) in 10000 cell composite spheroids at ratios of iPSC-EC:vSMC or iPSC-EC:ASC of 1:1; 1:5; 1:10; and 1:20 for the sprouting assay. Controls consisted of 10000 cell composite spheroids of HDMEC:vSMC/ASC at the same ratios. All combinations of iPSC-EC:vSMC/ASC spheroids produced near identical sprout structures to the control, with no digestion of the fibrin. Our preliminary findings show that the hyper-fibrinolytic activity of iPSC-EC are inhibited when co-cultured as spheroids with vascular support cells, such as vSMC or ASC, to levels comparable to that of HDMEC. This indicates that co-cultured iPSC-EC spheroids are a more suitable candidate for cell-based wound therapy than monocultured iPSC-EC. Further in-depth characterization in vitro and in vivo is ongoing to examine the potential of cell-based wound therapy using iPSC-EC and vascular support cells.

HEMATOPOIESIS/IMMUNOLOGY

F-1049

EXPANSION SYSTEM FOR PRODUCING A LARGE AMOUNT (>10^9 CELLS) AND HIGH PURITY (>90%) OF HUMAN CD3-CD56+NK CELLS FROM PBMCs AND THEIR THERAPEUTIC APPLICATION DUE TO ADCC ACTIVITY IN XENOGENIC MOUSE MODEL

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Human natural killer (NK) cells are one of attractive candidates for cell-based therapy against any cancers due to their strong cytotoxicity. However, there are few convenient and efficient method to obtain a large amount and high purity of functional NK cells from peripheral blood mononuclear cells (PBMCs) derived from a small amount of blood. Thus, we have developed a robust NK-cell expansion method using OK-432, IL-2 and RetroNectin® induced T (RN-T) cells as a stimulator. RN-T cells were prepared by previously established co-stimulation method using anti-CD3mAb and RetroNectin®, and treated to suppress the growth potential (modified RN-T cells). NK cells could be expanded from PBMCs stimulated with modified RN-T cells, OK-432 and IL-2, then cultured for more than 16 days. In our large-scale culture system using gas-permeable culture bag (CultiLife™215 and CultiLife™Eva), we could obtain 10^9 - 10^10 cells containing a high proportion (>90%) of CD3-CD56+ NK cells from 50mL of peripheral blood. Furthermore, almost all cells displayed

functional cell surface molecules such as NKG2D and CD16 implicated in cytotoxicity and antigen dependent cell cytotoxicity (ADCC). Thus, we investigated the antitumor effect of the expanded NK cells combined with Trastuzumab against HER2-positive human gastric cancer cell line NCI-N87 in hIL-2 Tg NOG mice (hIL-2-NOG mice; Central Institute for Experimental Animals). In this experiment, we used purified NK cells to reduce GVHD risk caused by human CD3+ cells including in the expanded cells. As a result, the combination of the NK cells and Trastuzumab dramatically enhanced the antitumor activity compared with each treatment alone. The chimerism of human NK cells in mouse peripheral blood was observed during the observation period without any GVHD symptoms and functional NK-cell surface markers such as CD16 and NKG2D also expressed in human NK cells. Furthermore, human NK cells were observed into tumor tissue even in 3 months after administration. Overall, we have established a robust NK-cell expansion system and the expanded cells showed strong antitumor activity in a xenogenic mouse model. It is considered that our expansion system could be used for chimeric antigen receptor (CAR)-NK cell processing or pluripotent stem cell derived NK-cell manufacture for future application.

F-1051

INFLAMMATION - ASSOCIATED CYTOKINES IGFBP1 AND RANTES IMPAIR THE MEGAKARYOCYTIC POTENTIAL OF HSCS IN PT PATIENTS AFTER ALLO - HSCT

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Prolonged isolated thrombocytopenia (PT) is a severe complication in patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Whether the megakaryoctic potential of hematopoietic stem cells (HSCs) in bone marrow is intact and what factors drive the pathological process of PT remain elusive. A retrospective study in patients (n=285) receiving HSC transplantation revealed that the occurrence of PT was approximately 8% and the number of platelets and megakaryocytes in PT patients is much lower compared to the control. To test whether the deficiency of thrombopoiesis was caused by the activities of HSCs, the megakaryocytic differentiation potential of HSCs before or after transplantation was assessed. Interestingly, a substantial decrease of megakaryocytic differentiation was observed two weeks after transplantation of HSCs in all of the allo-HSCT recipients. However, four weeks after transplantation, the ability of HSCs to generate CD41+CD42b+ megakaryocytes in SPE patients recovered to the same level as those of HSCs before implantation. In contrast, HSCs derived from PT patients throughout the post-implantation period exhibited poor survival and failed to differentiate properly. A protein array analysis demonstrated that multiple inflammation-associated cytokines were elevated in allo-HSCT recipients with PT. Among them, IGFBP1 and RANTES were found to significantly suppress the proliferation and megakaryocytic differentiation of HSCs in vitro. Our results suggested that the occurrence of PT might be attributed, at least partially, to the damage to HSC function caused by inflammation-associated cytokines after HSC transplantation. These findings shed light on the mechanism underlying HSC megakaryocytic differentiation in PT patients and might provide potential new strategies for treating PT patients after HSC transplantation.

F-1053

MYB IS AN ESSENTIAL REGULATOR OF THE PRIMITIVE HUMAN HEMATOPOIESIS

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MYB is well recognized to be a key regulator of definitive hematopoiesis that plays an important role in the maintenance and multilineage differentiation of hematopoietic stem cells (HSCs). In the vertebrate developmental context, MYB is widely regarded dispensable for primitive hematopoiesis but critically required for the development of definitive hematopoiesis. To explore the role of MYB in human hematopoietic development we have inactivated the gene by bi-allelic TALEN-supported gene targeting in several lines of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), and subjected these cells to hematopoietic differentiation in well-defined cell culture conditions. Venus gene reporter was inserted into the knock-in allele to monitor MYB expression during the course of the hESC/iPSC differentiation. The gene reporter system showed that MYB is specifically expressed during hematopoietic commitment in the earliest primitive blood cells. Moreover, the level of MYB expression was highest at the commitment stage of differentiation and significantly decreased at the maturations stage. We found that MYB was not required for initial hematopoietic commitment of nascent mesoderm and emergence of primitive, yolk sac-type human hematopoietic progenitors. However, inactivation

of MYB severely abrogated proliferation of the primitive erythroid and mixed erythroid-macrophage-megakaryocyte progenitors. In addition, MYB-negative hESC/iPSC lines demonstrated major defects in myeloid cell development and completely failed to generate mature granulocytes. Transposon-mediated rescue of MYB expression in MYB-null cells efficiently restored both the primitive hematopoietic progenitors and immature myeloid cells. Our data indicate that in contrast to its previously attributed exclusive role in definitive hematopoiesis, MYB is indispensable for primitive human hematopoiesis.

F-1055

HEMATOPOIETIC STEM CELLS ARE HIGHLY DEPENDENT ON THE MAINTENANCE OF PROTEIN HOMEOSTASIS

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Stem cells in adult tissues can persist throughout life to regenerate mature cells lost to turnover and injury. Diverse cell intrinsic and extrinsic mechanisms contribute to safeguarding stem cell longevity by ensuring genomic integrity, metabolic homeostasis and precise cell cycle control. One key regulator of longevity that has not yet been studied in adult stem cells is the regulation of protein homeostasis (proteostasis). Here we set out to compare protein quality within long-lived hematopoietic stem cells (HSCs) and short-lived restricted progenitors. We found that HSCs exhibited high protein quality, as they contained less ubiquitylated and unfolded protein as compared to restricted progenitors in vivo. Modest increases in protein synthesis reduced HSC protein quality in vitro and in vivo, indicating that HSCs depend upon low protein synthesis to maintain the integrity of their proteome. To test if the maintenance of proteostasis is essential for stem cell function, we examined *Aars*$^{sti/sti}$ mice that harbor a mutation in the alanyl-tRNA synthetase, which causes a tRNA editing defect that reduces translational fidelity and can lead to an accumulation of misfolded proteins. We determined that *Aars*$^{sti/sti}$ mice exhibit modestly reduced proteostasis that specifically impaired HSC maintenance and serial reconstituting activity in vivo, but did not impair restricted progenitors. HSCs sensed the accumulation of misfolded proteins because their ubiquitin proteasome system became overloaded, which resulted in stabilization and accumulation of c-Myc protein, increased cell cycle entry and stem cell exhaustion. HSCs are thus particularly dependent on the maintenance of proteostasis to regulate their self-renewal activity.

F-1057

INHIBITION OF RECEPTOR-MEDIATED ENDOCYTOSIS IMPAIRS LEUKEMIA STEM CELLS FUNCTION

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Current therapeutic regimen transiently reduce the tumour burden of patients with T-cell acute lymphoblastic leukemia (T-ALL), but fail to eliminate refractory leukemia stem cells (LSCs), which are responsible for relapse. Hence, the eradication of LSCs will be paramount for achieving long-term remission. These resistant LSCs are dependent on the growth factors produced by the microenvironment to develop and survive following chemotherapy treatment. Using the Lmo2 transgenic mouse model of T-ALL, we have shown previously that these LSCs have long-term self-renewal potential and are resistant to chemotherapy. We have also shown that growth factors produced by the thymic niche, like IL-7 and Notch1, are crucial for the development and maintenance of these LSCs during the early stage of the disease. In thymocytes, the activation of these signalling pathways is tightly controlled by receptor-mediated endocytosis, which is dependent on the GTPase Dynamin 2 (DNM2). We found that treatment of Lmo2 transgenic DN3 T-cell progenitors with the specific DNM2 inhibitor Dynole 34-2 blocked IL-7R and Notch1 internalization, which prevented the downstream activation of Stat5 and expression of Hes1, a canonical effector of Notch1 signalling. Inhibition of endocytosis with Dynole 34-2 significantly reduced the frequency and the engraftment of LSCs in transplantation assays. We then treated 2 month-old mice to address the importance of niche-mediated signalling in therapeutic resistance. Accordingly, we found that treatment with Dynole 34-2 sensitized LSCs to the current induction regimen used in the clinic for T-ALL. Finally, inhibition of DNM2 activity significantly

delayed leukemia onset in mice treated with standard therapeutic agents, suggesting that endocytosis is required for the maintenance of treatment-resistant T-ALL. These results provide the most convincing in vivo evidence that receptor-mediated endocytosis is crucial for the maintenance and therapeutic resistance of LSCs. Targeting endocytosis may represent an attractive therapeutic strategy for improving cure rates in refractory leukemias.

F-1059

ENABLING EFFICIENT EX VIVO EXPANSION OF LONG-TERM HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS FOR CELL THERAPY

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Umbilical-cord blood (UCB) is an important source of hematopoietic stem and progenitor cells (HSPC) for transplantation into patients lacking a suitable HLA-matched donor. However, due to the limited cell dose in each cord blood unit, individuals >60 kg are restricted from the use of UCB-based therapy. *Ex vivo* expansion of UCB CD34+ cells is one strategy employed to increase the HSPC dosage. A major limitation of current systems used for the expansion of HSPC is that *ex vivo* culture leads to expansion and differentiation of cells, at the expense of the most primitive pluripotent long-term stem cells. This has limited the clinical application of *ex vivo* expanded HSPC, since short-term progenitor cells only provide transient protection, ultimately reducing the positive health outcomes, increasing the duration of hospitalizations, and health care costs per patient. Development of a culture system that expands both short-term and long-term HSPC would facilitate immune protection during the early phase of recovery, and provides a suitable solution for transfusion-independent hematopoiesis. Therefore, we sought to develop a HSPC culture medium that enables the expansion of both long-term and short-term HSPC, while maintaining their functional properties. To this end, we conducted several iterative rounds of Design of Experiments (DOE) involving multifactorial analysis, and mathematical modeling methods. The DOEs allowed us to identify optimal combinations and concentrations of essential media components, small molecules, and growth factors. The performance of candidate HSPC expansion media were evaluated after 7 days of culture, upon which the CD34+ cells (total HSPC) and CD34+CD90+CD45RA- cells (long-term population) were quantified. The expression of aldehyde dehydrogenase was conducted to identify primitive stem cells, and colony-forming unit assays were performed to assess the *in vitro* differentiation capacity of expanded cells. We plan to determine whether the expanded cells are engraftable by transplanting the cells into immuno-deficient mice. Taken together, we seek to highlight our design philosophy in HSPC culture media development, and we believe our efforts are critical for the successful utilization of hematopoietic stem cell transplants in translational cell therapies.

F-1061

ZEB1 AND ZEB2 ACT AS KEY DETERMINANTS IN MYELOID DIFFERENTIATION: A POTENTIAL THERAPEUTIC TARGET IN ACUTE MYELOID LEUKAEMIA?

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The Zinc-finger E-box binding (ZEB) family of transcriptional regulators consists of two structurally related proteins: Zeb1 and Zeb2. Previous data has shown that Zeb1 is important for T-cell differentiation and acts as a tumour suppressor role in T-ALL formation using a murine global deletion/truncation model. We have reported that Zeb2 overexpression selectively transforms T-cells and results in ETP-ALL like disease indicating an oncogenic role for Zeb2 in T cells. Here we examined the precise roles of Zeb1/2 in hematopoietic stem cells (HSC) and leukemia stem cells (LSC) biology using the Cre recombinase transgenic mouse lines. Conditional Zeb1/2 gain/loss-of-function mice were crossed with Tie2-Cre (Cre-mediated deletion in endothelial and hematopoietic cells), Vav-iCre (Cre deletion limited to hematopoietic cells) or R26-Cre-ERT2 line (tamoxifen-inducible Cre deletion). All the strains were backcrossed to a C57Bl/6 genetic background for at least 6 generations. Hematopoietic over-expression of Zeb1 leads to abnormal monocyte differentiation skewing, while Zeb1 loss-of-function mice displayed differentiation defects in multiple myeloid populations including neutrophils and monocytes. Zeb2 overexpression resulted in an expanded immature myeloid population (CD11b+Grlow) whereas Zeb2 loss of function leads to enhanced granulopoiesis and the development of myeloproliferative syndrome. Given the fact that Zeb1 appears to act quite differently from Zeb2 in hematopoietic differentiation, we then investigated whether there was a functional synergy or antagonism between the two. In tamoxifen-inducible Zeb1 and Zeb2 double knock-out mice, flow cytometric analysis revealed sever dramatic defects in hematopoietic stem cell (HSC) compartment, indicating a synergistic effect of Zeb1 and Zeb2 in HSC differentiation. Our results reveal essential roles for Zeb family in hematopoietic differentiation. Unlike Zeb2, Zeb1 appears to be a positive regulator of

myeloid development. Both Zeb1 and Zeb2 levels have to be maintained to ensure myeloid lineage ontogeny and HSC differentiation potential. The precise role of Zeb1 and Zeb2 on AML initiation and progression will be further investigated using oncogenic MLL-AF9 and MLL-ENL models.

F-1063

HOXB5 REPROGRAMS B CELLS INTO FUNCTIONAL T LYMPHOCYTES

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Deletion of B cell master regulators reprogrammed B cells into T cells that were either functional defects or tumorigenic potential. Here we show that Hoxb5, which is expressed in uncommitted hematopoietic progenitors but absent in committed B and T lineages, could reprogram pro-pre-B cells into functional early T cell progenitors. The reprogramming started in bone marrow and completed in thymus, giving rise to T lymphocytes with transcriptomes, hierarchical differentiation, tissue distribution and immune functions closely resembling their natural counterparts. Hoxb5 repressed B cell master genes, activated T cell regulators and regulated crucial chromatin modifiers in pro-pre-B cells, ultimately driving B to T cell fate conversion. Our results provide a de novo paradigm for generating normal and functional T cells through reprogramming in vivo.

PANCREAS, LIVER, KIDNEY

F-1065

METABOLIC PROFILING OF HUMAN STEM CELL-DERIVED β CELLS REVEALS A BOTTLENECK IN GLUCOSE UTILIZATION AND INSULIN SECRETION RESPONSE

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Stem cell-derived β (SC-β) cells offer an unlimited source of material for the curative treatment of diabetic patients. We have reported that differentiation of SC-β cells produces transplantable endocrine organoids that respond to glucose in vitro. However, the magnitude and consistency of response is not as robust as observed in human islets. Studies of differentiated tissues have largely used gene expression to understand regulation of cell identity and tissue-specific function. However, the β cell response to elevated glycemic levels is a post-translational process coupled to tightly regulated metabolism of glucose unique to β cells. To specifically focus on glucose metabolism we have performed in-depth metabolic analysis of SC-β cells and human islets using the recently reported MIMOSA technique, combining carbon tracing and mass spectrometry analysis. Using MIMOSA we identified several differences in glucose metabolism between human islets and SC-β cells, including the inappropriate accumulation of citrate, which appears to inhibit normal β cell glucose sensing. Correcting or bypassing these metabolic deficiencies significantly improves SC-β response to nutrients and produces a cell type that recapitulates the function of bona fide human islets.

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F-1067

GALECTIN-EXPRESSING BETA-LIKE CELLS DERIVED FROM AUTOLOGOUS HEPATOCYTE REPROGRAMMING AMELIORATE AUTOIMMUNE DIABETES

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Type 1 diabetes mellitus (T1DM) is characterized by complete loss of beta-cells due to T-cell mediated autoimmune attacking. Islet transplantation is a potential way to sustainably control blood sugar levels of T1DM patients'. However, the shortage of donor islets and poor islet graft survival limit the potential use of islet transplantation to treat T1DM patients. We recently discovered combination of Pdx1 and Ngn3 can convert liver cells to insulin-producing beta-like cells that exhibit the characteristics of pancreatic beta-cells. Treatment with PDGF-AA was found to facilitate Pdx1 and Ngn3-induced reprogramming of hepatocytes to beta-like cells with the ability to secrete insulin in response to glucose stimulus. Importantly, this reprogramming strategy could be applied to adult mouse primary hepatocytes, and the transplantation of beta-like cells derived from primary hepatocyte reprogramming could ameliorate hyperglycemia in diabetic mice. The efforts provided solutions to donor shortages and poor islet graft survival and functions. However, patients received the allogeneic islet transplantation were still suffering from side effects of the immunosuppressive medications. Hence, the possibility of producing immune-tolerable beta-cells would be a key challenge for developing cell-based therapeutics for T1DM patients. Since the liver has immune privileged properties, we therefore hypothesize beta-cells derived from hepatocyte reprograming may possess immune-modulating capability. We then reprogrammed primary hepatocytes derived from Non-obese diabetic (NOD) mice with Pdx1/Ngn3/PDGFRa that resulted in efficient generation of glucose-responsive beta-like cells. Autologous transplantation of hepatocyte-derived beta-like cells to diabetic NOD mice significantly improved hyperglycemic status without needs of tolerogenic treatments. We further demonstrated induced expression of galectin-3 and galectin 9 by PDGF signaling in hepatocyte-derived beta-like cells could significantly suppress autoreactive T cells thus explain why hepatocyte-derived beta-like cells could ameliorate autoimmune diabetes. The findings raise the possibility of developing cell therapeutic strategies for patients with type 1 diabetes via autologous hepatocyte reprogramming.

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F-1069

OPTIMISING HUMAN KIDNEY ORGANOIDS FOR SCREENING NEPHROTOXIC DRUGS

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Kidneys are uniquely vulnerable to drug toxicity due to their high metabolic rate and their role in filtering substances from the blood. Proximal tubule cells in the kidney are the most frequently affected, as they express transporters and receptors which can increase their uptake of drugs relative to other cell types. The in vitro assays and animal tests currently used to screen drugs in development for nephrotoxicity are not sufficiently predictive because they have decreased expression of these transporters compared to mature human proximal tubules in vivo. We have recently reported protocols for the generation of human kidney organoids from pluripotent stem cells. Such organoids contain patterning and segmenting nephrons. Hence, kidney organoids may be useful for in vitro nephrotoxicity screening during drug development if they show sufficient nephron maturation. Whole kidney organoids have increased expression of key proximal tubule transporters compared to the gold standard of primary proximal tubule cells (RPTECs) cultured in vitro. Staining and FACS for the epithelial marker EpCAM and CD13 yielded a double-positive population which was enriched for the expression of OAT1 and CUBN (an organic anion transporter and endocytotic receptor complex molecule respectively) compared to RPTECs, showing that the proximal tubules within organoids were more functionally mature. The improved maturity of organoid proximal tubules compared to RPTECs suggests that they will recapitulate the in vivo toxic injury response more accurately and will be a more sensitive and specific in vitro screening platform.

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F-1071

SINGLE CELL ANALYSES OF HUMAN ISLET CELLS REVEAL DE-DIFFERENTIATION SIGNATURES

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Human pancreatic islets containing insulin-secreting β cells are notoriously heterogeneous in cell composition. Since β cell failure is the root cause of diabetes, understanding this heterogeneity is of paramount importance. Recent reports have catalogued human islet transcriptome but not compared single β cells in detail. Here we scrutinized *ex vivo* human islet cells from healthy donors and show that they exhibit de-differentiation signatures. Using single cell gene expression and immunostaining analyses, we found healthy islet cells to contain polyhormonal transcripts, and INS^{+} cells to express decreased levels of β cell genes but high levels of progenitor markers. Rare cells that are doubly-positive for progenitor markers/exocrine signatures, and endocrine/exocrine hormones were also present. We conclude that *ex vivo* human islet cells are plastic and can possibly de-/trans-differentiate across pancreatic cell fates, partly accounting for β cell functional decline once isolated. Therefore, stabilizing β cell identity upon isolation may improve its functionality.

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F-1073

OVER-EXPRESSION OF THE STEM CELL FACTOR LIN28 LEADS TO CYSTIC KIDNEY DEVELOPMENT AND ABERRANT CILIA FORMATION

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Lin28 is a RNA binding protein that regulates gene expression via inhibition of Let7s microRNA maturation and also by Let7 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 of 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 Lin28 overexpression in the entire kidney during embryonic kidney development prevents the final wave of differentiation of the cap-mesenchyme cells, and leads to the formation of a tumor similar to human Wilms tumor. Here we show that, by contrast to this developmental phenotype, Lin28 overexpression in the adult nephrons leads to the formation of cystic kidneys. Analysis of the Lin28 derived cystic kidneys point to some levels of similarities to the cystic kidney phenotype of ADPKD (autosomal dominant polycystic kidney disorders) patients. For example, the distribution of the cysts along the entire nephron, the increased tubular epithelial cell proliferation and the relatively low levels of fibrosis are typical for ADPKD. The vast majority of cystic kidney disorders involve aberrant cilia formation/maintenance. Indeed, the Lin28 transgenic kidneys have significantly less cilia compered to normal kidneys. To verify and explore the effect of Lin28 on the cilia we introduced an inducible Lin28A over-expression cassette into the mIMCD-3 cell line. Using this transgenic cell line, we showed a significant (cell cycle independent) decrease in the number of ciliated cells upon Lin28 over-expression. So far the effect of Lin28 over-expression was studied mainly in respect to abnormal embryonic development and tumorigenesis. Our new data demonstrate a much broader effect of Lin28 over-expression. Elucidating the connection between Lin28 over-expression, abnormal cilia formation and the cystic kidney phenotype may significantly advance our understanding regarding the cellular and molecular mechanisms underlying the development of polycystic kidneys.

F-1075

CONDITIONED MEDIA DERIVED FROM ADIPOSE TISSUE MESENCHYMAL STROMAL CELLS IMPROVED HEPATOCYTE MAINTENANCE

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Recent advances in cell therapy encouraged researchers to provide an alternative for treatment and restoration of liver by using hepatocytes. However, these cells quickly lose their capabilities in vitro. Here, we aim to use the secretome of mesenchymal stromal cells (MSCs) to improve in vitro maintenance of hepatocytes. After

serum deprivation, human adipose tissue derived MSCs (hAT-MSCs) were cultured for 24 hours under normoxic (N) and hypoxic (H) conditions. Their conditioned media (CM) were subsequently collected as N-CM (normoxia) and H-CM (hypoxia). Mouse liver was perfused by collagenase, after which the isolated hepatocytes were cultured in hepatocyte basal (William's) medium supplemented with 4% N-CM or H-CM. As controls, William's and hepatocyte specific media (HepZYM) were used. Finally, we evaluated the survival and proliferation rates, as well as functionality and gene expressions of cells. MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay showed a significant increase in viability of hepatocytes in the presence of N-CM and H-CM compared with HepZYM on day 5. Indocyanine green (ICG) uptake of hepatocytes in the H-CM and HepZYM groups at days 3 and 5 also suggested that this CM supported the hepatocytes at the same level as hepatocyte specific medium. The HepZYM group demonstrated significantly higher albumin (Alb) and urea secretions compared to the other groups (0.0001). However, there were no significant differences in cytochrome activity as well as cytochrome gene expression profiles among these groups. We found a slightly higher concentration of vascular endothelial growth factor (VEGF) in the H-CM group compared to the N-CM group (p=0.063). The enrichment of William's basal medium with 4% hAT-MSC derived CM improved some of the hepatocyte's physiologic parameters in a primary culture. Hepatocyte, Mesenchymal stromal cell, Regenerative medicine, Conditioned medium

F-1077

STEM CELL DERIVED PANCREATIC TISSUE FOR TRANSPLANTATION INTO THE ANTERIOR CHAMBER OF EYE

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Type 1 Diabetes is a rapidly expanding endocrine disease affecting millions of people worldwide. Patient with type1 Diabetes can be successfully treated with transplantation of new pancreatic islets, but this approach is limited by shortage of donor pancreatic tissue and low viability of transplanted donor pancreatic islets. Transplantation of pluripotent stem cell 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. To generate islet-like cells for ACE transplantation, we differentiated human embryonic stem cells (hESCs) into PDX1+/NKX6.1+ pancreatic progenitor cells. The progenitor cells were FACS purified and further differentiated towards pancreatic islet cells as 3D aggregates cultured in suspension. The hESC-derived cell aggregates were then 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. The results show that the injected aggregates were embedded into the iris tissue and formed duct-like structures that resemble native developing pancreas. Expression of markers for the three major pancreatic tissues, endocrine (Synaptophysin), exocrine (Trypsin), and ductal (CK19), were confirmed by immunohistochemistry. However, few Insulin+ beta cells were identified, indicating that in vivo maturation into mature beta cells requires more than one month. Together these results suggest that it would be beneficial to differentiate hESCs into more mature and restricted endocrine cells before ACE transplantation.

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F-1079

THE GENERATION OF LIVER-HUMANIZED RATS USING FAH-/-RAG2-/-IL2RG-/- RATS

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Due to host tropism of hepatotropic pathogen and the known species differences in liver metabolism, liver-humanized mice generated by transplanting human hepatocytes are an absolute necessity to study virus infection and drug toxicology. Rats have been known to be more close to humans than mice in terms of various physiological and pathological aspects. Moreover, rats, as a medium-sized laboratory model, are capable of providing greater biological sample including hepatocytes. Here, we have generated Fah-/-Rag2-/-IL2rg-/- (FRG) rats using CRISPR/Cas9 system and demonstrate they are severely immune-deficient rats with abnormal lymphoid development and can be xenotransplanted with human induced pluripotent stem cells and liver cancer cells. The extensively repopulation of mouse hepatocytes in FRG livers within two months after transplantation further proves FRG rats can act as a liver repopulation model. Finally, we successfully transplant human hepatocytes to FRG rats. The chimera liver with efficient reconstitution of human hepatocytes

secretes a large amount of human albumin and shows the expression of human mature hepatocyte markers including zonal position-specific enzymes and human specific cytochrome P450 enzymes. These results suggest FRG rats may be an alterative liver-humanized animal model and can be bioreactors to provide large quantities of highly functional human hepatocytes.

EPITHELIAL TISSUES

F-1081

GENERATION OF NKX2.1+ BRONCHIAL PROGENITORS FROM IPSC: CRITICAL ROLE FOR PRIOR ADAPTATION TO SINGLE CELL DISSOCIATION

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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. Induced pluripotent stem cells (iPSC) is a promising alternative, but lack of robust differentiation protocol of into airway epithelium is still of concern. Recent reports show that it is possible to reconstitute in vitro a complex and functional airway epithelium displaying all the characteristic features described in vivo from hiPSC. Our aim was to establish a robust and reproducible step-wise differentiation protocol of hiPSC into the bronchial progenitors, by mimicking the developmental process of the human lung. Adjustment of developmental signaling pathways such as canonical TGF-beta pathway, Wnt and BMP allowed the generation of NKX2.1+ bronchial progenitors. Efficiency was evaluated at each step by flow cytometry and immunofluorescence. CXCR4/SOX17 expression were used as a marker of definitive endoderm, and NKX2.1 of bronchial progenitors ate the stage of ventralized anterior foregut Endoderm (VAFE). We observed that the combined action of activin and the WNT agonist CHIR99021 lead to the highest CXCR4+/SOX17+ induction rate of definitive endoderm (DE), with more than 90% CXCR4+ DE cells in 6 different iPSC lines). Of note, initial medium supplementation with Y27632 was mandatory, at least in part to prevent dissociation-induced apoptosis, as well as several prior passages to adapt the iPSC lines to single cell dissociation. Then, culture in serum free medium without cytokines during 4+/- 2 days drives CXCR4+ cells differentiation into NKX2.1+ VAFE progenitors in a high purity rate (mean value = 78% n =6, 5 iPSC lines). VAFE progenitor cells were characterized by flow cytometry and immunofluorescence by the presence of NKX2.1 and the lack of hepatic marker alpha foeto-protein. RT-q PCR confirmed the expression of NKX2.1 at the mRNA level, and the lack of neuronal and thyroid markers (PAX8/TUJ1 and PAX6/thyroglobulin respectively). In conclusion, we describe a method to produce with high efficiency NKX2.1+ bronchial progenitors. These NKX2.1+ progenitor cells can be used in several differentiation models (2D Air Liquid Interface or 3D models) to further obtain a mature and functional airway epithelium.

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F-1083

FUNCTIONAL SIMILARITIES OF MICRORNAS ACROSS DIFFERENT TYPES OF TISSUE STEM CELLS IN AGING

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Adult tissue stem cells (TSCs) are impaired in their functions with age. TSC dysfunctions and decreased regenerative capacity are involved in, at least a part of, disturbances of tissue homeostasis, e.g., inefficient muscle repair, reduced bone mass, neurodegenerative diseases, and dysregulation of hematopoiesis. Therefore, restoration of tissue homeostasis by controlling stem cell aging is a promising therapeutic approach for geriatric disorders. The molecular mechanisms underlying age-related dysfunctions of specific types of TSCs have been studied, and various microRNAs (miRNAs) were recently reported to be involved. However, the central roles of miRNAs in stem cell aging remain unclear. We found that miRNA-regulating stem cell competence is one of the critical molecular machinery for the regulation of various developing stem cell properties. We could improve diminished differentiation potential and senescence-associated secretory phenotype (SASP) by upregulating the specific miRNA in multiple aged TSCs. These functional similarities of miRNAs indicate that there is the central molecular machinery underlying the stem cell aging and the control of it would enable to recover the TSC functions.

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F-1085

JUNB GUARDS EPIDERMAL HOMEOSTASIS THROUGH RESTRICTING ABERRANT LINEAGE DIFFERENTIATION

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Transcription factors ensure skin homeostasis via tight regulation of distinct resident stem cells. Here we uncovered the previously unreported role of JunB, a redox transcription factor belonging to the AP1 family, to act as a gatekeeper for epidermal differentiation mainly by the suppression of epidermal progenitors differentiation. This together with the JunB-dependent regulation of Notch gene expression ensures epidermal differentiation and, in consequence, maintenance of a protective epidermal barrier. Deficiency of JunB in basal keratinocyte and their progenitors result in a dermatitis-like syndrome resembling seborrheic dermatitis with enlarged sebaceous glands, epidermal hyperplasia, lack of epidermal differentiation and inflammation. Of note, full thickness wounds revealed delayed healing in K14 JunB mutant mice. These data imply a master role for JunB in the control of epidermal stem cells plasticity and appendage specification and will help to design novel therapeutic approaches for sebaceous gland disorders.

F-1087

THE ROLES OF GRAINYHEAD-LIKE 2 AND 3 IN INTESTINAL HOMEOSTASIS, REGENERATION AND CANCER

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The intestinal epithelium is the fastest regenerating tissue in mammals, and has a great ability to repair itself following damage caused by disease (Beumer and Clevers, 2016). The epithelial monolayer of small intestine and colon is organised into invaginated crypts, which contain proliferating epithelial cells (Clevers, 2013). Crypt base columnar (CBC) cells act as stem cells that constantly maintain the epithelium, while other cell types can act as reserve cells that replenish the CBC stem cell pool following damage. These cells are regulated by multiple interacting proteins and signalling pathways during homeostasis and regeneration. The Grainyhead-like (Grhl) proteins are a family of transcription factors that control the formation and differentiation of the epithelium (Boivin and Schmidt-Ott, 2017). Mammals have three evolutionarily conserved homologues: Grhl1, Grhl2 and Grhl3. Despite their similarities in sequence structure, the Grhl proteins lack functional redundancy and it has been suggested that each family member plays unique roles. However, the individual roles of Grhl proteins and their interactions in intestinal stem cell regulation and colorectal cancer are yet to be elucidated. Pilot data from our laboratory has shown that Grhl2 and Grhl3 are expressed in the intestinal epithelial stem cells. We are investigating the roles of Grhl2 and Grhl3 in intestinal homeostasis and repair in mice using an inducible, conditional intestinal knockout approach for Grhl2 and/or Grhl3. Intestinal tissue samples from knockout mice are being utilised for histological, immunohistochemical and molecular analyses. The ability of tissue from these models to drive growth of intestinal organoids is being examined using in vitro growth assays. To further investigate the roles of Grhl2/3 in intestinal repair, we will study the capacity of the intestinal epithelium to regenerate following injury induced by administration of a commonly used chemotherapeutic agent 5-fluorouracil (5-FU). These studies will reveal the individual roles of Grhl2 and Grhl3 in regulating intestinal stem cell-mediated growth and regeneration.

F-1089

PTEN LOSS IN LGR5+ HAIR FOLLICLE STEM CELLS PROMOTES CELL ACTIVATION, MIGRATION AND TRANSFORMATION

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Pten acts as a tumor suppressor gene largely through the inhibition of its phosphatase protein product to the AKT signaling pathway. Mutations of this gene are found in the development of many cancers. Here we examined the influence of Pten loss on the behavior of $Lgr5^{+}$ hair follicle stem cells. We generated Lgr5-Cre(Lgr5-GFP-Cre-ERT2):$Pten^{flox/flox}$ mice. Tamoxifen treatment of the

mice (7 weeks old) markedly induced phosphorylation of AKT in Lgr5^{+} hair follicle stem cells and increased the number of the cells expressing Ki67, resulting in a much shortened second telogen phase. When the skin of Lgr5-Cre:Pten$^{flox/flox}$ mice which were pre-treated with tamoxifen, was wounded, there was a dramatic increase in number of Lgr5^{+}(GFP^{+}) cells in the upper portion of the hair follicle adjacent to the wound at day 3 post wounding and in the re-epithelized epidermis at day 5 post wounding, compared with the numbers seen in untreated mice (without tamoxifen treatment). These data indicate that *Pten* loss enhances Lgr5^{+} hair follicle stem cell activation and migration to the epidermis following wounding. To further study the consequence of *Pten* loss in Lgr5^{+} cells in young mice, we treated Lgr5-Cre:Pten$^{flox/flox}$ mice aged 20-22 days with tamoxifen; the result showed that *Pten* loss in Lgr5^{+} cells caused marked hyperplasia of the hair follicle, which was greatly enlarged and composed of increased number of epithelial cells. In addition, when the mice received topical treatment with dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA), the mice showed earlier occurrence and increased incidence of skin papilloma (100% of mice with *Pten* loss in Lgr5 cells developed papilloma at 9 weeks post TPA treatment vs 93.3% of control mice developed papilloma at 22 weeks post TPA treatment). Moreover, 57.1% of mice with *Pten* loss in Lgr5 cells developed invasive skin carcinoma by 27 weeks post TPA treatment, compared to 14.3% in control mice. Thus our data indicate that *Pten* loss in Lgr5^{+} hair follicle stem cells can induce the cell activation and enhance the cell migration toward the wounded epidermis, and *Pten* loss in Lgr5^{+} stem cells in young mice enhances the susceptibility to oncogenes.

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F-1091

MimEX™ GI, A NOVEL AND ROBUST 3-D GASTROINTESTINAL TISSUE MODEL APPLICABLE FOR TOXICOLOGY, DRUG DISCOVERY, AND DISEASE MODELING

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Advancements in 3-D in vitro models of the gastrointestinal system are leading to their adoption as reliable tools for toxicology, drug discovery, and disease modeling. 3-D in vitro models, such as organoids and spheroids, provide a more accurate representation of the actual tissue microenvironment when compared to 2-D cell culture models. Some of the major issues encountered with current 3-D gastrointestinal models include variability, tissue viability, and experimental accessibility. To overcome these obstacles, we introduce MimEX™ GI, an innovative human "open book" gastrointestinal tissue model system. This system utilizes the unique characteristics of adult "ground state" (GS) stem cells to generate 3-D gastrointestinal epithelial tissue on a 2-D surface. Using this technology, GS stem cells can be isolated from the epithelium of any region of the adult gastrointestinal tract and clonally expanded in 2-D culture. Subsequently, using MimEX™ GI Differentiation Media and Reagents, a high-density monolayer of region-specific gastrointestinal stem cells differentiate into a 3-D tissue mimicking their respective tissue of origin ex vivo. The differentiated tissue obtained is uniform and oriented such that the apical surface of the mucosa is accessible to experimental manipulation. Here we characterize GS stem cells and their differentiated derivatives using a variety of techniques including immunostaining, automated Western blot (Wes™), Single-Cell Western blot (Milo™), and RNA in situ hybridization (RNAscope®) to analyze the protein and RNA expression of the GS stem cells post-isolation and differentiation. Additionally, MimEX™ GI tissue is used in a high-throughput permeability assay. The results obtained demonstrate the barrier function ability of ex vivo-generated tissue and its response to different molecules, demonstrating the flexibility of this tissue in high-throughput drug screening.

EYE AND RETINA

F-1093

HUMAN UMBILICAL CORD DERIVED-MESENCHYMAL STEM CELLS PROMOTES GENERATION OF SPHERES, CHARACTERISATION AND CELLULAR MIGRATION OF HUMAN CORNEAL EPITHELIAL CELLS

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Transplantation of cornea stem cells is a contemporary cellular therapy for ocular surface regeneration. The difficulty in maintaining the cell barrier is due to insufficiency of the local microenvironment. Cells in spherical cultures exhibit extensive cell-cell contacts

mimicking the ultrastructure of the native environment thus may improve stem cell functions and engraftment. Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) which have great regenerative potentials were introduced to human telomerase-immortalised corneal epithelial cells (hTCECs) to improve its characteristics, proliferation and cellular migration. This project aims to investigate the effects of hUC-MSCs on cornea stem cell expression, cellular function and three-dimensional spherical cultures. The hUC-MSCs and hTCECs were co-cultured in indirect culture using transwell with 3.0 µm pore membrane insert. The protein and gene expression were characterised by immunocytochemistry and RT-PCR. The cells were cultured in direct and indirect co-culture and scratch migration assay was performed to investigate the effect on cell migration after 8 hours. Cellular proliferation was evaluated using Ki67 staining. Spheres were generated from hTCECs alone and co-culture with hUC-MSCs for three days in Defined Keratinocyte Serum Free Medium and DMEM with 10% foetal bovine serum using EZsphere plate with microwell coated with low cell attachment. Immunofluorescence revealed that hTCEC in direct and indirect co-culture with hUC-MSCs maintained the expression of corneal markers; p63, ABCB5 and ABCG2 without expression of differentiation marker cytokeratin 3. RT-PCR showed that gene expression of Np63, ABCB5, and ABCG2 also improved in indirect co-culture of hTCEC with hUC-MSCs. Cellular migration and proliferation of hTCEC significantly showed improvement in direct co-culture with hUC-MSCs compared to indirect co-culture. hUC-MSCs promotes generation of spheres in co-culture compared to generation of spheres in hTCEC alone. In conclusion, hUC-MSCs maintain corneal stem cell markers expression in hTCECs. hUC-MSCs enhanced cellular migration and generation of hTCECs spheres. Direct cell-to-cell contact of hUCMSCs in co-culture improves its effects on cellular migration and proliferation.

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F-1095

TREATEMNT OF MACULAR DEGENERATION USING HUMAN SOMATIC CELL NUCLEAR TRANFER EMBRYONIC STEM CELL DERIVED RETINAL PIGMENT EPITHELIUM: PRELIMINARY RESULTS IN AN ASIAN PATIENT

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Here, we report safety and tolerability of the first clinical application of somatic cell nuclear transfer(SCNT) embryonic stem cell derived cells in human. Our precedent clinical trial results have been published showing treatment of macular degeneration using human embryonic stem cell-derived retinal pigment epithelium(hES-RPE) was feasible and safe for a year. We have established patient SCNT-hES from skin fibroblasts. RPE cells have been derived from SCNT-hES at GMP facility. SCNT-hES-RPE met GMP standards consistent to the clinical grade hES-RPE. Korean ministry of food and drug safety authorized the clinical trial. Subretinal transplantation of SCNT-hES-RPE in an Asian female patient with advanced dry age related macular degeneration(AMD) was performed. No immunosuppressant was administered. The patient was closely monitored in terms of systemic and ophthalmologic safety for 16 weeks. Pigmentations were noted at subretinal and preretinal area without definite evidence of rejection, no evidence of adverse proliferation, and ectopic tissue formation. An epiretinal membrane with dark brown pigmentation developed at 2 weeks. The epiretinal membrane enlarged causing minimal thickening of the underlying retina, and the pre-retinal pigmentation area increased and darkened by 16 weeks. Best corrected visual acuity(BCVA) was 21 letters read on Bailey-Lovie chart at screening and stable through 16 weeks after transplantation(22 letters read). No serious adverse events related to transplanted cells were noted for 16 weeks. Corneal abrasion, nausea were adverse events related to the surgical procedure and general anesthesia that recovered without sequela. Tremor, decrease of serum magnesium level, and dizziness were systemic adverse events considered as unrelated to the study procedure by the neurology and otorhinolaryngology specialists. Tremor and serum magnesium level improved with observation in 2 weeks. Dizziness improved with medical therapy(betahistine mesylate, flunarizine and prednisolone). According to these results, we would like to present preliminary safety and tolerability of SCNT-hES-RPE therapy. Long term observation and further investigation in larger number of participants are necessary to confirm these results.

F-1097

GENERATION OF FUNCTIONAL RETINAL PIGMENT EPITHELIUM FROM iPSC- DERIVED 3-D EMBRYOID BODIES

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The loss of vision in age-related macular degeneration (AMD) is associated with progressive degeneration of retinal pigment epithelium (RPE). RPE secretes and provide nutritional factors, phagocytose sheds of photoreceptor outer segment (POS), forms a barrier between blood and retina, acts as anti-oxidative and also maintain choroidal circulation by secretion of

vascular endothelial growth factor (VEGF). Thus, the transplantation of RPE is being established for the cell-replacement therapy in AMD. In recent years, the transplantation of RPE derived from patient-specific induced pluripotent stem cells (iPSC) is under translation in clinics with the better outcome evident from Phase I clinical trials. Here, we develop the RPE from keratinocytes-derived iPSC. In the study, the human epidermal keratinocytes were transduced with non-integrating Sendai virus and maintained in feeder-free conditions. The iPSC colonies were observed in 12 days which showed the expression of pluripotency markers. The iPSC was self-induced to embryoid bodies (EB) which later expressed the markers for germ layers. The neuroectodermal differentiation process was performed by plating the EB in the vitronectin coated plates and maintained in the retinal differentiation media containing B27 for differentiation into RPE. The RPE formation was observed within 30 days with characteristic flat hexagonal morphology. In long-term culture, the RPE showed beginning of pigmentation, formation of tight junctions (ZO1), and expression of RPE markers (MITF, CRALBP, Bestrophin, and RPE65). In addition, monolayer culture of iPSC-derived RPE showed formation of vectorial fluid-filled sac and evident the phagocytic function. In the line, the functional RPE have been generated from the normal human keratinocytes for further transplantation studies.

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STEM CELL NICHES

F-1099

MESENCHYMAL STEM CELLS SELF-REGULATE HLA-I SURFACE EXPRESSION IN INFLAMMATORY MICRO-ENVIRONMENT

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The human leukocyte antigen class I(HLA-I) is supposed to be expressed low on mesenchymal stem cells(MSCs), while it can be affected by inflammatory environment. This study was designed to investigate the phenomenon and underlying mechanism of the HLA-I surface expression on MSCs within inflammatory micro-environment. Here, we found the MSCs had inconsistent expression of total HLA-I and surface HLA-I under IFN-γ treatment. The MSCs can downregulated the surface expression automatically, even though the total HLA-I molecules were increased continually. Subsequently, gene chip data indicated the transport vesicle was upregulated in MSCs after IFN-γ treatment. Interestingly, we found MSCs can accelerate the endocytosis rate of surface HLA-I molecules to maintain low surface expression of HLA-I in inflammatory micro-environment. Further study identified the MSCs endocytose surface HLA-I through a Clathrin-independent Dyanmin-dependent pathway. Our study discovered the ability and mechanism of MSCs self-regulating surface HLA-I expression to maintain low immunogenicity.

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F-1101

THE ADAPTIVE REMODELING OF STEM CELL NICHE IN STIMULATED BONE MARROW COUNTERACTS THE LEUKEMIC NICHE

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Accumulating studies have shown the cellular nature of hematopoietic stem cell (HSC) niche in bone marrow (BM) and their degenerative changes under leukemic conditions. However, the dynamic adaptation of niche cells to changes in physiological stimulatory signals remains largely uncharacterized. Here, we have established a niche stimulation model induced by 5-fluorouracil. This model reveals a rapid and reversible conversion of mesenchymal cells into niche-like stromal cells, which exhibit a platelet derived growth factor receptor-alpha^{+}/ Leptin receptor^{+} (PL) phenotype. These cells selectively induce the niche signaling molecule, Jagged-1, but not CXCL12, to initiate a stimulation-induced regeneration of HSCs in a Jagged-1 dependent manner. Conversion of mesenchymal cells into niche-like cells occurred independently of mitotic activation. The conversion was accompanied by the acquisition of primitive mesenchymal cell characteristics, including the rapid induction of stage specific embryonic antigen-3 and the acquisition of clonogenic potential. The stimulation-induced remodeling of the BM niche resulted in a positive stimulatory effect on the regeneration of normal HSC, but exerted inhibitory effects on leukemic cells, leading to a competitive advantage for normal HSCs in the BM niche and prolonged survival of mice engrafted with leukemic cells. Thus, the reactive conversion of mesenchymal stroma into niche-like cells reveals the adaptive changes of the BM microenvironment to stimuli, and provides insight on the remodeling of niche towards pro-normal/ anti-leukemic microenvironment, which can counteract the progressive pro-leukemic changes driven by the leukemic niche. Our study raises the potential for anti-leukemic niche targeting therapy.

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F-1105

ENVIRONMENTAL ENRICHMENT ENHANCES G-PROTEIN-REGULATED INDUCER OF NEURITE OUTGROWTH (GPRIN) IN THE NEURAL STEM CELL NICHE OF ADULT MOUSE BRAIN

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Environmental enrichment (EE) enhances motor performance and neurogenesis. However, the underlying therapeutic mechanism remains unclear. Therefore, we attempted to unveil the long-term effects of EE on neurogenesis in the subventricular zone (SVZ) and striatum. At 6 weeks of age, CD-1 mice were randomly housed either in EE condition or standard cages for two months. Proteomic studies on samples of the basal ganglia were conducted. Differentially expressed proteins (DEPs) were identified, and upregulated proteins were selected for further analysis. To further validate the upregulated proteins, western blotting (WB) and immunohistochemistry (IHC) were performed. The WB analysis confirmed that the expression of brain derived neurotrophic factor (BDNF), G-protein regulated inducer of neurite outgrowth (GPRIN1), and alpha 7 nicotinic acetylcholine receptor (α7 nAChR) was significantly increased in the striatum of EE mice compared to control mice. Moreover, IHC further validated the increased expression of GPRIN1 in the SVZ of EE mice, corresponded with the increased number of neural stem/progenitor cells indicated by NESTIN and of migrating neuroblasts indicated by doublecortin(DCx). Surprisingly, GPRIN1 is colocalized with early neuronal markers (NESTIN and DCx) and α7 nAChRs but not with a mature neuronal marker (NeuN) in the SVZ. Likewise, in an in vitro study, the overexpression of GPRIN1 significantly increased the number of viable cells compared to the Naïve cell group and GFP infected cell group. In conclusion, the upregulation of GPRIN1 following the long-term exposure to EE is related to neuroblast activation especially in the SVZ of adult mouse brain, and the close interplay among BDNF, α7 nAChR, and GPRIN1 may be responsible for adult neurogenesis and the therapeutic mechanism of EE.

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F-1107

CHRONIC SENESCENCE IS INDUCED IN MESENCHYMAL STEM CELLS AFTER EXPOSURE TO THE CHEMICAL WARFARE AGENT SULFUR MUSTARD

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The banned chemical warfare agent sulfur mustard (SM) is still a threat to soldiers and civilians as revealed by verified and suspected incidents in the last years. SM induces the characteristic painful skin blisters, which lead to chronic wound healing disorder. We hypothesize that bone marrow derived mesenchymal stem cells (MSCs) might be involved in this process due to their essential role in wound healing. MSCs in chronic senescence persist over long time periods and display a senescence-associated secretory phenotype (SASP), which might be the underlying mechanism for the disrupted wound healing after SM exposure. MSCs were isolated from bone marrow of donor femoral heads and the efficiency was confirmed by cell surface markers via FACS. MSCs were exposed to single doses or continuously with SM and senescence-associated β-galactosidase (SA-β-gal) was stained with X-gal substrate. Expression levels of proteins were determined by Western blot. Senescence was observable one week after single dose (10 - 40 µM SM) and continuous exposure (0.5 - 1 µM SM). The percentage of senescent cells as well as the intensity of the staining increased until week 2. At this time, almost 100 % of all cells were senescent and this state was not reversible. In contrast, 1 µM SM single dose and 0.1 µM SM continuous exposure did not result in a stable senescent phenotype. Morphological changes like cell size increase as well as elevated expression of senescence markers such as p16INK4a were observed in senescent MSCs. In conclusion, SM exposed MSCs were positive for senescence markers such as SA-β-gal and p16INK4a expression. This demonstrates that single dose or continuous SM exposure resulted in senescence in MSCs in a concentration and time dependent manner. The SM-induced chronic senescence provides a new pathway in the pathomechanism of chronic wounds after SM exposure.

F-1109

POTENTIAL REGENERATIVE MECHANISMS OF SPERMATOGONIAL STEM CELL NICHE RECOVERY BY HUMAN MESENCHYMAL STEM/ STROMAL CELL CONDITIONED MEDIUM IN RAT ABDOMINAL CRYPTORCHIDISM MODEL

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Couple infertility is a severe medical problem worldwide, and the impact of male factor becomes substantial or even prevailing in some regions. In general, male infertility emanates from primary spermatogenic failure caused by conditions other than obstruction of male genital tract or hypothalamic-pituitary malfunction. Different components of spermatogonial stem cell (SSC) niche can be involved in the development of male infertility. The niche itself is supported by complex intercellular interactions including a plethora of paracrine signals. So far there is a lack of effective therapy for the majority of male infertility cases. Thus, the research and development of novel therapies to recover spermatogenesis is actual. Multipotent mesenchymal stem/stromal cells (MSC) conditioned medium (CM) could be a promising therapeutic tool as MSC secrete a wide spectrum of paracrine factors supporting proliferation, survival and differentiation of cells in different resident stem cell niches. Importantly, according to our previous studies MSC secrete molecules supporting functions and viability of SSC, Leydig and Sertoli cells. We developed the combined drug based on human MSC CM products and collagen as a protective depo for MSC secreted components. Rat abdominal cryptorchidism model of spermatogenesis failure was used. We determined that either combined biomaterial containing MSC CM or MSC cell therapy recovered injured SSC niche. Importantly, the total and moving spermatozoa fractions reflecting the reproductive potential had increased after the therapy. Using histologic and immunohistochemical analyses we assessed the involvement of several regenerative mechanisms such as increase of Sertoli and Leydig cells number, proliferation of SSC and angiogenesis in recovery process at different time points after the injury (1 and 3 months). By analyzing serum concentrations of androgens we revealed the indirect connection between Leydig cells' function and SSC niche recovery. According to our results the application of combined biomaterial based on human MSC CM and collagen gel can be promising, and it could be more preferable to cell therapy with MSC.

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F-1111

ROLE OF STEM CELLS AND MICRO-NICHES IN THE INITIATION AND PROGRESSION OF WOUND-INDUCED SQUAMOUS CELL CARCINOMA IN MOUSE SKIN

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Defining the unique populations of cells capable of initiating carcinoma is essential in designing new and efficacious cancer therapies. Here we used three different mouse cancer models to explore the cellular and molecular events associated with the initiation and progression of Squamous Cell Carcinoma (SCC). Based on the expression of specific markers, subpopulations

of stem (progenitor) cells have been assigned to different anatomical locations of the epidermis. Three of such markers; Lgr6, Lgr5, and Gli1 were used here to investigate how the stem cell microenvironment influences tissue regeneration and carcinogenesis. To that end, we induced mutations (KrasG12D and p53R172H) conditionally in unique stem cells, which can occupy different micro-niches (Lgr6+ cells: IFE, isthmus, and bulge, Gli1+ cells: isthmus and touch dome) also, different stem cells sharing the same micro-niche (Lgr5+ and Gli1+ cells: lower bulge, and Lgr6+ and Gli1+ cells: isthmus) in mouse skin using Cre-recombinase (CreERT2). We lineage traced the recombined cells by incorporating ROSA26 td-Tomato. Using flow cytometric analysis, we quantified the number and locations of recombined cells in each model. Mice expressing oncogene developed spontaneous tumors at several sites including the nose, lips, perianal, ventral, and dorsal skin two months after Tamoxifen treatment. Tumorigenesis was drastically enhanced in the dorsal skin when experimental wounds were made. Lineage tracing of healing wounds showed that all three stem cell compartments could contribute to wound healing in an equally efficient manner. To study how the niche influences the gene expression profile of early cancer-initiating cells, we performed whole mRNA sequencing, and the data demonstrated significant interactions between the niche and tumor-initiating cells. This study delineates the tumorigenic potential of three stem cells in mouse skin in the context of wounding.

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F-1113

SMALL MAF ACTS AS A REDOX SENSOR WHICH REGULATES GERMLINE STEM CELLS IN THE DROSOPHILA TESTIS

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Reactive oxygen species (ROS) are byproducts of normal cellular metabolic activities, and redox signaling has been shown to play important role in the maintenance of stem cell homeostasis. However, the downstream effectors of ROS signaling that govern stem cell behavior remain poorly characterized. Here, we utilized the Drosophila testis as an in vivo model to identify and characterize molecules involved in high ROS-mediated germline stem cell (GSC) differentiation. In Affymetrix microarray analysis, 152 genes were classified to be differentially expressed during high ROS-induced GSC differentiation. Remarkably, 31% of these genes had no predicted biological function, suggesting that our microarray assay identified numerous uncharacterized genes with a possible role in ROS-induced stem cell differentiation. As a follow-up investigation, we examined the possible role of Maf-S in GSC maintenance, whose expression was found to be downregulated in response to high ROS. We showed that Maf-s can regulate ROS levels in the Drosophila testis, in which inhibition of Maf-S significantly elevated intracellular ROS levels but overexpression of Maf-S reduced ROS levels. Notably, Maf-S inhibition resulted in a loss of GSC by promoting GSC differentiation, whereas overexpression of Maf-S caused a significant induction of GSC-like cells positive for the mitotic marker phospho-Histone H3. In addition, our study showed that Maf-S genetically interacts with Keap1/Nrf2 signaling in the Drosophila testis to maintain GSC homeostasis. Taken altogether, our study suggests that Maf-S plays a key role in the maintenance of GSC in the Drosophila testis by regulating redox signaling.

F-1115

DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO FEMALE GERM CELLS USING HUMAN CUMULUS CELLS CONDITIONED MEDIUM

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Human embryonic stem cells (hESCs) are pluripotent stem cells that can differentiate to three germ layers derivatives and germ cells. Because of ethical and technical problems, it is difficult to study early germ cell development in human. In vitro differentiation of pluripotent stem cells to germ cells is a proper way to help to infertile couples and as a model for scientific researches. Cumulus cells are nursing cells that support oocyte during development by secretion of some factors like growth factors. Conditioned medium from cumulus cells may contain this factors that can effect on differentiation of hESC to female germ cells. Embryoid bodies (EBs) from Yazd4 (female hESC line) were divided into 2 groups: a) spontaneously differentiation; and b) treated with human cumulus cells conditioned medium. EBs from both groups were cultured for 14

days. Pluripotency and germ cells genes expression were examined by Q-PCR at the time of days 0, 4, 7 and 14. Q-PCR results showed that in both groups NANOG expression decreased from day 0 to day 14. Unlike NANOG, VASA expression increased from day 0 to day 14. VASA expression in group a was more than group b. Unlike VASA, NANOG was expressed more in group b. Further works with more markers and techniques are in progress to be able to come out with a precise interpretation. So far, our data confirm the previously reported studies regarding the ability of hESCs to form germ cells in both spontaneously and cumulus cell conditioned medium cultures.

Funding Source: This work was supported by Yazd Reproductive Sciences Institute for a PhD project by Miss Somayyeh Sadat Tahajjodi.

NEURAL DEVELOPMENT AND REGENERATION

F-2001

MAKING DO AND MAKING NEW: HOW THE ZEBRAFISH RAPIDLY REGENERATES FOLLOWING CENTRAL NERVOUS SYSTEM INJURY

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The zebrafish is a remarkable vertebrate due its spontaneous and successful regeneration following traumatic central nervous system injury (CNS). The cellular and molecular mechanisms regulating this regeneration are largely unknown. We have used in vivo imaging and behavioral approaches in a zebrafish spinal cord injury model to pin-point specific mechanisms and signals that control successful regeneration. Surprisingly, we identified two temporally and mechanistically distinct waves of cellular regeneration in the spinal cord. The initial wave of regeneration relies on cellular migration of pre-existing neuronal progenitors to the lesion site that enables rapid functional recovery. The second wave of regeneration involves activation of quiescent neural stem cells and regenerative neurogenesis. Remarkably, the cell production compensates for lost tissue at injury site as well as the cells depleted from proximal areas due to migration. While these mechanistic waves of regeneration are temporally distinct, we have found that they share strong spatial overlap. Indicating that neurogenic programs are controlled by molecular and mechanical signals. The two waves of regeneration demonstrates how zebrafish are able to rapidly regain motor function within days after severe injury and also replenish lost tissue complexity over time. Furthermore, we have identified molecules and pathways that enhance neural regeneration in the spinal cord. These molecules act to alert the system of tissue injury and lead to the appropriate activation of downstream regenerative mechanisms. Activation of such pathways in mammals may be relevant for future therapies since the key cell types enabling neural and axonal regeneration in zebrafish have direct counterparts in mammals.

F-2003

COMPREHENSIVE PROTEOMIC CHARACTERISATION OF HUMAN OF AIDPOSE STEM CELLS NEURAL DIFFERENTIATION

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The central nervous system is comprised of billions of neurons and it is difficult to promote regeneration of these in vivo. It is therefore not surprising that there is significant interest in utilisation of stem cells as neuronal regenerative therapies. However, there is a lack of evidence supporting the use of human adipose stem cells as treatments for neurodegenerative diseases and neurological disorders. Despite this, patients are still seeking these high-risk treatments. It is therefore vital that these human adipose stem cells, and their ability to undergo neural differentiation are thoroughly investigated. This project characterises cellular and secreted proteins of human adipose stem cells undergoing neural differentiation to allow the characterisation of the cells phenotype. Functional complexity of these cells were highlighted through the proteome as it differs both spatially and temporally. This was achieved through the development of a comprehensive spectral library that was utilised for data independent acquisition mass spectrometry with a Q Exactive™ Plus Orbitrap Mass Spectrometer. The resulting data set provides unique insight into the neural differentiation capabilities of human adipose stem cells and also investigated proteins present in extracellular vesicles. Furthermore, 27 secreted cytokines were also analysed using a Multiplex Immunoassay and provide unique insight as cytokines are utilised for cellular communication and are known to contribute to brain functions such as synaptic plasticity. This research into the plasticity of human adipose stem cells, and specifically their ability to undergo neural differentiation, provides a comprehensive library of proteins. This will assist in providing knowledge on the neural capabilities of human adipose stem cells and assist in shaping the way future neural regenerative therapies are approached.

F-2005

PRECISE NANOINJECTION DELIVERY OF PLASMID DNA INTO A SINGLE FIBROBLAST FOR DIRECT CONVERSION OF ASTROCYTE

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Direct conversion is a powerful approach to safely generate mature neural lineages with potential for treatment of neurological disorders. Astrocytes play a crucial role in neuronal homeostasis and their dysfunctions contribute to several neurodegenerative diseases. Using a single-cell approach for precision, we describe here a robust method using optimized DNA amounts for the direct conversion of mouse fibroblasts to astrocytes. Controlled amount of the reprogramming factors Oct4, Sox2, Klf4, and cMyc was directly delivered into a single fibroblast cell. Consequently, 2,500 DNA molecules, no more or less, was found to be the optimal amount that dramatically increased the expression levels of the astrocyte-specific markers GFAP and S100b and the demethylation gene TET1, the expression of which was sustained to maintain astrocyte functionality. The converted astrocytes showed glutamate uptake ability and electrophysiological activity. Further, we demonstrated a potential mechanism whereby fibroblast was directly converted into astrocyte at a single-cell level; this was achieved by activating BMP2 pathway through direct binding of Sox2 protein to BMP2 gene. This study suggests that nanotechnology for directly injecting plasmid DNAs into cell nuclei may help understand such a conversion at single-cell level.

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F-2007

NEUROGENIC DIFFERENTIATION BY HIPPOCAMPAL NEURAL STEM AND PROGENITOR CELLS IS BIASED BY NFIX EXPRESSION

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Our understanding of the transcriptional program underpinning adult hippocampal neurogenesis is incomplete. In mice, under normal physiological conditions, adult hippocampal neural stem cells (AH-NSCs) generate neurons and astrocytes, but not oligodendrocytes. The factors limiting oligodendrocyte production, however, remain unclear. Here, we reveal that the transcription factor NFIX plays a key role in this process. NFIX is expressed by AH-NSCs, and its expression is sharply upregulated in adult hippocampal neuroblasts. Conditional ablation of *Nfix* from AH-NSCs, coupled with lineage tracing, transcriptomic sequencing and behavioural studies collectively reveal that NFIX is cell autonomously required for neuroblast maturation and survival. Moreover, a small number of AH-NSCs also develop into oligodendrocytes following Nfix deletion. Remarkably, when *Nfix* is deleted specifically from intermediate progenitor cells and neuroblasts using a Dcx creER^{T2} driver, these cells also display elevated signatures of oligodendrocyte gene expression. Together, these results demonstrate the central role played by NFIX in neuroblasts within the adult hippocampal stem cell neurogenic niche, with this transcription factor promoting the maturation and survival of these cells, while concurrently repressing oligodendrocyte gene expression patterns in these neuronally lineage-restricted cells.

F-2009

SINGLE-CELL RNA-SEQ SURVEYS A DEVELOPMENTAL LANDSCAPE OF THE HUMAN PREFRONTAL CORTEX

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The mammalian prefrontal cortex (PFC) constitutes a corporation of highly specialized brain areas with billions of cells, serves as center of the highest-order cognitive functions, such as memory, cognitive ability, decision making, social behavior, etc.. Although neural circuits are built in the late stage of human embryo development and even after birth, the diverse classes of functional cells begin to generate and migrate to the appropriate location to play essential roles earlier. In addition, accumulating data indicate that the dysfunction of PFC contributes to the cognitive deficits or most of neurodevelopmental disorders; thus, detailed knowledge of the PFC development is required. However, identifying cell types of the developing human PFC and distinguishing their developmental features are still lacking. Here, to address these challenges, more than 2,300 single cells from the developing human PFC, ranging from gestational weeks 8-26, were analyzed via RNA-seq. We identified 35 subtypes of cells within 6 main classes and depicted the developmental trajectory of these cells. Detailed analysis of neural progenitor cells (NPCs) illustrated new marker genes and unique

developmental features of intermediate progenitor cells. We also mapped the neurogenesis timeline of PFC excitatory neurons and identified the presence of interneuron progenitors in early developing PFC. Moreover, we revealed the development-dependent intrinsic signals regulating neuron generation and circuit formation using single-cell transcriptomic data analysis. Our screening and characterization approach provides a blueprint for understanding human PFC development in the early and mid-gestational stages with which to systematically dissect the cellular basis and molecular regulation of PFC function in humans.

F-2011

ENRICHMENT OF MOUSE SUBCEREBRAL PROJECTION NEURONS USING L1CAM

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Cell-based therapy is a promising approach to treat neurodegenerative disease. Similarly, reconstruction of corticospinal tract by cell transplantation is expected as a treatment for stroke and brain injuries. In mouse studies, fetal cortices can survive and project their fibers into host brain by point-to-point manner. However, donor cells inevitably contain inappropriate cells. To eliminate the unwanted cells, we focus on purifying subcerebral projection neurons from mouse cortices. When we transplanted mouse cortices, only CTIP2-positive cells could project their fibers into host spinal cord. For this reason, we generated CTIP2 knock-in mouse embryonic stem cells (mESCs), and we induced neurons by the addition of wnt inhibitor and transforming growth factor beta inhibitor. To identify a cell surface marker of subcerebral projection neurons, we compared gene expression profiles between CTIP2:GFP+ cells vs. CTIP2:GFP- cells. We identified L1cam as a marker for CTIP2+ cells. When we performed cell sorting of fetal mouse cortices based on L1CAM expression, CTIP2+ cells were more frequently observed in positive population compared with unsorted and negative populations (75.8% vs. 36.4% vs. 13.9%). Moreover, mRNA levels of proliferative cells such as Pax6+ and Tbr2 was lower in L1CAM+ cells than L1CAM- the one. In support of these results, L1CAM+ graft volume was smaller than L1CAM- the one, but the L1CAM+ fibers were more frequently observed through the corticospinal tract. In conclusion, a cell surface marker for subcerebral projection neurons, L1CAM, can provide a tool for regeneration of cortical circuits.

F-2013

EFFECT OF HYPOXIA ON HUMAN FETAL NEURAL STEM CELLS: AN IN VITRO DISEASE MODEL TO ELUCIDATE THE PATHOGENESIS OF CEREBRAL PALSY

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Cerebral Palsy (CP) is a neurological disorder that impairs motor function in young children. Cerebral ischemia and maternal intrauterine infections in preterm fetuses result in CP. Premature oligodendrocytes (OL) are vulnerable to perinatal hypoxic injury. We aim to develop an *in vitro* model of CP by inducing Fetal Neural Stem Cells (FNSCs) to differentiate into oligodendrocytes (OL), followed by exposing them to hypoxia. With permission from the Institute Ethics Committee and informed consent from the mothers undergoing medical termination of pregnancy for maternal indications, aborted fetuses (n=3) were collected under aseptic conditions from the Department of Obstetrics and Gynaecology, AIIMS, New Delhi, India. FNSCs were isolated from the sub-ventricular zone of the fetal brain and expanded in culture. The isolated FNSCs were characterized using immunocytochemistry (ICC) and flow cytometry. FNSCs were exposed to normoxia (20% oxygen) and hypoxia (0.2% oxygen) for 48 hours, and exposure to hypoxia was validated by a panel of markers (CA9, VEGF and PGK1) using qPCR. The effect of hypoxia on cell death, in FNSCs, was analysed using Annexin V assay. Microarray was done using Agilent whole genome 4x44K array slides to study transcriptomic changes between FNSCs exposed to normoxia and hypoxia. Data was analysed using Flow Jo, Gene Spring GX13 and GeneGO MetaCore. FNSCs were differentiated into OL using specific inducers and OL specific markers were evaluated by ICC and qPCR. Nestin and Sox2 were found to be expressed by FNSCs by ICC and flowcytometry. The expression of CA9, VEGF and PGK1 (hypoxia markers) were found to be up regulated by qPCR, in FNSCs that were exposed to hypoxia, compared to FNSCs exposed to normoxia. The FNSCs that differentiated into OL showed positive

expression for OL specific Myelinating Oligodendrocyte Glycoprotein (MOG) by ICC and NG-2 by qPCR. Gene expression analysis revealed novel genes which were expressed in FNSCs exposed to hypoxia. Our findings indicate that hypoxia does not cause cell death in FNSCs, however, transcriptomic changes do occur, and that some interesting pathways are modulated under hypoxic conditions. We are currently validating these pathways and exploring them further and aim to establish a cell culture based model of CP using the differentiated OL from FNCSs.

Funding Source: Department of Biotechnology (DBT), Govt. of India

F-2015

ANTEROPOSTERIOR GRADIENT OF WNT AND RETINOIC ACID-INDUCED INTERCELLULAR ADHESION PROPERTIES VIA ECM INDUCTION IN DEVELOPING MOUSE SPINAL CORD

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During the development, mammalian embryos exhibit a transition from head morphogenesis to trunk elongation to meet the demand of axial elongation. Caudal neural tube (NT) is formed with neural stem cells (NSCs) derived from neuromesodermal progenitors (NMPs) localized at the tail tip. However, molecular and cellular basis of elongating NT morphogenesis is poorly understood. Here, we discovered that cell-cell adhesion properties of NSCs are strongest in the caudal NT and decrease gradually along the anteroposterior (AP) axis in mouse embryo and human cellular models. Strong cell-cell adhesion of caudal NCSs causes collective migration, allowing AP alignment of NSCs depending on their birthdate. We further validated that this gradient of cell adhesion affinity is established in response to graded Wnt signaling emanating from tail-buds, and antagonistic retinoic acid (RA) signaling. Together, these results suggest that progressive reduction of NSCs' adhesion affinity along the AP axis establishes cellular basis of NT development.

F-2017

NEURONAL DIFFERENTIATION OF NEUROSPHERES-DERIVED HUMAN DENTAL PULP STEM CELLS FOLLOWING CO-CULTURE WITH CHICKEN AUDITORY BRAINSTEM SLICES

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The organotypic co-culture system as a useful model to study the regeneration of neurons for cell-based therapy. Here we present the neuronal differentiation ability of neurospheres-derived human dental pulp stem cells (hDPSCs) following co-culture with chicken auditory brainstem slices (E12). The neurospheres-derived hDPSCs can survive after co-cultured with chicken auditory brainstem slices for 2 weeks. Immunohistochemistry analysis revealed the differentiation of neurospheres into neuronal cells. Interestingly, they were highly expressed βIII-tubulin (Tuj1), a mature neuronal marker, since 1st - 2nd weeks of the co-cultured period. The positive staining of Tuj1 was detected in neurosphere-derived differentiated cells as a fiber extension near the chicken auditory brainstem slices. The overall results demonstrated that the co-cultured neurospheres-derived hDPSCs differentiate into neuronal cells. Moreover, they are capable of integrating into host central nervous system circuitry.

Funding Source: Science Achievement Scholarship of Thailand, SAST

F-2019

BASIC CHARACTERISTICS OF INJURY-INDUCED MULTIPOTENT STEM CELLS IN HUMAN BRAINS AND FUTURE PROSPECTS

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We previously demonstrated that injury/ischemia-induced stem cells developed within the post-stroke area in a mouse model of cerebral infarction. Because they had the characteristics as neural progenitors, we initially named them injury/ischemia-induced neural stem/progenitor cells (iNSPCs). However, subsequent studies showed that these stem cells could differentiate

into various cells other than neural lineage. Thus, we now call them injury-induced multipotent stem cells (iSCs). We also demonstrated that brain microvascular pericytes acquired the multipotency following cerebral ischemia in mice and that they could function as iSCs. To examine the traits of human iSCs, brain samples were obtained from post-stroke areas in patients requiring both decompressive craniectomy and partial lobectomy for diffuse cerebral infarction (The Ethics Committee of our college reviewed and approved this study). After several passages through needles, brain samples were subjected to cell culture by adherent conditions. Polymerase chain reaction analysis revealed that the putative iSCs did not express astrocytic and endothelial lineage markers. However, they expressed pericytic and neural crest lineage markers, suggesting that human iSCs likely originate from brain pericytes, consistent with mouse iSCs. By immunohistochemistry, it was found that iSCs could differentiate into neural and mesenchymal lineages. These results indicated that iSCs are cell populations sharing the characteristics of neural stem cells and mesenchymal stem cells. Recent analysis by ours also demonstrated human iSCs have potential to differentiate into functional neurons with electrical activities. Because they can differentiate into various cells including neurons, iSCs have the potential to repair the damaged CNS following ischemic stroke. For clinical application by iSCs, we now propose two strategies. The first approach is the way to transplant the cultured-autogenous iSCs in stroke patients during subacute-chronic phase. The second option is the one to administer bioactive molecules which can contribute to survival, proliferation, migration, and neuronal differentiation into endogenous iSCs. Thus, iSCs may be a promising cell source for patients suffering from cerebral infarction.

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F-2021

TRANSPLANTATION OF SENSORY NEURAL PROGENITORS DERIVED FROM FRIEDREICH ATAXIA INDUCED PLURIPOTENT STEM CELLS INTO ADULT DORSAL ROOT GANGLIA

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Friedreich ataxia (FRDA) is a disease characterised by neurodegeneration and cardiomyopathy. FRDA is due to insufficiency of the mitochondrial protein, FRATAXIN (FXN) which leads to mitochondrial dysfunction, cell toxicity and cell death, particularly within the nervous system and cardiac tissue. The peripheral sensory nervous system is one of the primary and most significant sites of degeneration occurring in FRDA. Cell replacement therapies still remain an 'ideal' approach for a cure, in terms of replacing mutated FXN cells. These studies show 'proof of concept' the capacity of donor FRDA iPS-derived neural progenitors to differentiate, survive and integrate into the adult nervous system. Recently, we have developed an efficient system to differentiate iPS cells to peripheral sensory neurons of the dorsal root ganglia (DRG). We have shown that FRDA iPS cells are able to differentiate to sensory neurons with similar efficiency to control stem cells. In this study, we show a method to transplant FRDA and control iPS-derived sensory DRG neural progenitors directly into the DRG regions. Using a combination of immunohistochemistry and patch clamp analyses, we evaluated the capacity of these cells to survive, and functionally integrate into the DRG of adult rodents. Our data shows survival and integration of human iPSC-derived neural progenitors into DRG region of adult rats following transplantation. The donor cells show expression of sensory neuronal markers, demonstrating their capacity to differentiate to sensory neuronal lineages in vivo. These studies will address the possibility of developing cell replacement therapies for treating neurodegeneration occurring within the peripheral sensory nervous system in FRDA.

Funding Source: Stem Cells Australia, Friedreich's Ataxia Research Alliance USA and Friedreich Ataxia Research Association Australasia, Melbourne International Research Scholarship, Melbourne International Fee Remission Scholarship

F-2023

THE NEUROGENIC POTENTIAL OF NEOCORTICAL ASTROCYTES IN RESPONSE TO STAB-WOUND INJURY

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Adult neural stem cells may be useful for replacing neurons lost to injury. Their contribution to self-repair, however, is limited by their restricted population size. Parenchymal astrocytes could present an alternative, more abundant source of latent neurogenic cells, whose potential has recently been demonstrated in the mouse striatum after an ischemic lesion. Notch signalling depletion triggers neurogenesis by striatal astrocytes, but has proven insufficient for initiating a neurogenic program in most other regions of the adult mouse brain, such as the somatosensory cortex. This prompts questions around the extent of the intrinsic neurogenic potential of astrocytes throughout the brain and the experimental manipulations that are necessary to uncover it. Our latest investigations have revealed that a small portion of astrocytes in the somatosensory cortex can undertake a neurogenic program and generate neuroblasts, when subjected to Notch signalling depletion and stab-wound injury. We are currently carrying out single cell RNA sequencing experiments, which will help us better characterise molecular dynamics and cell state checkpoints that describe lineage fate modifications as cortical astrocytes transition to neural progenitors. Ultimately, we aim to unveil molecular mechanisms that promote astrocyte-mediated regeneration across the damaged adult brain.

F-2025

COAXING OF HUMAN MESENCHYMAL STEM CELLS INTO DOPAMINERGIC NEURONAL CELLS IN VITRO: A POTENTIAL THERAPEUTIC APPROACH FOR MEDICAMENT OF PARKINSONISM

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Parkinson's disease (PD) is a neurodegenerative movement disorder, characterized by a loss of midbrain dopaminergic neurons. Pharmacological management of the disease with L-DOPA is helpful only at initial stages; hence, it further requires the use of cell based treatment to manage the disease in a better and efficient manner. Considering this point, several stem cell researchers have explored the neurogenic potential of Mesenchymal Stem Cells (MSCs) and their effects in improving the behavioural functions in PD rat model. MSCs from various tissue sources like bone marrow, adipose tissue, dental pulp, Wharton's jelly, etc. have been explored for their neurogenic potential under both in vitro and in vivo conditions. However, bone marrow derived Mesenchymal Stem Cells have been highly explored stem cell candidates in this regard. Hence, the current study was planned to explore a) The functional behaviour of differentiated BM-MSCs using FGF2 and FGF2+22-hydroxycholesterol (HC), and b) The difference in the neurogenic potential of BM-MSCs in naive (N=03) and differentiated state (N=03 for each group) up on transplantation in Parkinson's disease Wistar rat model. PD rat model was prepared by creating unilateral lesion by injecting 6-OHDA stereotaxically in the substantia nigra pas compacta region of the midbrain. Established PD rat models, after their behavioural assessment (Rotarod and beam tests), were considered for further studies. Post characterization of the differentiated BM-MSCs, they were transplanted in the brain at the same coordinates as the lesion. Neuroregenerative effect of the transplanted BM-MSCs was assessed 4 weeks after transplantation on the basis of behavioural changes, H & E staining and immunohistochemistry analysis for the expression of MAP2 and TH proteins. To ensure the homing of the transplanted cells, FISH was performed with human X- probes. Transplanted cells showed no inflammatory symptoms in the rats, also confirmed by H & E staining. It was observed that BM-MSCs which were pre- primed into cells of dopaminergic neuronal lineage had better neuroregenerative effect after transplantation into PD rat models. With the current study, it may also be concluded that BM-MSCs primed with FGF2+22-HC had better neuroregenerative potential as compared to those primed with FGF2 alone.

Funding Source: Departmental funding from Department of C.T.V.S., All India institute of Medical Sciences, New Delhi

NEURAL DISEASE AND DEGENERATION

F-2027

FUNCTIONAL PHENOTYPIC SCREENING OF PATIENT IPSC-DERIVED MOTOR NEURONS - IN VITRO HTS DISEASE MODELING WITH MICRO ELECTRODE ARRAYS COUPLED WITH AI-BASED ANALYSIS METHODS

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One of the major challenges of drug discovery is decreasing attrition rates which requires developing more predictive pre-clinical in vitro models. Human induced pluripotent stem cell-derived (hiPSC) neuronal cultures promise higher physiological relevance and thus, better translation to the in vivo situation. Patient-derived iPSC models have been designed for various indications. We focused on investigating motor neuron diseases (MND) such as Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), which cause the loss of motor neurons. Our aims were to phenotypically describe the consequence of the genetic variation present in ALS and SMA patient iPSC-derived motor neurons on the functional activity and network connectivity. We further elucidated how functional ALS and SMA phenotypes separated from controls during network establishment to enabled compound testing to rescue the disease phenotypes. We cultured patient iPSC-derived motor neurons and controls on multiwell micro-electrode arrays (MEA) for multiple weeks to analyze their functional network activity patterns by multi-parametric analysis. Our results showed reproducible spontaneously active motor neuron networks with synchronized activity on the majority of the electrodes. We identified disease-specific functional phenotypes and showed how reference compounds can affect them. In conclusion, we show that hiPSC-derived motor neurons are able to produce meaningful functional in vitro phenotypes and that these phenotypes can be associated with known motor neuron diseases. By using artificial intelligence-based multivariate MEA data analyses combined with reproducible physiologically relevant iPSC neuron models we provide a functional phenotypic assay platform for high throughput compound screening.

F-2029

APPLICATION OF MODIFIED MICRORIBBON HYDROGELS IN SPINAL CORD INJURY FOR DELIVERY AND RETENTION OF HUMAN STEM CELL DERIVED NEURAL CELLS AND INJURY SITE MODULATION

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Accelerating treatments in spinal cord injuries (SCI) requires advancing methods that help bring uniformity and reproducibility to comparative analysis and optimization. Towards this goal we are designing biocompatible hydrogel platforms with dual roles in the retention of healthy transplanted cells as well as in modulation of the microenvironment by chondroitinase to block inhibitory signals. This dual spinal treatment enhancing platform (2STEP) approach will deliver cells and promote regenerative repair pathways to reestablish neural connectivity. The overarching goal is to promote a generalizable favored strategy that will more coherently bring together exciting research in the SCI field by many laboratories to create synergistic rapid advances. This work is part of a larger study to address cellular regeneration and repair of SCI by combining stem cell modulation and nanotechnology methodologies in a hemicontusion and behavioral animal model of SCI. The current work presents findings with human neural stem cells, spinal motor neurons and oligodendrocytes in hydrogels towards this goal and brings together expertise in pluripotent and neural human stem cell biology, nanotechnology and materials engineering, coupled with additional relevant expertise in the neuronal microtubule cytoskeleton, ECM and electrophysiology.

Funding Source: NY State Spinal Cord Injury Review Board (NYSCIRB) funded project

F-2031

VERIFICATION OF AUTOMATED CELL CULTURE PROCESS OF IPS CELLS FOR PARKINSON'S DISEASE TREATMENT

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Cost reduction of cell production and maintenance of sterility are the main challenges to the spread of regenerative medicine. An automated closed cell culture system is considered to be a platform that can be used to address these two challenges. We have been developing an automated closed cell culture equipment for iPS cells that can highly maintain sterility. In this study, we have developed a new prototype (iACE1) in order to automate the process of amplification for undifferentiated iPS cells and induced differentiation to dopaminergic progenitor cells, which are prepared for treatment of Parkinson's disease. We successfully performed the amplification and differentiation steps automatically. Proliferation rate under automated condition was equal to or more than that under manual condition. Expression of undifferentiation marker NANOG and OCT4 were maintained after amplification step by the equipment equally to those cultured manually. Floor plate marker CORIN positive rate under the automated culture was more than 30% as with the manual culture. These results show that amplification of iPS cells and differentiation to dopaminergic progenitor cells can be proceeded by the automated closed culture equipment with equal quality and efficiency to manual culture. We will make the process robust so as to produce higher quality cells more efficiently in the future.

Funding Source: This research is supported by the "Project focused on developing key evaluation technology: Evaluation for industrialization in the field of regenerative medicine" from Japan Agency for Medical Research and development, AMED.

F-2033

KNOCKING OUT TERT IN HUMAN PLURIPOTENT STEM CELLS AND NEURAL DERIVATIVES TO MODEL NEURONAL AGING

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The differentiation of pluripotent stem cells (PSCs) to neuronal lineages can simulate disease modeling and investigate the pathogenesis of neurodegenerative disorders where suitable animal models are lacking. However, protocols often produce a fetal-like state of cells rather than the fully mature state, in which most adult diseases inflict. Telomere shortening is typically established as a hallmark of aging. In this study, we abolished telomerase reverse transcriptase (TERT) activity using CRISPR/Cas9-mediated knock-out of the hTERT gene. The TERT knock-out (TERT-KO) iPSCs were then differentiated into spinal motor neurons to investigate the acceleration of age-related phenotypes such as those present in Amyotrophic Lateral Sclerosis (ALS) motor neurons. Our preliminary data shows increased ER stress in TERT-KO motor neurons compared to their isogenic controls. From this project, we expect to recapitulate age-related disease onset of this neurodegenerative diseases and elucidate the mechanisms involved. The findings from this research project will have important implications for in vitro disease modeling of age-related neurodegenerative diseases in the future.

F-2035

UIPSC STUDIES OF AN AUTISTIC CHILD WITH PHOTOGRAPHIC MEMORY

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Autism spectrum disorders (ASDs) are a heterogeneous group of complex neurodevelopmental disorders that have some core symptoms, including social interaction impairment, communication deficits, restricted interest, and repetitive behaviors. Here we generated urinary iPSCs (UiPSCs) from an autistic child with photographic memory and an unrelated healthy control. Exfoliated renal epithelial cells presenting in the children's urine samples were reprogrammed with the human OSKM transcription factors using the Sendai-virus (SeV) deliver system. Exome sequencing studies of the sporadic autism case have identified multiple autism risk genes reported in the previous researches, including TBR1, MECP2, SCN2A, NRXN1 and CHD8, duplicating in the copy number variations (CNVs). Neurodevelopmental disorders are often characterized by cellular defects apparent at early stages in life. Especially ASDs are early-onset neurodevelopmental disorders. In the maturation process of the UiPSCs-derived neurons, we found TBR1 protein expression in the autistic UiPSCs-derived neurons was different with the controls. However, there were no significant difference between the basic electrophysiological properties of the autistic UiPSCs-derived neurons and control ones at the neuron maturity phase. Our study suggests that disrupted TBR1 expression might play the roles in the maturation process of the autistic UiPSCs-derived neurons.

F-2037

ENTERIC NEUROPATHY ASSOCIATED WITH INTESTINAL INFLAMMATION: EFFECTS AND POTENTIAL MECHANISMS OF MESENCHYMAL STEM CELL THERAPY

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Damage to the enteric nervous system caused by inflammation underlies long-term intestinal dysfunction in Inflammatory Bowel Disease (IBD) patients. The Winnie mouse model of spontaneous chronic colitis resulting from a mutation in the Muc2 gene is one of the best available models of IBD. The aim of this study was to test the therapeutic potential of human bone marrow-derived mesenchymal stem cells (MSCs) to alleviate enteric neuropathy and intestinal dysfunction associated with chronic intestinal inflammation. Winnie mice receiving multiple MSC treatments demonstrated a significant reduction in disease activity, including reduced diarrhoea, weight loss, colonic hypertrophy, rectal bleeding and prolapse. Long-term inhibition of colitis was confirmed by a non-invasive biomarker for intestinal inflammation. Treatment with MSCs restored changes in gastrointestinal motility in chronic colitis. This was accompanied by decreased infiltration of leukocytes to the level of myenteric ganglia, damage to neuronal processes projecting to the mucosa and loss of myenteric neurons observed in the inflamed colon. The role of oxidative stress in myenteric neuropathy was investigated in ex vivo and in vivo experiments. In ex vivo organotypic cultures of myenteric neurons, oxidative stress induced cytoplasmic translocation of redox sensitive high mobility box group 1 (HMGB1) which correlated with neuronal loss. HMGB1 translocation and neuropathy were prevented by MSCs in a paracrine manner suggesting that the MSC secretome can directly protect myenteric neurons. In the Winnie model of chronic colitis, high levels of mitochondria-derived superoxide and HMGB1 translocation were observed in myenteric neurons which correlated well with neuronal loss in vivo; these were all attenuated by MSC therapy. Treatment with the HMGB1 inhibitor, Glycyrrhizin, partially replicated the effects of MSCs in mice with chronic colitis by reducing HMGB1 translocation, neuropathy and diarrhoea. We have identified numerous neuroprotective factors released by MSCs. Our studies are the first to demonstrate the therapeutic potential of MSCs in spontaneous chronic colitis. MSC therapy alleviates intestinal dysfunction and enteric neuropathy through mechanisms that involve the inhibition of oxidative stress and HMGB1 translocation.

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F-2039

FUNCTIONAL ASSAY USING MICROELECTRODE ARRAY SYSTEM FOR NERURONS DERIVED FROM IPSCS 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. 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. Patient-derived induced pluripotent stem cells (iPSCs) promise unique insight into the true pathophysiology of DS. Recently, we generated iPSCs (D1 iPSCs) derived from a DS patient with a c.4933C>T substitution in SCN1A predicted to cause truncation in the fourth homologous domain of the protein (p.R1645*). In this study, 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. We succeeded in the generation of such iPSCs by either correcting the mutation in the patient-derived iPSCs or introducing the mutation into iPSCs derived from a healthy individual. Excitatory/inhibitory (E/I) imbalance in the cerebral cortex can cause central nervous system disorders, such as DS (or epilepsy). In this poster, we present data comparing excitatory or inhibitory neurons derived from healthy (WT), DS (D1), and isogenic control (D1 TALEN) iPSCs that were measured using multi-electrode arrays

(MEA). We generated excitatory or inhibitory neurons by employing direct in vitro conversion of iPSCs through the overexpression of neuronal lineage markers as a novel approach for neuronal differentiation. 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-2041

NEURAL CREST DERIVED MESENCHYMAL STEM CELLS: A NOVEL CELLULAR THERAPY FOR NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY

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Hypoxic-ischemic encephalopathy (HIE) is one of the most serious birth complications happening in neonatal and infants. MSC-based therapy has been emerging as a promising strategy in the treatment of HIE in recent years. However, the application of MSCs derived from mesenchymal tissue sources such as bone marrow, umbilical cord and adipose tissue etc. are limited by several scientific hurdles, such as poor homing ability, inefficient neuronal differentiation and poor survival in vivo and insufficient number of transplantable cells. Neural Crest Stem Cells (NCSCs) are multipotent stem cells with trans-differentiation abilities, i.e. they can give rise to mesenchymal tissue, despite their ectodermal origin. In this study, we exploit the hNCSC-derived MSCs (hNCSC-MSCs) in the neonatal HIE rats. Our results showed that hNCSC-MSCs exhibited a much enhanced anti-inflammatory effects compared to human umbilical cord-derived MSCs (hUC-MSCs). The expression levels of glial markers such as GFAP and Vimentin, as well as the microglial marker IBA-1 were dramatically decreased in hNCSC-MSC-treated animals compared to PBS- or hUC-MSC-treated animals. In addition, hNCSC-MSCs promoted endogenous neurogenesis more than hUC-MSCs did, as the expression levels of Nestin, Sox-2 and NeuN were dramatically increased in the hNCSC-MSC-treated brains 10 days after treatment. More importantly, hNCSC-MSCs had a better function on alleviating the brain damage as demonstrated by much decreased apoptosis and reduced lesion size compared to hUC-MSCs. At molecular level, hNCSC-MSCs clustered more closely with hUC-MSCs than hBM-MSCs, indicating that hNCSC-MSCs represent a more primitive stage compared to hBM-MSCs. Gene ontology analysis revealed that genes associated with neurogenesis and immune modulation were differentially expressed between hNCSC-MSCs and hUC-MSCs. These data suggest that hNCSC-MSCs may function as a modulator of the neurogenic niche via its neuroprotective and neuro-regenerative effects, thus ideal for HIE treatment.

F-2043

DISEASE-IN-A-DISH: DRUG DISCOVERY USING PATIENT-DERIVED STEM CELLS IN HEREDITARY SPASTIC PARAPLEGIA

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Hereditary spastic paraplegia (HSP) is a neurological disorder characterised by degeneration of corticospinal tract axons leading to lower limb weakness and spasticity. We identified cell functions altered in SPAST HSP patient-derived olfactory-neurosphere derived (ONS) cells and induced pluripotent stem (iPS) cells differentiated into cortical neurons. SPAST encodes spastin, which regulates microtubule dynamics. Patient-derived ONS cells and neurons have reduced spastin, reduced acetylated α-tubulin (a marker for stabilised microtubules), altered cellular distribution of mitochondria and peroxisomes, and slower peroxisome transport speeds. Patient ONS cells were more sensitive to oxidative stress induced by hydrogen peroxide, which is normally metabolised by peroxisomal catalase. We screened ONS cells and identified low doses of tubulin-binding drugs that restored acetylated α-tubulin levels to control levels, restored peroxisome transport to control speeds and reduced the oxidative effects of hydrogen peroxide treatment. We generated eleven iPS cell lines from fibroblasts of three SPAST HSP patients and three healthy controls and differentiated them into cortical neurons. Patient-derived axons had characteristic swellings, similar to corticospinal tract pathology in patients, with peroxisome transport deficits similar to patient-derived ONS cells, including reduced retrograde transport. Patient axons were more sensitive to hydrogen peroxide-induced degeneration than control-derived axons. The tubulin-binding drugs restored peroxisome transport speeds and reduced axonal degeneration in patient axons. By reducing stable microtubules SPAST mutations impair peroxisome trafficking and turnover thereby increasing oxidative stress, axon degeneration

and cell death. Tubulin-binding drugs rescue these disease-specific defects. Our research demonstrates the potential of multiple sources of patient-derived cells for disease modelling and drug discovery for brain diseases.

Funding Source: Hereditary Spastic Paraplegia Research Foundation Incorporated and the Spastic Paraplegia Foundation Incorporated

F-2045

TRACKING INP/ZNS QUANTUM DOTS LABELED OLFACTORY ENSHEATHING CELLS IN SPINAL CORD INJURY RAT MODEL

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Olfactory ensheathing cells (OECs) transplantation has provided a huge therapeutic potential for the treatment of spinal cord injury (SCI). However, the fate of cells in vivo remains unclear. In order to evaluate the migration of transplanted cells in SCI rat model by intravenously injection via the tail vein, OECs were labeled with InP/ZnS quantum dots (QDs) and then injected at 24 hours after the establishment of hemisection SCI model. Rats were sacrificed after injection, respectively, at 10 min, 1 hour, 24 hours, 3 days, 7 days, 14 days and 28 days. The spinal cords were quickly isolated and then imaged under fluorescent imaging (FLI) system. The data showed that the peak signals presented in 7 days after cell-infusion, related to the accumulation of QDs labeled OECs around the injury site. At 28 days after injection, the fluorescence signal was still observed in the spinal cord but with a reduced intensity. The uninjured groups were employed for a comparison, the fluorescence signal was as low as background level regardless of the time point investigated. These results indicated that intravenously injected OECs could reach the parenchyma of spinal cord by penetrating from the lesion site.

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CANCERS

F-2049

DEFINING CELLS-OF-ORIGIN IN MELANOCYTIC TRANSFORMATION IN MOUSE

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Melanocyte stem cells are amelanotic and thought to regenerate functional melanotic melanocytes in a hierarchical manner. In human or mouse, the hair follicle bulge is a major stem cell niche for melanocytes in hairy skin, while the secretory portion of the eccrine sweat gland is proposed to be such a niche in glabrous skin. Genetic or epigenetic changes in melanocytes can cause dysfunction of the hierarchy, leading to pathophysiological skin conditions, including melanoma, which develop distinctly according to anatomical origin. For this reason, understanding the developmental lineage of melanocytes is important. In comparison to other cell systems, the molecular mechanisms through which melanocytic development is controlled are not well characterized because of limited methods to prospectively isolate phenotypically and functionally distinct melanocytes. Here we show purification of melanocytes directly from hairy skin and glabrous skin by using the melanocyte-associated marker c-kit. In mice, we observed flow cytometrically detected side (SSC)- and near infra-red (IR)-scattering of light by intracellular melanin pigment, the canonical marker of melanocytic differentiation. We found that pigmented cells (SSC-high, IR-high) within unfractionated melanocyte subpopulations have decreased clonogenic activity and differentiation potential, compared to non-pigmented cells, dependent on anatomical site, culture conditions and the presence of underling perturbations in NRas signaling. These findings will enable elucidation of the cells-of-origin of melanoma, leading to improved disease prevention and treatment.

F-2051

EVALUATION OF PANCREATIC CANCER IN IMMUNE DEFICIENT MICE MODELS TREATED WITH RABBIT UMBILICAL CORD STEM CELLS LOADED WITH INTERFERON BETA

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Pancreatic cancer is one of the most aggressive forms of cancer to occur in humans. Upon diagnosis, the typical five-year survival rate is 6.7%. There are numerous treatments available, but none can effectively target the cancer cells directly without harming nearby healthy cells, causing unwanted side effects to the patient. The objective of this study is to determine how effective rabbit umbilical cord stem cells (rUCSC) are at targeting Pan02 pancreatic cancer cells and delivering interferon-beta (IFN-beta) to kill the cancer cells in vitro and in vivo. We investigated the trophic ability of rUCSC towards Pan02 cells. This study showed that as Pan02 cell concentration increased, the amount of stem cell migration increased as well. We then investigated the anti-cancer ability of rUCSC-IFN-beta on Pan02 cells as compared to regular rUCSC. Pan02 cells both alive and dead were counted. This study showed that wells with rUCSC-IFN-beta had higher amounts of dead Pan02 cells compared to the control wells with rUCSCs. We are currently investigating the trophic effects of rUCSC-IFN-beta on severe combined immune deficient (SCID) mice tumor models by examining tumor size and the localization of injected stem cells by examining various tissues.

F-2053

THE ROLE OF UROKINASE RECEPTOR IN EPITHELIAL-MESENCHYMAL TRANSITION IN MOUSE NEUROBLASTOMA CELLS

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Neuroblastoma is the most common extracranial solid tumor of infancy, arising from the neuroblasts of the sympathetic nervous system (pluripotent sympathetic cells). These tumors can regress spontaneously or differentiate into a benign ganglioneuroma. However, in most of the cases neuroblastoma have extensive growth or metastatic spreading with a highly invasive cell phenotype and overall poor prognosis. The molecular basis of neuroblastoma progression is still obscure. Previously it was shown that the activation of urokinase system (urokinase (uPA) and its receptor uPAR) stimulates cell migration and invasion in many cancers. Here we show a new uPAR-dependent mechanism of the epithelial-mesenchymal transition (EMT) in murine Neuro 2A neuroblastoma cells. We employed CRISPR/Cas9 nickase system to target uPAR gene in Neuro 2A cells. The designed constructs effectively suppressed uPAR expression in the total population of transfected cells and in the selected clones after single cell plating. uPAR overexpression in Neuro 2A cells was carried out using plasmid transfection and antibiotic stable cell line establishment. uPAR suppression had a great impact on cell morphology: uPAR-deficient cells (Neuro2a-ΔuPAR) exhibited 4-folds ($p<0.05$) increase in the cell size compared to the control cells. In a scratch wound assay, 12 hour uPA administration stimulated a 1.5-fold increase ($p<0.05$) in cell migration of Neuro 2A-ΔuPAR cells compared to the control and to uPAR-overexpressing cells (Neuro 2A-uPAR). After 24 hours Neuro2a-ΔuPAR cells almost completely (up to 79%, $p<0.05$) recolonized the scratch wound, while control and Neuro 2A-uPAR cells recolonized it to a much lesser extent (9 and 12%, correspondingly). Moreover, control cells and cells overexpressing uPAR were characterized by a clonogenic growth type, while Neuro2a-ΔuPAR cells actively migrated and formed a uniform monolayer. uPAR deficiency was accompanied by a significant increase in IL-6 expression - a highly potent migratory and EMT inducing factor in neuroblastomas. These results suggest a novel mechanism of uPAR-dependent epithelial-mesenchymal transition in neuroblastoma cell.

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F-2055

IDENTIFICATION OF PUTATIVE MEDIATORS OF DRUG RESISTANCE TO BET BROMODOMAIN INHIBITORS IN ACUTE MYELOID LEUKEMIA VIA HIGH-THROUGHPUT CRISPR SCREENING

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Acute Myeloid Leukemia (AML) is a cancer of the blood and bone marrow characterized by accumulation of immature, dysfunctional white blood cells. If untreated, the disease progresses rapidly and is typically fatal within weeks or months. Dysregulation of epigenetic mechanisms can facilitate cancer initiation, progression and drug resistance. AML cells are dependent on BRD4, a member of the bromodomain and extra terminal (BET) protein family. It acts as an epigenetic reader and a transcriptional coactivator by binding acetylated lysines on histones and recruiting factors, which interact with RNA polymerase to facilitate gene expression. Hence, BETs are promising targets for therapeutic interventions

because of the unique dependency of AML cells on BRD4. JQ1 is a BET inhibitor that displaces the BRD4 fusion oncoprotein from chromatin and exerts anti-proliferative effects in BRD4-dependent cell lines and patient-derived xenografts. Structural analogs of JQ1 are currently being assessed in clinical trials. Evaluation of resistance mechanisms towards BET inhibitors is crucial for optimization of their clinical efficacy. Therefore, the aim of this project was to identify putative mediators of resistance towards JQ1 in AML. To this end, we deployed a positive selection CRISPR screen, in which a lentiviral library comprising of 1390 single guide RNAs (sgRNAs) targeting 238 epigenetic readers, writers and modifiers was delivered into human AML MOLM-13 cells constitutively expressing Cas9 to generate knockouts. Cells were then treated with JQ1 or DMSO for twenty doublings to allow depletion and enrichment of harmful and beneficial sgRNAs respectively. The abundance of each sgRNA was compared between the two conditions using next generation sequencing. The screen yielded 8 hits: EZH2, TRIM33, SUV420H2, TRIM24, EHMT2, SIRT6, ATRX and EHMT1. Those were validated by infecting cells with individual sgRNAs-GFP and tracking their depletion from culture using flow cytometry. EZH2 and TRIM33 are published mediators of resistance towards BET inhibitors in mouse models of AML. The other six hits are novel candidates for drug tolerance genes. Since epigenetic modification are tissue specific and reversible, pharmacological inactivation of hit genes might re-establish drug sensitivity and improve AML treatment outcome.

F-2057

THE PROTEASE CATHEPSIN D ATTRACTS MESENCHYMAL STEM CELLS TO TUMOUR SITES

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Bone marrow derived mesenchymal stem cells (MSCs) have been identified at tumour sites where they have both pro- and anti-tumorigenic effects. The specific signalling molecules are secreted by the tumor cells that mobilise MSCs to the sites of tumours are still unclear. Here, we describe the role of the proteases cathepsin D (cath D), in the homing of MSCs to the tumour sites, using in vitro studies. Breast and colon cancer cell lines MDA-MB-231 and HT29 or their conditioned media were co-cultured with MSCs in a Boyden chamber with or without protease inhibitors to analyse the migration (collagen I coated) and invasion (through Matrigel) of the MSCs. The migration and invasion of MSCs was induced by both the cancer cells and their three day conditioned media. As increased levels of proteases could play a significant role in the regulation of the tumours, we focussed on proteases which attract MSCs to the tumour cells. Migration of MSCs was decreased with a wide range of protease inhibitors (pepstatin A and GM6001 $P<0.05$ n=3). With pepstatin A, a potent inhibitor of cath D, consistently decreased the migrations of MSCs in both the MDA-MB-231 and HT29 cell lines. Furthermore, immunoblotting studies confirmed that cath D expression was upregulated in tumour cells when compared to the MSCs. The effects of the protease on MSC mobilisation was confirmed by siRNA mediated knock down of cath D, which showed a decrease in migration (MDA-MB-231 cells and HT29 cells, $P<0.001$ n=3) and invasion (MDA-MB-231 and HT29 cells $P<0.001$ n=3). Immunoblotting confirmed activation of the MAPK/ERK signalling pathway in the MSC cells, which was reversed by the addition of pepstatin A. We demonstrated for the first time that the secretion of cath D by breast and colon cancer cells increases migration and invasion of MSCs via the activation of the ERK signalling pathway. These findings provide the first evidence that cathepsin D plays a role in attracting MSCs to tumour sites providing new mechanism that can be exploited for novel cancer therapies.

Funding Source: University of Otago Funds

F-2059

DOWNREGULATION OF CANCER STEM CELL PROPERTIES AND PROMOTION OF APOPTOSIS VIA THE FAS-P53 AND WNT CALCIUM PATHWAYS BY THE WNT ANTAGONIST, SFRP4, IN HUMAN GLIOMABLASTOMA CELL LINE

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Our previous findings demonstrated that the addition of sFRP4 protein hampered cancer stem cell properties like chemo resistance, migration and invasion. To understand the molecular mechanism of sFRP4 in cancer cells, we show that overexpressed sFRP4 (sFRP4 OE) in the glioma cell line U87 induces apoptosis. Localization of sFRP4 in nucleus and further downstream ChIP-sequencing of sFRP4- pull down DNA revealed the presence of a key homeobox protein Cphx2, which is related to ETS2, a key regulator of senescence. Furthermore, apoptosis inducing miRNA885 which acts via p53 was upregulated in sFRP4 OE cells. Whole transcriptome RNA sequencing analysis suggested that sFRP4 mediated apoptosis is via the Fas-p53 pathway and probably by activating the Wnt-calcium and ROS pathways. Interestingly, sFRP4 OE cells had a decreased expression stemness markers CD133, ABCG2 and pluripotent markers, Nanog and Oct4, the effect of which was dramatically reversed in RNAi mediated knockdown of sFRP4. To extend this observation, when sFRP4 was knocked down in multipotent mesenchymal stem cells, there was induction of pluripotentiality with concomitant upregulation of the Wnt-b catenin pathway. Collectively,

these study indicates sFRP4 promote apoptosis by possible activation of DNA damage machinery and induction of several pro-apoptotic pathways including caspase activated Fas-FasL-p53 signaling cascade. It also suggests that Wnt antagonism maybe operational in regulating pluripotentiality.

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F-2061

PI3K/MTOR DUAL INHIBITOR VS-5584 TARGETS HUMAN ORAL SQUAMOUS CELL CARCINOMA STEM-LIKE CELLS

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Increasing evidence suggests that head and neck squamous cell carcinoma (SCC) follows the cancer stem cell (CSC) model. We isolated a population of tumorspheres from surgically resected human oral SCC. The tumorspheres fulfilled the definition of CSC. Using the tumorsphere as a model, we studied VS-5584, a dual PI3K-mTOM inhibitor, aiming to find effective therapies for oral cancer. VS-5584 in vitro treatment reduced the tumorsphere cell's viability. In parallel, the chemotherapeutic drug cisplatin had an effect similar to VS-5584 on tumorsphere's viability. After VS-5584 or cisplatin treatment, viable cells were harvested for clonal growth assay, sphere-initiation and cancer-initiation analysis without further VS-5584 or cisplatin treatment. The VS-5584 treated cells showed 50-80% clonal growth reduction, 2- to 6-fold decrease in sphere-initiation efficiency and 5- to 11-fold decrease in cancer-initiating cell frequency. While cisplatin-treated cells demonstrated 30-50% clonal growth enhancement, 2- to 4-fold increase in sphere-initiation efficiency and 2- to 5-fold increase in cancer-initiating cell frequency. To investigate VS-5584 on pre-established tumor, we injected the tumorspheres subcutaneously into NOD/SCID mice. Upon formation of palpable tumors, we started the mice with in vivo treatments. It was revealed that VS-5584 or cisplatin alone led to reduced tumor volumes as compared to vehicle treatment. While the treatment of VS-5584 plus cisplatin ended with the smallest tumor volume as compared to other treatments. Furthermore, at the end of in vivo treatments tumors were harvested for exploring mechanisms that mediated the above outcomes. It was found that all the treatments caused increases of $TUNEL^+$ cells with the VS-5584 plus cisplatin treated tumor showing the highest level of $TUNEL^+$ cells, and that tumor cells from the VS-5584 alone and the VS-5584 plus cisplatin treated mice demonstrated decreases in clonal growth, sphere-initiation efficiency and cancer-initiating cell frequency as compared to vehicle or cisplatin alone treatment. Together, our findings suggest that VS-5584 is effective in targeting oral CSCs, and that the combination of VS-5584 and cisplatin represents a new modality for oral cancer by targeting both CSCs and bulk tumor cells.

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CHROMATIN AND EPIGENETICS

F-2065

CELL CULTURE-GENERATED NEURONS SHOW NON-PHYSIOLOGICAL DNA METHYLATION SIGNATURES

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Patterns of cytosine methylation in genomic DNA occur in a cell-specific manner and thereby are highly effective markers for a cell's identity, with DNA methylation being associated with transcriptional repression in certain contexts. In most cells in mammals, DNA methylation is predominantly found in the CG sequence context (mCG), where the majority of CG dinucleotides are methylated. Interestingly, in the central nervous system, especially in neurons, DNA methylation is also found in non-CG context (mCH, where H = A, C or T), which increases from birth to adulthood and ultimately becomes the most abundant form of methylcytosine in the genome of adult neurons. Since these brain-specific mCH patterns are established in the first weeks after birth, coinciding with a wave of synaptogenesis, we queried whether mCH would be a suitable indicator for the maturation status of neurons. As neuronal cell culture systems are widely used to study brain physiology and disease mechanisms, a consistent marker for mature neurons would greatly improve the usability of such cell culture

systems. We used whole genome bisulfite sequencing to define the methylation state of mouse cell cultures, starting from ES cells to mature neurons, and compared it to in vivo datasets of different developmental stages. Levels of mCH similar to adult brain were first seen in cell culture-generated neurons at day 38. Deep sequencing revealed that although global methylation values are comparable, mCH patterns are dramatically different throughout the genome. While neurons generated in cell culture are similar to embryonic brain neurons in the CG context, their CH methylation patterns do not resemble any population or time point found in vivo. Differentially methylated regions are enriched for genes involved in neurodevelopment and -maturation or are associated with disorders of the autism-spectrum. We postulate that differences in CH methylation may affect the ability of cell-culture generated neurons to fully mature to a state comparable to the situation in vivo.

F-2067

DERIVATION OF ANDROGENETIC HUMAN EMBRYONIC STEM CELLS FOR STUDYING MOLECULAR AND DEVELOPMENTAL IMPLICATIONS OF GENOMIC IMPRINTING

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Genomic imprinting is an epigenetic phenomenon by which parent-of-origin dependent DNA methylation confers parental-specific differential regulation of a subset of genes in the genome. The non-equivalence of parental genomes due to 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 challenging. Here we derived six completely-paternal human androgenetic embryonic stem cell (aESC) lines to investigate molecular and functional aspects of imprinting. Sperm injection into human oocytes with removal of the maternal genome resulted in efficient preimplantation development and subsequent derivation of homozygous pluripotent aESC lines. Analyzing aESCs alongside completely-maternal parthenogenetic embryonic stem cells (pESCs) from unfertilized oocytes and bi-parental embryonic stem cells from in vitro fertilization (IVF-ESCs), allowed the establishment of a single cell-type experimental system of different parental backgrounds. Comparative RNA-seq analysis of aESCs, pESCs and IVF-ESCs uncovered both known and novel candidate imprinted genes with monoallelic expression in IVF-ESCs. Methylome analysis readily identified known imprinted regions, where DNA methylation is either germline-inherited or gained during embryogenesis, and also pointed to several new candidate loci. We next utilized the pluripotency of our different cell lines to study the implications 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 liver differentiation. We thus differentiated aESCs and pESCs in vitro into trophoblast cells and hepatocytes, aiming to study the mechanistic roles of specific imprinted genes that may underlie these phenotypes. Our results emphasize the potential of pluripotent cells with different parental origins for studying the impact of imprinting on human development and disease.

F-2069

TISSUE ORIGIN INFLUENCES THE EPIGENOME OF MOUSE MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) were originally identified as stem/progenitor cells that can differentiate into three mesenchymal lineages, osteocytes, adipocytes, and chondrocytes. It has also clarified that MSCs have a unique differentiation potential towards a broader range of cell types and an anti-inflammation activity; these features position MSCs as a promising tool in regenerative medicine. Although each MSC line is known to have unique characters including differentiation potential or growth rate, likely influenced by tissue origin or culture conditions, but tools that can identify the molecular mechanisms of how each MSC acquires unique features are not established. Here, we prepared mouse MSCs from four different tissues (bone marrow from femur or vertebra, adipose, and lung) as examples of distinct MSCs, and analyzed their gene expression patterns and epigenomes. After confirming the expression of MSC makers (CD106, CD29, CD44, and Sca-1), we performed RNA-seq on the established MSCs. RNA-seq analysis highlighted cell type specific gene expression programs and the feature accurately clustered the MSCs with reflecting the tissue origin. To analyze the epigenome, we further performed ATAC-seq (Assay for transposase-accessible chromatin using sequencing) which identifies open chromatin regions. Interestingly, the open chromatin status identified by ATAC-seq, particularly when focusing on the non-promoter region (defined as a region excluding +/- 1kb from transcription start sites), clustered the established MSCs with much higher power compared to the clustering with RNA-seq data, suggesting the epigenome as a

useful marker to distinguish MSCs with different origins or properties. Furthermore, we have successfully identified transcription factor motifs that were accessible in a cell type specific manner, suggesting that those transcription factors may establish cell type specific epigenomes and gene expression patterns. Thus, our study provides the foundation of effective identification of distinct MSCs using the epigenome and a basis for understanding molecular mechanisms of how certain MSCs acquire unique features.

F-2071

REVEALING NON-CANONICAL FUNCTIONS OF CHROMATIN REGULATORS IN MOUSE EMBRYONIC STEM CELLS BY USING HIGH-THROUGHPUT DATA MINING

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In eukaryotic chromatin, as epigenetic information is encoded and decoded through the combinations of multiple chromatin regulators, revealing the functional mechanisms of chromatin regulators is of great significance to understand the regulation of gene transcription. During the past twenty years, many chromatin regulators have been discovered as writers, erasers or readers of certain histone modifications, providing the panoramic views of canonical functions of chromatin regulators. Interestingly, a few cases have been reported for non-canonical functions of several chromatin regulators, i.e. functions independent to their known substrate or products, by profiling their genomic occupancies, revealing the complexity of functional mechanisms of chromatin regulators. With the accumulation of high-throughput biological data, it has become possible to systematically identify the non-canonical functions of chromatin regulators through data mining, especially the cooperation mechanisms between chromatin regulators and co-factors. Here, we developed a novel algorithm by applying Elastic Net model to predict non-canonical functions of chromatin regulators based on the genomic co-occurrence matrix of chromatin regulators, histone modifications and transcription factors. Dozens of potential non-canonical functions of chromatin regulators were predicted in ChIP-seq data-rich cell cells / types. We confirmed the predicted non-canonical function of CBX7, a known component of PRC1 complex, in mouse embryonic stem cell (mESC). We found that CBX7 co-localizes with NANOG at genomic loci without H3K9me3 signals, and the depletion of CBX7 reduced the pluripotency of mESCs.

ORGANOIDS

F-2073

HIGHLY EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO LONG-TERM EXPANDABLE "MINI-GUT" ORGANOIDS

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The differentiation of human pluripotent stem cells (hPSC) into intestinal organoids represents an attractive tool for disease modelling, drug screening, and cell-replacement therapy. We developed the STEMdiff™ Intestinal Organoid Kit, which is a serum-free, defined medium that efficiently and reproducibly promotes the differentiation of human embryonic stem (hES) and induced pluripotent stem (hiPS) cells through stages of definitive endoderm, mid-/hindgut, and small intestine. Monolayers were first generated from multiple hES (WA07, WA09) and hiPS (WLS-1C, STiPS-MOO1) lines maintained on Corning® Matrigel® in mTeSR™1 and then induced to the next step of definitive endoderm. This is followed by the induction of mid-/hindgut, which results in morphological changes of the 2-D culture, formation of epithelial tubes and budding of 3-D hindgut spheroids. Detached spheroids were harvested from the supernatant, embedded into Corning® Matrigel® and cultured in STEMdiff™ Intestinal Organoid Growth Medium (OGM) to generate small intestinal organoids. Samples were removed at each developmental stage for analyses by flow cytometry, ICC and/or qPCR to confirm differentiation efficiency by lineage-specific marker expression. Endoderm formation was highly efficient with 81.6% 3 8.6% (n=21) of cells co-expressing FOXA2 and SOX17. Furthermore, differentiation into posterior endoderm to promote the formation of mid-/hindgut resulted in 71.4 3 8.5% (n=13) of cells expressing the hindgut marker CDX2. The detached spheroids, embedded into Corning® Matrigel® and cultured in STEMdiff™ Intestinal OGM generated intestinal organoids composed of a polarized, CDX2+/E-cadherin+ intestinal epithelium patterned into villus-like structures and a surrounding, niche factor-producing, vimentin-expressing mesenchyme. Organoids cultured for > 25 days in vitro consisted of enterocytes (villin+), goblet cells (MUC2+), Paneth cells (lysozyme+), and intestinal stem cells (LGR5+) as confirmed by the

respective marker expression. These organoids could be dissociated, passaged and expanded in STEMdiff™ Intestinal OGM or cryopreserved for future applications. In summary, STEMdiff™ Intestinal Organoid Kit supports the derivation of human intestinal organoids from hPSC in a highly efficient and reproducible manner.

F-2075

CULTURE AND DIFFERENTIATION OF MOUSE HEPATIC ORGANOIDS USING HEPATICULT™ SERUM-FREE MEDIUM

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Liver organoids are miniature three-dimensional (3D) cell culture systems for studying liver cell biology. Liver organoids retain many features of in vivo hepatocytes, including a polarized epithelium, and represents a more physiological system than conventional 2-dimensional cell culture for studying hepatic development, regeneration, detoxification, metabolism, and disease. We have developed a novel, serum-free HepatiCult™ Organoid Growth Medium and protocols for establishing and expanding hepatic progenitor organoids derived from mouse liver tissue. Mouse livers were enzymatically treated to isolate the putative liver stem cell niche contained in hepatic ducts. The ducts were then further dissociated into single cells to derive clonal organoids. Liver organoids formed within 4 - 7 days from hepatic ducts or single cells that were embedded in Corning® Matrigel® and cultured in HepatiCult™ (n = 148 mice). The organoids were passaged every 5 - 7 days at split ratios between 1:10 and 1:30 and could be maintained in culture for > 2 years, indicating the presence of self-renewing hepatic stem cells. Cells within the organoids expressed genetic markers representative of hepatic stem and progenitor cells (Prom1, Axin2, Sox9, Cd44), ductal cells (Krt19, Hnf1b) and hepatocytes (Hnf4a, Afp), and were primed for downstream differentiation into mature functional hepatocytes. Differentiation was easily induced using a published protocol. Production of the organoids could be scaled up by culturing them on an orbital shaker in a dilute suspension of Matrigel® in HepatiCult™. Methods were also established to cryopreserve the organoids for long-term storage. Our results demonstrate that HepatiCult™ promotes the establishment, expansion, long-term propagation and banking of mouse hepatic organoids that maintain their capacity for differentiation.

F-2077

MODELLING HIRSCHSPRUNG DISEASE WITH HUMAN PLURIPOTENT STEM CELL DERIVED COLONIC ORGANOIDS

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The recent breakthrough in stem cell research allows disease modeling in vitro, which is particularly useful for studying the genetic basis of congenital diseases. We have previously used Hirschsprung (HSCR)-specific induced pluripotent stem cells (iPSC) to generate enteric neural crest cells (ENCCs) and unveil the biological impacts of genetic lesions in HSCR pathogenesis. In this study, we established human iPSC-based colonic organoids (HCO) to further illustrate how the gut environment and mesenchymal signals may influence the ENCC development and disease development. Our organoid model comprises two major components: ENCCs and gut endodermal cells. These two populations of cells were generated from hiPSCs, by sequentially activating the ACTIVIN and WNT pathways to promote the formation of definitive endoderm and the hindgut spheroids, respectively. The hindgut spheroids were then caudalized further by treatment of BMP2. The ENCCs, on the other hand, were generated using the dual SMAD inhibition differentiation method and enriched by FACS with HNK1 and p75NTR antibodies. hiPSC-ENCCs were then co-cultured with the hindgut spheroids in a three-dimensional matrigel culture, in which ENCCs received patterning signals from the gut endoderm and developed together with the endodermal cells. By thirty days, crypt-like structure was observed in the HCOs in which a distinct layer of gut epithelium (VILLIN+, CDH1+) expressing colonic specific marker (SATB2+), endocrine (CHGA+) and Goblet cells (MUC2+) were found. Intriguingly, ENCCs could be able to self-pattern and aligned with the epithelium and started differentiating into neurons (TUJ1+). After transplanting into the kidney capsule of immunodeficient mice, HCOs underwent morphogenesis and formed mature tissues with defined crypts and colonic epithelium, while ENCCs gave rise to nerve cells residing proximity to the submucosal and myenteric layers of smooth muscle fibers. Upon electrical-field stimulation, the engrafted tissues exhibited a sustained wave of contractions, which can be inhibited by the blockade of neuronal activity with tetrodotoxin. This model is currently being used to generate “HSCR colon” for a better understanding HSCR pathogenesis.

F-2079

GENERATING INNER EAR ORGANOID LIKE STRUCTURE BY CO-CULTURING WITH MESENCHYMAL STEM CELLS AND HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Hearing loss is the most common form of sensory impairment in humans and the main reason for the permanence of these chronic disorders is that mammalian cochlear hair cells do not regenerate. Although, our inner ears have around 15,000 cochlear hair cells, they are sensitive to mechanical and chemical insults which may affect people of any age from mild to complete deafness. Lots of works have been done in order to understand the enhanced view of the development of this organ. One is achieved by generating organoids which are known as 3D structures derived from pluripotent stem cells, in which cells self-organise into differentiated functional cell types and recapitulate the microanatomy of the considered tissue. In our study, otic progenitor cells were directly differentiated with stepwise protocol from human induced pluripotent stem cells within 14 days by expressing specific markers such as PAX8, AP2, ECAD, NCAD and SOX2. Afterwards, human inner ear organoid like structures were generated by co-culturing the otic progenitor cells with only human mesenchymal stem cells (MSCs) and also with human umbilical vein endothelial cells (HUVECs) and MSCs on growth factor reduced matrigel with 1:5 and 1:5:5 cell ratios respectively. Both cellular combinations were capable to generate compact organoid like structures after 24 hours. The formed organoid-like structures could be manipulated physically and were mechanically stable. At the end, the organoid like structures were removed and histologically analyzed. Furthermore, by immunostaining, the results showed the endothelial markers (CD31) in our organoid and other specific markers of considered tissues. It seems that the cross talk between these cells and otic progenitor cells is necessary for further maturation and transplantation. In our knowledge this is the first time that human inner ear organoid like structure has been achieved by co-culturing otic progenitor cells, MSCs and HUVECs.

F-2081

MODELING THE HUMAN DIENCEPHALON USING PLURIPOTENT STEM CELLS

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The human diencephalon is an important relay and integration center that modulates sensory input, motor output and higher cognitive activity. In contrast to the laminated development of the cortex, formation of the diencephalon is compartmental, with structural and functional clusters of neurons (called nuclei) within each of these compartments. Disruptions in parts of the diencephalon, including thalamus and hypothalamus, have been implicated in various neurological disorders. A human model of the diencephalon will enable us to study its role in regulating normal brain function and its contribution to neuropsychiatric conditions. Based on available literature on mammalian brain development, we have developed a reliable and reproducible protocol to differentiate human pluripotent stem cells (PSCs) into a 3D model of the caudal forebrain. We characterized these diencephalon-like organoids by immunostaining for caudal forebrain progenitor markers like Otx2, and thalamic cell type specific markers such as Tcf7l2 and Olig3 and show that they self-organize into distinct layers of cell types corresponding to different stages of diencephalic development and maturation. Interestingly, by staining for the mutually exclusive midbrain marker Pax7, we also found distinct caudal forebrain versus midbrain regionalization within individual organoids. In addition, we injected dissociated organoids in vivo into immunocompromised mouse diencephalon, and found the cells survived and differentiated into neurons. We are currently following their distribution and axonal trajectory in the injected mouse brains. Our preliminary data suggests that the human PSC-derived diencephalic organoids recapitulate atleast some molecular features of their in vivo mouse counterpart.

F-2083

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

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Progenitor-like cells from adult human pancreas could be a potential source of therapeutic beta-like cells for treating end-stage 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 our semisolid medium, a single pancreatic cell that multiplies and forms a group of cells, or a colony, is defined as a pancreatic colony-forming unit (PCFU). Differentiation of PCFUs into pancreatic lineages was determined by assaying gene expression by qRT-PCR and protein expression by immuno-fluorescent staining. Self-renewal of PCFUs was determined by dissociating colonies into single cells, re-plating and observing the formation of new colonies. Adult human PCFUs gave rise to cystic colonies, also known as organoids, three weeks after plating. Single-colony gene expression revealed that some colonies expressed markers for the three major pancreatic lineage cells: duct, acinar and endocrine. These data suggest that some adult human PCFUs were tri-potent. However, the expression levels of the endocrine lineage markers, including insulin, were low. Addition of a Notch signaling inhibitor (DAPT) to 10-day-old colonies improved endocrine differentiation, as seen by increased expression of NGN3, an endocrine progenitor cell marker. Colonies treated with DAPT and placed into the subcutaneous space of diabetic mice developed into insulin-expressing cells 10 weeks post-transplantation. Addition of a Notch ligand and a ROCK inhibitor enhanced human PCFU self-renewal in vitro. These results demonstrate that progenitor-like cells isolated from adult human pancreases are capable of in-vitro self-renewal and differentiation. Our results have implications in cell replacement therapy for end-stage T1D patients.

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F-2085

SELF-ORGANIZED SYNCHRONIZATION IN HUMAN NEURONAL NETWORK ACTIVITY DERIVED FROM CEREBRAL ORGANOID

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The cerebrum is a major center for brain function, and its activity consists from some assembly of activated cells in a neural network. The neural functions depend on network activity of living neurons through activation of some combined cell assemblies and dysfunction of the network activity causes neuropsychiatric disorders, however, it is currently not available to study cerebral network activity derived from human cells in single cell resolution, which makes it difficult to study neuropsychiatric disorders using human-derived cells. Here, using cerebral organoid, we report self-organized and complexed human neural network activity and its analysis in single cell resolution. Self-organized neuronal network was formed by dissociation culture of human embryonic stem cell-derived cerebral organoids. We found spontaneous individual and synchronized activity of the network by calcium imaging, and the analysis with a whole-new algorithm enabled to examine cell activity pattern in single cell resolution with raster plot, cluster analysis, and cell distribution at the same time. Finally, we demonstrated drug induced dynamics of network activity. Thus, comprehensive functional analysis of human neuronal network may offer a way to access human brain function and neuropsychiatric diseases.

TISSUE ENGINEERING

F-2087

ONTOGENIC COMBINATORIAL CONDITIONING FOR PHENOTYPIC MATURATION OF HUMAN PLURIPOTENT STEM CELL-DERIVE 3D VENTRICULAR MUSCLE STRIPS AND PUMPS

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Cardiomyopathy is the leading cause of morbidity and mortality worldwide. Human pluripotent stem cell-derived ventricular cardiomyocytes (hPSC-VCMs) provide a species-specific platform for disease modeling, cardiotoxicity screening and drug discovery. However, hPSC-VCMs typically display immature phenotypes including poor calcium handling, immature

electrophysiological properties, a lack of ordered sarcomeric organization, and weak inotropic effects in response to β-adrenergic agonists. In this study, hPSC-VCMs were fabricated into three-dimensional (3D) human cardiac tissue strips (hCTSs, n=36) and human cardiac organoid chambers (hCOCs, n=24), and were assessed for their phenotypic maturation during extended culture. In order to investigate the effects of ontogenic combinatorial treatment, these 3D tissues were also treated with the thyroid hormone triiodothyronine (T3) and/or electro-mechanical conditioning. In hvCOCs, measurements of developed pressure and cardiac output increased with prolonged culture time from 10 days to 30 days. Upon treatment of hvCOCs with T3 (100nM) for 10 days, increases in developed pressure and cardiac output comparable to hvCOCs cultured for 30 days were observed. In addition, positive chronotropic and inotropic responses to β-adrenergic agonists including isoproterenol (1μM) and dobutamine (1μM) were enhanced. In hvCTSs, prolonged culture increased developed tension up to 26 days post-fabrication. Upon combinatorial conditioning with T3 and electrical pacing (continuously at 1Hz, 2.5 V/cm and 5 ms duration) for 11 days, hvCTSs elicited stronger contractile forces by 3-fold compared to non-treated controls. Calcium transient kinetics also improved with a shortened upstroke time and an increased conduction velocity, along with upregulation of crucial Ca2+ handling genes including PLN, ATP2A2 and RYR2. Furthermore, the combinatorial conditioning significantly upregulated the expression of β1-adrenergic receptor, which increased the sensitivity of hvCTSs to catecholamines. In conclusion, T3 and electrical conditioning promote functional maturation of human pluripotent stem cell-derived 3D tissues, offering a straightforward approach for creating in vitro assays more representative of the native adult myocardium.

F-2089

CONSTRUCTION OF DEXAMETHASONE-CONJUGATED PEI COMPLEXES WITH POLYCISTRONIC SOX6, SOX9 AND SHANGPTL4 GENES FOR THE TREATMENT OF OSTEOARTHRITIS

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Transfection of a cocktail of genes into cells has recently attracted attention in stem cell differentiation. However it is not easy to control the transfection rate of each gene. To regulate gene delivery into human mesenchymal stem cells (hMSCs), multicistronic genes coupled with a non-viral gene carrier system can be proposed as an ideal method. Dexamethasone (DEX), a long-acting glucocorticoid, is used widely for the induction of osteogenic, adipogenic, and chondrogenic differentiation. As a powerful gene carrier, polyethylenimine (PEI) has been studied transfection reagent. The SOX trio (SOX5, 6, and 9) are transcription factors that belong to the same group of regulatory molecules. SOX9 is expressed in all chondrogenic cells, with the exception of hypertrophic chondrocytes. SOX6 is a co-factor of SOX9, which is required for perfect chondrogenesis. And ANGPTL4 increased expression of Col2α1 and Aggrecan. In this study, we performed the gene transfection by introducing SOX9/SOX6 and ANGPTL4 genes into DEX-conjugated PEI for the treatment of osteoarthritis. Covalently crosslinked DEX-PEI had a diameter of 190.1386.2 nm. The uptake of DEX-PEI-SOX9/SOX6/shANGPTL4 in hADSCs was 52.5%. Thereafter, overexpression of SOX6 and SOX9 and down-regulation of shANGPTL4 was observed. pMC_SOX9/SOX6 expressed more than control, and pMC_SOX9/SOX6/shANGPTL4 was more than pMC_SOX9/SOX6. The expression of Col2α1 and Aggrecan in hADSCSOX9SOX/6/shANG showed significantly higher than those of DEX-PEI-SOX9/SOX6 complexes-transfected hADSCSOX9/SOX6. After Safranin O staining and Immunofluorescense, it was identified that RDbSOX9 / SOX6 / shANGPTL4 expressed more than control and TGF-BMP. The OARSI and Mankin scores of hADSCSOX/9SOX/6/shANG-injected rats were significantly lower than those of vehicle and vector control. In this study, DEX-PEI was an efficient gene carrier of SOX9/SOX6 and ANGPTL4 genes to hADSCs. The significantly suppressed OA progression in rats by ADSCSOX9/SOX6/shANG compared with vehicle and vector control indicated the effectiveness of DEX-PEI and SOX9/SOX6/shANGPTL4 genes combination. These results suggest the necessity of DEX-PEI complexes as a non-viral gene carrier system for transfection of hADSCs which may offer the improved potency for the treatment of osteoarthritis.

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F-2091

BIOENGINEERING AN ELECTRO-MECHANICALLY FUNCTIONAL MINIATURE VENTRICULAR HEART CHAMBER FROM HUMAN PLURIPOTENT STEM CELLS

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Tissue engineers and stem cell biologists have made exciting progress toward creating simplified models of human heart muscles or aligned monolayers to help bridge a longstanding gap between experimental animals and clinical trials. However, no existing human in vitro systems provide the direct measures of cardiac performance as a pump. Here, we developed a next-generation in vitro biomimetic model of a pumping human heart chamber, and demonstrated its capability for pharmaceutical testing. From human pluripotent stem cell (hPSC)-derived ventricular cardiomyocytes (hvCM) embedded in collagen-based extracellular matrix hydrogel, we engineered a three-dimensional (3D) electro-mechanically coupled, fluid-ejecting miniature human ventricle-like cardiac organoid chamber (hvCOC). Structural characterization showed organized sarcomeres with myofibrillar microstructures. Transcript and RNA-seq analyses revealed upregulation of key Ca2+-handling, ion channel, and cardiac-specific proteins in hvCOC compared to lower-order 2D and 3D cultures of the same constituent cells. Clinically-important, physiologically complex contractile parameters such as ejection fraction, developed pressure, and stroke work, as well as electrophysiological properties including action potential and conduction velocity were measured: hvCOC displayed key molecular and physiological characteristics of the native ventricle, and showed expected mechanical and electrophysiological responses to a range of pharmacological interventions (including positive and negative inotropes). We conclude that such "human heart-in-a-jar" technology could facilitate the drug discovery process by providing human-specific preclinical data during early stage drug development.

F-2093

NOVEL DEGRADATION RESISTANT SELF ASSEMBLING BETA AMINO ACID PEPTIDE HYDROGEL TO IMPROVE STEM CELL THERAPY FOR CHILDBIRTH INDUCED VAGINAL DAMAGE: RODENT MODEL STUDY

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Vaginal childbirth causes overstretching of the vaginal wall, ligaments and pelvic floor muscles that impact maternal health immediately or many years later. Pelvic organ prolapse (POP) is a debilitating pelvic floor disorder that affects 25% of all women, often diagnosed after menopause. Recently, surgical treatments using transvaginal mesh for POP have been associated with serious complications; inflammation, pain and erosion. Transvaginal meshes were banned in Australia and New Zealand in 2017. At present, there are no therapies that target early repair of childbirth induced vaginal wall damage. We hypothesise that repair of vaginal wall soon after childbirth will prevent the progression to future POP. Previously, our team discovered SUSD2+ Mesenchymal stem cells in the endometrium (eMSCs) and showed their potential in vaginal wall repair in pre-clinical animal models. Herein, we report for the first time a novel, degradation resistant self-assembling peptide hydrogel (SAPH) using beta amino acids for eMSC proliferation and delivery. For the first time, we show that this SAPH supports eMSC proliferation without any alterations to its regular protein expression profile. This novel SAPH forms nanostructured fibrils in the range of 30-50nm which mimics the native collagen fibril size in human vagina. In mice, the SAPH alone remained in the same injected location 4 weeks after injection. eMSCs injected alone survived only half of that time. This presentation will highlight our novel findings regarding the ability of b-amino-acid SAPH to improve cellular retention in a damaged rat vagina and the immunobiological response triggered by this novel SAPH, particularly its effect on the polarization of the macrophages. Our study holds significant potential as a novel minimally invasive therapy for the repair of the vaginal wall following childbirth in the form of an injectable secondary prophylaxis for vaginal birth induced POP.

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F-2095

ANTI-INFLAMMATORY PROPERTIES OF CORNEAL STROMA-DERIVED STEM CELLS: POTENTIAL AS A TOPICAL THERAPY FOR THE OCULAR SURFACE

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Corneal stroma-derived stem cells (CSSC) show potential as a stem cell source for corneal regeneration and wound healing, by acting as bi-directional sensory "factories" that secrete trophic factors in response to an injured microenvironment. Delivering CSSC topically to an injured corneal surface, using a substrate such as amniotic membrane (AM), represents a novel cellular therapy for severe keratitis conditions that can potentially lead to blindness. In this study, we optimised an in vitro inflammation model using human corneal epithelial cells (hCEC) treated with combinations of ethanol, lipopolysaccharide, and pro-inflammatory cytokines, interleukin 1-β and tumour necrosis factor-α. The effect of this combined injury was assessed for effect on hCEC viability and proliferation, cytotoxicity, cell lysis, and further expression of pro-inflammatory cytokines. To assess the anti-inflammatory potential of the CSSC, a co-culture system was used, with and without cells seeded on AM. Expression of anti-inflammatory trophic factors by CSSC was analysed using protein arrays and ELISAs. Co-culture of the optimised hCEC injury model with the CSSC cell therapy led to increased hCEC viability and proliferation, decreased cytotoxicity and cell lysis, and decreased levels of proinflammatory cytokines, when compared to injury alone, demonstrating the anti-inflammatory potential of the CSSC. CSSC could be easily cultured on the AM, establishing a promising method of applying the cells topically to the cornea. CSSC demonstrate an anti-inflammatory effect with potential to be clinically translated into a topical therapy for the injured ocular surface, using a carrier such as amniotic membrane.

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F-2097

INDUCIBLE PARACRINE RELEASE OF IGF-1 IMPROVES HEART MUSCLE THICKNESS IN DIFFERENT GEOMETRICALLY ARRANGED ENGINEERED HUMAN HEART MUSCLE AND FUNCTION

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Human engineered myocardium is emerging as a viable option for clinical applications in heart failure repair. Optimal graft survival and function will be key to ensure therapeutic efficacy. We hypothesized that cardiomyocyte survival and function in various geometrically arranged engineered human myocardium (EHM) can be enhanced by the co-application of fibroblasts, genetically engineered to release IGF-1 in a drug-controlled manner. Human foreskin fibroblasts (HFF) were lentivirally transduced to express hIGF-1 under the control of a tetracycline-inducible promotor (TetOn). Cardiomyocytes (CM) were derived from human embryonic stem cells (HES2) by directed differentiation followed by metabolic selection. ELISA studies confirmed enhanced IGF-1 release upon doxycycline stimulation in HFFIGF1 vs HFFWT (3.3x10-6 vs. 8.3x10-8 ng/ml/cell/day; n=3/3). Biological activity of secreted IGF-1 was assessed by Akt phosphorylation analyzes (2.430.6-fold increase vs HFFWT; n=3/3) and quantification of IGF-1 receptor phosphorylation in HFF-cardiomyocyte co-cultures (24-fold increase vs HFFWT). EHMs comprising defined mixtures of CM and HFFIGF1 (70:30%) developed significantly higher twitch forces than CM:HFFWT EHMs (0.4330.04 vs. 0.3230.04 mN; n=10). Administration of doxycycline for induced IGF-1 release further enhanced twitch force in CM:HFFIGF1 EHM (0.5230.04 mN; n=10). Single cell analysis from EHMs demonstrated an enhanced cardiomyocyte content (1832x104 vs 1032x104 cardiomyocytes enzymatically isolated from EHMs; n=6/6) and cardiomyocyte hypertrophy (1.0030.07 vs 0.8630.04; sarcomeric actinin fluorescence intensity by FACS; n=6/6) in EHM supplemented with HFFIGF1 vs HFFWT. To further enhance EHM contractile performance, a layering method was developed yielding EHM with an average cross sectional area thickness of 13.3132.1 vs 2.3330.3 mm2 in tri-layered vs single EHMs (n=10/10). Tri-layered EHM supplemented with HFFIGF1 demonstrated a markedly enhanced force of contraction. Collectively, our data provides proof-of-concept for pharmacologically controlled paracrine support of cardiomyocyte survival and growth in a thick tissue context. Ongoing studies investigate whether IGF1-enhanced layered EHM show improved cardiomyocyte survival and therapeutic efficacy in vivo.

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F-2099

CARTILAGE ENGINEERING FROM PAEDIATRIC AUTOLOGOUS FAT FOR RECONSTRUCTION IN ACQUIRED AND CONGENITAL NASAL DEFORMITY

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Congenital or acquired nasal deformity can have an overwhelming effect on the patient's physical and psychological well-being. Patients often require extensive, repeated surgical procedures commonly using autologous cartilage grafts harvested from the patient's own rib. Although rib provides excellent quality and volume of cartilage, it is far from ideal due to significant morbidity at the chest donor site including post-operative pain, risk of infection, risk of pneumothorax and scarring. Alternatives to rib grafting, such as silicone implants, are associated with high risk of infection and extrusion. Bioengineering cartilage tissue using autologous stem cells would allow surgeons to avoid such invasive procedures and eliminate donor site morbidity. Our work focuses on tissue engineering nasal septal cartilage with similar biomechanical properties to native septum. Adipose tissue offers a solution for this surgical challenge as it is easy to obtain through a minor surgical procedure. We have exploited the differentiation potential of paediatric adipose tissue-derived stem cells (pADSCs) and developed conditions for generating mature cartilage in vitro. We have isolated, expanded and differentiated pADSC along the chondrogenic lineage under different 3D (3-dimensional) conditions. Seeding pADSC onto collagen I extracellular matrix (ECM) scaffolds allows cartilage generation in 3D. Over several weeks, a cartilage like appearance is observed, and maturation of the ECM demonstrated by positive collagen type II staining. We have also assessed the ability of pADSC spheroids to differentiate chondrogenically and secrete ECM in a scaffold free environment. We demonstrated pADSC chondrogenic differentiation and deposition of Collagen II using modified tissue clearing protocols that allow immunocytochemical staining of these dense spheroids without the need to section them. Finally, we are investigating the behaviour of pADSC following 3D bioprinting with collagen bioinks as this will allow high fidelity printing of anatomical shaped nasal septum. Our initial results demonstrate good pADSC viability within the printed construct.

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F-2101

3D BIOFABRICATION WITH HUMAN ADIPOSE DERIVED STEM CELLS SUSPENDED IN A NOVEL MODIFIED PLANT-DERIVED MACROMER BIOINK

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3D biofabrication (3D bioprinting, or biological additive manufacturing) enables the fabrication of cell-laden synthetic scaffolds with complex architectures and geometry that could not be practically crafted through conventional means. "Bioink" materials for 3D biofabrication of cell-laden structures must satisfy a wide range of key properties, including cytocompatibility as well as suitable mechanical and rheological properties both at the time of fabrication and in the finished construct. Temperature-dependent and shear-dependent rheology allows the formulation of a bioink that can practically be extruded whilst supporting its own weight during the biofabrication process. Crosslinking during and after fabrication using either divalent cation solutions or photo-induced crosslinking of methacryloyl moieties on the macromolecule backbone during printing using UV LED and near-UV laser radiation turns the relatively weak printed structure into a more rigid structure, holding itself together as a matrix for further cell proliferation and differentiation. This research addresses the preparation of a plant-derived macromolecule gel as the novel basis of a bioink suitable for practical biofabrication, its combination with other bioink components such as GelMA and alginate, and the preparation of its methacryloyl derivative combined with VA-086 as the basis of a photoactive bioink. This bioink is seeded with human adipose derived stem cells (ASCs) and used to print three-dimensional cell-laden constructs followed by tissue culture and live/dead staining to quantify cell viability in this bioink following extrusion and crosslinking, showing that strong cell viability is maintained in this novel bioink base, before and following the cytotoxic stresses of the biofabrication process.

F-2103

THE IMPORTANCE OF ENERGY METABOLISM IN THE MATURATION OF HUMAN PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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Human pluripotent stem cell derived-ventricular cardiomyocytes (hPSC-CMs) display fetal-like cell properties, which limit their translatability into clinical use. Compared to their adult counterpart, hPSC-VCMs exhibit a different metabolic profile. In mature adult CMs, 95% of their ATP comes from oxidative phosphorylation, in particular fatty acid β-oxidation, whereas fetal and hPSC-CMs are highly dependent on glycolysis, with only 60% of its ATP deriving from oxidative metabolism. During this perinatal metabolic transition, the serum level of thyroid hormone T3 also increases. Apart from its metabolic effects, T3 is known for its maturational effects on the developing heart. In

2D culture, T3 has been shown to increase the yield of CMs from hPSC, and promote maturation after differentiation. Thus, we hypothesize that metabolic maturation of hPSC-CMs can drive their functional maturation. In this study, we investigated the effect of T3 on metabolism and function in both 2D culture and engineered tissues. In human engineered cardiac tissue strips (hvCTS), mitochondrial genes that are responsible for fatty acid oxidation were significantly increased after T3 treatment. In addition, T3 treatment also led to improvements in calcium handling and contractile properties; To further investigate, we directly induce the up-regulation of the fatty acid β-oxidation in hPSC-VCMs by activating the peroxisome proliferator-activated receptors (PPAR) pathway with its endogenous agonist, oleic acid (OA), as well as a synthetic activator GW 7647. Treatment of hPSC-VCMs in 2D cultured by OA resulted in an increase in mitochondrial potential and the upregulation of key metabolic enzymes. In hvCTS, treatment with OA resulted in an increase in ATP synthesis and the expression of key metabolic genes, including those not directly regulated by PPAR. More importantly, we observed an increase in developed force after PPAR activation, concomitant with an increase in the expression of key calcium handling genes including ATP2A2, RYR2, and CACNA1C. In conclusion, our results show that metabolic maturation alone is enough to drive functional maturation in hPSC-CMs both in 2D culture and 3D engineered tissues. These findings build a strong foundation for our understanding of the role of energy metabolism in the maturation of hPSC-CMs.

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F-2105

EVALUATION OF THE FUNCTIONALITY OF 3D OSTEOCYTIC NETWORK IN DIFFERENT HYDROGELS

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Osteocytes reside as three-dimensionally (3D) networked cells in the lacunocanalicular structure of bones. Nowadays they have been shown to play multiple roles in bone (mechanic-transductors, bone metabolism regulators). The multi-functionality of osteocytes makes them intriguing targets for bone tissue engineering. Due to the osteocyte network being deeply embedded within hard bone tissues ex vivo, it has been extremely challenging to access and characterize these cells. So far, evaluation of osteocyte activity and regulation in vitro have mainly been conducted in 2D systems, with only very few studies conducted in a 3D environment. Three-dimensional (3D) cultures are attractive as they better mimic the physiological features of the ECM surrounding the cell. Biomaterials have been employed to mimic the native extracellular matrix (ECM) in a 3D environment, which plays a key role in tissue regeneration. Due to their easily tunable chemical, physical and mechanical properties, hydrogels represent an interesting way to create a 3D environment. Then we use a hydrogel developed by our collaborator as based system to build the 3D network.We used human MSC to process 3D culture in different stiffness (low/high) hydrogel system with osteogenic differentiation medium for 6 weeks. Through ALP staining, real time PCR, IHC, microCT detection, we found that MSC can osteogenic differentiation and formation of mineralization in both groups, and better interconnected network between cells was significantly increased within low stiffness hydrogel, compared to cells within high stiffness matrices at the same cell densities. These results proved our 3D culture system is worked, and can be used to investigate the mimic bone 3D structure.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

F-2109

REPAIRING THE CELL-TRANSPLANT PARADIGM FOR PARKINSON DISEASE: SCIENCE AND ETHICS

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Parkinson Disease has proven to be both very difficult to address through cell transplantation and yet still beckons to researchers as putatively low-hanging fruit in the cell transplantation world. Fetal ventral mesencephalic (FVM) cell transplants were successful in reducing symptoms in a small number of cases, but only in the hands of a small number of clinician-scientists whose results have been difficult to replicate. Possible explanations include variation in the quality (for clinical purposes) of the fetal tissues used, variation in mode, site, and mechanism of delivery, and the number of cells / size of sample delivered; additionally, there were concerns about the methods for measuring success, questions about the mode of action, and issues associated with inclusion and exclusion criteria for participants. Concerns on many of these fronts needed to be alleviated in order to proceed with the cell transplant paradigm for treating PD. FVM research dwindled, but the cell transplant paradigm was rescued twenty years ago when there was much excitement about cultivating human pluripotent stem cells (hPSCs) for transplantation. Using cells derived from hPSC research would address the first of the possible explanations of the demise of FVM transplants: the clinical quality of the cells to be transplanted. No longer would surgeons be transplanting gross fetal tissue that might vary from fetus to fetus (as from surgery to surgery); instead, hPSC scientists imagined creating clinical grade cells for transplantation. Were everyone to use the same calls, or well-characterized variants thereof, rapid clinical progress might be achieved on the path to cell-based

treatments for PD. And yet another two decades have passed without a successful cell-transplant treatment for PD. One possible explanation is that research has been hampered by ethical restrictions on deriving and studying hPSCs from human embryos. A more probable explanation is that the almost-exclusive reductionistic focus on the cellular material to be transplanted has led to the neglect of all of the other issues originally identified in FVM research. As we move into a new round of attempts in the cell-transplantation paradigm, it is imperative that we step back and systematically explore more system-level issues to make sure we do not repeat past mistakes.

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

F-2113

ALLOGENEIC STEM CELLS VERSUS MICROFRACTURE FOR CARTILAGE REPAIR IN OSTEOARTHRITIS

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This phase 3 trial compared the safety and efficacy of allogeneic human umbilical cord blood-derived MSCs (hUCB-MSCs)-4% hyaluronate(HA) hydrogel with microfracture. Patients with multiple, symptomatic, full-thickness, degenerative cartilage defects of the knee (n=103; associated with subchondral sclerosis or edema and multiple lesions consistent with osteoarthritis) were enrolled in a randomized, open-label, multicenter trial. A single (most symptomatic) lesion was treated with hUCB-MSCs (n=50) or microfracture (n=53). The primary endpoint was proportion of subjects improved by ≥1 International Cartilage Repair Society (ICRS) grade at 48 weeks. Secondary endpoints included histology, pain visual analogue scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and International Knee Documentation Committee (IKDC) scores. Extended follow-up was 60 months. Average defect sizes were 4.9 cm2 (range, 2.0–9.0 cm2; hUCB-MSC) and 4.0 cm2 (range, 2.0–8.0 cm2; microfracture). Improvement by ≥1 ICRS grade was found in 97.7% of the hUCB-MSC group versus 71.7% of the microfracture group (P=0.001); hUCB-MSCs provided superior results for larger lesions and lesions in older subjects (P<0.05). While clinical scores did not differ between groups at 48 weeks, at month 60, pain scores were significantly better with hUCB-MSC versus microfracture (P<0.01); at 36 and 60 months, WOMAC and IKDC scores were significantly better in the hUCB-MSC group (P<0.05). No differences in the incidence/nature of adverse events between groups were found. hUCB-MSC implantation is safe and more effective than microfracture leading to durable cartilage restoration of full-thickness lesions in 97.7% of treated subjects with knee osteoarthritis.

F-2115

PROGRESS TOWARDS ESTABLISHING AN AUSTRALIAN CORD BLOOD-DERIVED IPSC HAPLOBANK FOR CLINICAL USE

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National and global banks of human induced pluripotent stem cells (iPSC) are being developed across the world. Cord blood (CB) is an ideal source of starting cells for iPSC generation, as a rich source of immature cells that are highly proliferative. We aim to establish a clinically relevant GMP compliant bank of cord blood derived iPSC lines for cellular therapies in Australia and globally, increasing Australia's attractiveness for clinical trial research. The cell lines in these iPSC banks will have specially selected homozygous profiles of HLA markers, chosen from donors whose combined haplotypes can cover over 50% of the target population. Starting from the ground up we are building a collaboration between the iPSC core facility at MCRI, the BMDI Cord Blood Bank and Sydney Cord Blood Bank, experts in HLA and statistical genomics and experts in GMP and international regulatory compliance to develop (i) the technical expertise to produce clinically compliant GMP quality IPSC lines with long term stability (see abstract by Tian et al) and (ii) identify homozygous haplotypes best suited for covering a significant percentage of the Australian population as well as haplotypes of interest to the global community. HLA tissue typing data from CB banked at the BMDI Cord Blood Bank was interrogated using a purpose-written algorithm; from a total of 13,679 records interrogated at least 143 cord blood units with homozygous haplotypes, with at least 30 cord

blood units having unique haplotypes. The identified haplotypes would be sufficient to cover over 50% of the Victorian population, while some of the less common haplotypes could be of interest to other countries. We are also working on ethical issues of consent/ re-consent, use of the created banked lines, education and co-operation with the repositories, issues that are critical when dealing with previously collected and banked samples. Some of the regulatory challenges include QA and QC, freedom to operate and the standards required to operate potentially as part of a global network; goals are being achieved by working in collaboration with bodies such as the Global Alliance for iPSC Therapies (GAiT) in their efforts to establish international networks and standards. Here we present our progress, ongoing challenges and the considerations ahead.

F-2117

GRAFTING OF HUMAN IPSC-DERIVED MGE-LIKE PROGENITOR CELLS INTO THE HIPPOCAMPUS EASES CHRONIC TEMPORAL LOBE EPILEPSY

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Temporal lobe epilepsy (TLE) is drug-resistant in ~35% of patients, and drug therapy does not alleviate cognitive and mood dysfunction seen in TLE. An alternative therapy that is efficient for reducing spontaneous recurrent seizures (SRS) and improving cognitive and mood function is therefore needed. While medial ganglionic eminence (MGE) cell grafts derived from the fetal brain or embryonic stem cells can restrain SRS in animal models of TLE, availability and/or ethical issues limit the use of such cells in clinical applications. Application of human MGE (hMGE) progenitors expanded from human induced pluripotent stem cells (hiPSCs) resolves ethical concerns as well as promotes patient-specific cell therapy. We examined the efficacy hiPSC-derived hMGE cells grafted into the hippocampus of F344 rats exhibiting kainate-induced chronic TLE. Chronically epileptic rats (CERs) exhibiting similar SRS were classified into 4 groups, which received hMGE cell grafts into the hippocampus and cyclosporine injections (grafted group), sham-grafting surgery (sham group), cyclosporine injections (cyclosporine alone group) or no treatment (epilepsy alone group). Continuous video-EEG recordings performed 4 months after grafting showed substantially reduced and less intense SRS in CERs receiving grafts, in comparison to control CER groups. The frequency of all SRS, stage-V SRS and the amount of time spent in SRS activity were reduced by 71-80%. A battery of behavioral tests also revealed alleviation of impairments in object location memory, novel object recognition, pattern separation, and mood function in the grafted group. Analyses of grafts revealed robust yield of graft-derived cells with pervasive migration. Graft-derived cells differentiated mostly into interneurons expressing GABA (70%) including subclasses expressing parvalbumin (21%), neuropeptide Y (12%), somatostatin (6%) and calretinin (13%). Only 1% of graft-derived cells were proliferating, and none expressed markers of pluripotent stem cells. Analysis of the host hippocampus revealed decreased glutamate concentration associated with an increased GABA concentration. Thus, hiPSC-derived hMGE cell grafting into the epileptic hippocampus leads to greatly reduced SRS and improved cognitive and mood function in a model of chronic TLE.

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F-2119

TREATMENT OF ALZHEIMER DEMENTIA IN OLDER DOGS WITH AUTOLOGOUS SKIN-DERIVED NEUROPRECURSOR CELLS: INITIAL RESULTS FROM A PHASE I VETERINARY CLINICAL TRIAL

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Dementia affects 50M individuals with estimates for over 130M by 2050. Medical management has not progressed for 20 years - it remains an incurable, progressive and fatal disorder. A major challenge is that dementia is not adequately modelled in transgenic mice. We have therefore studied and indeed clinically defined Canine Cognitive Dysfunction (CCD) in older pet dogs. Dogs with CCD: display amnesia, disorientation, sleep-wake disturbance and agitation; prevalence doubles every 2-years after 10 years; express both Alzheimer and non-Alzheimer pathology; and veterinary trials recapitulate

poor outcomes seen in humans. CCD is therefore an important translational model of Alzheimer dementia. In parallel, our group developed and recently published a clinically-relevant protocol for replicable isolation and expansion of homogenous P75+/CD133+/Nestin+ neural precursors sourced from mature canine skin without genetic manipulation. We bring this research together in the DOGS+CELLS veterinary clinical trial. Dogs are screened for CCD using our validated CCD rating scale, assessed for general and neurological health and brain scans conducted to plan neurosurgery and exclude non-CCD aetiology. A small skin-sample is taken and cells cultured over 3-weeks until release criteria are met. 250,000 neuroprecursor cells are injected stereotaxically into the hippocampi using MRI-guidance. Here we report on clinical outcomes of the first N=3 dogs to reach our primary endpoints: safety and global clinical function at 3-months post treatment. We found a trend for decline in global CCD ratings: PRE mean (SD) 50.7 (9.5) vs POST 34.7 (18.9); paired T-test = 2.7, df=2, p= 0.1. At the individual level, this arose from transformative clinical responses in the first two patients (45-50% reductions in global ratings) that were stable for >20 months. Objective spatial memory testing corroborated these outcomes. The third patient did not respond clinically (CCD rating 60 pre and 56 post) and so was euthanized by the owner. Importantly, we observed no safety concerns or reportable adverse events in these complex geriatric canine patients. Overall, this trial demonstrates that in-principle it may be possible to safely treat a naturalistic dementia-like syndrome using patient-specific skin-derived neural precursors.

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F-2121

IMPROVEMENT OF QUALITY OF LIFE IN CHILDREN WITH CEREBRAL PALSY AFTER BONE MARROW MONONUCLEAR CELLS TRANSPLANTATION

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Quality of life (QOL) is an important factor when evaluating the effectiveness of treatment in children with cerebral palsy (CP). The aim of this study was to evaluate the improvement in QOL of children with CP after transplantation of autologous bone marrow mononuclear cells (BM MNCs). Thirty children with CP aged from 2 to 15 years of age received two intrathecal infusions of BM MNCs, one at baseline and the second three months later, at Vinmec International Hospital from December 2015 to December 2016. The motor function and muscle tone of the patients were evaluated using the Gross Motor Function Measure (GMFM)-88 and Modified Ashworth Score, respectively. Their QOL was assessed at baseline and six months after the first BM MNC transplantation using the Cerebral Palsy Quality of Life Questionnaire for children (CP QOL-Child) comprised of seven domains. A paired t-test and multivariate analyses were conducted to evaluate the total QOL score and to identify the key factors correlated with the QOL score. Gross motor function and muscle spasticity were significantly improved after treatment, as demonstrated by the GMFM-88 total score, each of its domains, the GMFM-66 percentile and the muscle tone. QOL of the children with CP was noticeably improved six months after BM MNC transplantation, accompanied by improvements in gross motor function and muscle tone. Trial registration: ClinicalTrials.gov Identifier: NCT02574923. Registered on December 7, 2015.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

F-2123

HUMAN DERIVED TROPHECTODERM CELL LINES. A TIME LAPSE IMAGING REPORT FROM DAY-6 BLASTOCYSTS

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The derivation of human embryo trophectoderm cells from day-6 blastocysts has not been reported. This study was designed for video monitor trophectoderm cell derivation during 20 days of cell culture. Study trophectoderm cells were obtained from embryos determined to be aneuploid after undergoing preimplantation genetic screening (PGS). After embryos deemed nonviable based on PGS, patients consented to have subject embryos re-biopsied for this IRB-approved study. A total of 25 frozen-thawed aneuploidy blastocysts were studied. An average of 6.2 cells was obtained from the re-biopsy of tested blastocysts. Trophectoderm cells were cultured in presence of human fibroblast growth factor-4 in a time lapse incubator (Embryoscope) for 20 days. A controlled environment of 5% O2, 5% CO2 and 37°C were used to provide the cell growth

conditions. All 25 specimens were individually cultured using time-lapse dishes in a small (25 µl) aliquot of RPMI 1640 medium supplemented with 20% HSA under a confluent oil cover. Culture media was changed every 24 hours. Cells were settled at the bottom of the wells in a central depression with a diameter of 0.2 mm. The time between video acquisitions was 10-minute cycle time for 7 focal planes for 20 days. A sample of 4-5 µl aliquot of cells was taken for 16 specimens and analyzed by VeriSeq (high resolution Next Generation Sequencing) to confirm the chromosomal analyses of the new cells. Trophectoderm cell proliferation initiated within 30 minutes of culture for all tested cells. Individual small cells were released from the biopsied mass of cells. Cell activity was noted in all tested cells during the 20 days of cell culture. Resulted trophectoderm cells were counted with a mean of 700 cells and a range of 500 to 1500 cells. Chromosomal analyses resulted genetic information in all 16 trophectoderm tested samples. Time lapse system provided an excellent tool for observation of cell development, while high quality images were acquired. Furthermore, this study exposes the possibility to derive newly formed trophectoderm cells in vitro from a day-6 human blastocyst. These results further develop our understanding of initial trophectoderm populations by demonstrating the capability to self-renew these cells in vitro.

F-2125

NEW EMBRYONIC PLURIPOTENCY FACTORS IDENTIFIED FROM MOUSE EMBRYONIC MASS SPECTROMETRY

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Pre-implantation embryo development is an intricate and precisely regulated process orchestrated by maternally inherited proteins and newly synthesized proteins following zygotic genome activation. Some of these factors may play important roles in the differentiation of blastocyst and pluripotency of embryonic stem cells (ESCs), however the protein landscape during this process remains unexplored. Using quantitative mass spectrometry, we identified nearly 5,000 proteins from 8,000 mouse embryos of each stage (zygote, 2-cell, 4-cell, 8-cell, morula, and blastocyst).We applied weighted gene co-expression network analysis (WGCNA) to obtain a system wide understanding of groups of proteins whose co-expression patterns are highly correlated during embryonic development. We identified the protein groups that highly expressed in the blastocyst and further hypothesize the proteins with high connectivity which might be indispensable in blastocyst formation. We selected Zcchc8 and several core factors which are related with RNA processing but poorly studied in early embryogenesis or in ESCs. We found lack of Zcchc8 which is a member of nuclear exosome targeting(NEXT) complex may impact blastocyst development. We then found the Zcchc8 knockout mice have difficity to get birth with shorter life span and smaller size. Moreover, defects of Zcchc8 could impact the pluripotency of ESCs and cause differentiation disability in vitro and in vivo. Our study provides an invaluable resource for further mechanistic studies and deification of core factors in regulating embryogenesis and stem cell pluripotency.

F-2127

RETINOIC ACID INDUCED HUMAN AMNIOTIC FLUID STEM CELLS HAVE A POTENTIAL TO GERM CELLS

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Retinoic acid is a metabolite of vitamin A that mediates the functions of vitamin A required for growth and development. Previous studies showed that the stepwise approach to induce female germ cells formation, which were authenticated by phenotypic changes, expression of oocyte-specific markers, DNA content as well as the physiological function by adding porcine follicle fluid, from human amniotic fluid stem cells (hAFSCs). Here we found that the CD29+/CD38- hAFSCs triggered by RA showed the fibroblastoid morphology and expressed both stem cell markers (OCT4, NANOG and SOX2) and germ cell markers (DAZL, DDX4 and STELLA). Cells were first cultured in the conditioned medium supplemented with RA for 40 days. The monolayer cells were changed to from fibroblast-like cell to spherical cells, which with the morphological characteristics of early germ cells for 5 days, The cumulus-oocyte-complexes like structures, oocyte like cells and sperm like cells were observed, and some small cells, which characteristic were similar to germline stem cells were also derived from condition medium. Further studies should be needed to verify the expression of genes and proteins with germ cell.

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TECHNOLOGIES FOR STEM CELL RESEARCH

F-2129

MAPPING CELLULAR REPROGRAMMING VIA POOLED OVEREXPRESSION SCREENS WITH PAIRED FITNESS AND SINGLE CELL RNA-SEQUENCING READOUT

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Understanding the complex effects of genetic perturbations on cellular state and fitness in human pluripotent stem cells (hPSCs) is critical to discovering novel reprogramming factors for differentiation to diverse lineages, and to study the subtle and heterogenous effects of reprogramming. This has been challenging using traditional pooled screening techniques which typically rely on unidimensional phenotypic readouts. Here, we use barcoded open reading frame (ORF) overexpression libraries with a coupled single-cell RNA sequencing (scRNA-seq) and fitness screening approach, a technique we call SEUSS (ScalablE fUnctional Screening by Sequencing), to establish a comprehensive assaying platform. Using this system, we perturbed hPSCs with a library of developmentally critical transcription factors (TFs), and assayed the impact of TF overexpression on fitness and transcriptomic cell state across multiple media conditions. We further leveraged the versatility of the ORF library approach to systematically assay mutant gene libraries and whole gene families. From the transcriptomic responses, we built genetic co-perturbation networks to identify key altered gene modules. Strikingly, from the network analysis we found that KLF4 and SNAI2 have opposing effects on the pluripotency gene module, while from the fitness responses we identified ETV2 as a driver of reprogramming towards an endothelial-like state. These findings highlight the effectiveness of SEUSS in understanding the effects of genetic perturbations, furthering our understanding of reprogramming, and in discovering novel methods of differentiating hPSCs.

F-2131

EVALUATION OF PAIN RESPONSES IN HUMAN IPSC-DERIVED SENSORY NEURONS USING MEA SYSTEM

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Functional evaluation assays using human induced pluripotent stem cell (hiPSC)-derived sensory neurons are expected to predict the pain-related toxicity of drugs and the pharmacological effects. However, evaluation assays in hiPSC-derived sensory neurons has not been established, and electrophysiological response to pain-related molecules are not known. In this study, we aimed to evaluate the physiological responses aginst pain-related molecules including anti-cancer drugs in cultured hiPSC-derived sensory neurons using multi-electrode array (MEA) system. Human iPSC-derived sensory neurons were cultured on MEA chips, and the electrophysiological responses against capsaicin, menthol, allyl isothiocyanate (AITC), anti-cancer drug vincristine and oxaliplatin were measured by the MEA system. To confirm the responses depending on each receptor, we examined the responses in presence of each receptor antagonist. We also examined whether the increase of cold sensitivities occur in presence of anticancer drug in vitro hiPSC-derived sensory neurons. We firstly confirmed the expression of typical sensory marker Nav1.7, TRPV1, TRPM8, and TRPA1 using immunostaining in culture hiPSC-derived sensory neurons at 8 weeks culture. Evoked responses against capsaicin, menthol, and AITC were detected. As the responses disappeared with each channle bloker, these responses were confirmed to be channel specific responses. The evoked responses against anticancer drug vincristine and oxaliplatin were also detected. Furthermore, we found that the responses against AITC increase in presence oxaliplatin and with the concentration of oxaliplatin. We have succeeded in detecting the electrophysiological pain reponses against capsaicin, menthol, allyl isothiocyanate (AITC), anti-cancer drug vincristine and oxaliplatin in hiPSC-derived sensory neurons using MEA system. We found that the increase of cold sensitivities in vivo phenomenon was also detected in vitro hiPSC-derived sensory neurons.

F-2133

QUANTITATIVE ANALYSIS OF CYTOTOXICITY USING IPSC-DERIVED CARDIOMYOCYTES, NEURONS, AND HEPATOCYTES

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Cell-based phenotypic imaging assays have become an increasingly attractive alternative to traditional in vitro and in vivo testing in pharmaceutical drug development and toxicological safety assessment. The time- and cost-effectiveness of automated imaging assays, combined with the organotypic nature of human induced pluripotent stem cell (iPSC)-derived cells, open new opportunities to employ physiologically relevant in vitro model systems to improve testing for potential chemical toxicities. In this study, we used three human iPSC types, cardiomyocytes, neurons, and hepatocytes, to test various imaging assays and end point combinations for their applicability in a multi-parametric assay format. We used automated cell imaging with the ImageXpress Pico system to simultaneously determine cell viability, cytoskeletal integrity, apoptosis, and mitochondrial function in different cell types. Effects on cardiomyocyte beating frequency were characterized by measurements of calcium oscillations. For neuronal cells, automated imaging was used to evaluate the extent and complexity of neural networks and determine effects of compounds on neurite outgrowth, number of branches and processes. For hepatocytes, imaging was also used to evaluate cytotoxicity and cytoskeletal integrity, as well as mitochondrial integrity, and the potential for lipid accumulation. Kinetic analysis indicated that mitochondria potential assessment and the effect on calcium oscillations are best detectable 30-60 min following initial treatment, whereas cytotoxic effects were most stable after 24 h. Overall, our results demonstrate how a compendium of assays can be utilized for quantitative evaluation of chemical effects in iPSC-derived cardiomyocytes, neurons, and hepatocytes and enable rapid and cost-efficient multidimensional biological profiling of toxicity.

F-2135

NANOSYSTEMS TO ENABLE RAPID AND EFFICIENT GENERATION OF GENE-MODIFIED STEM CELL-BASED THERAPIES

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Dramatic advances in gene-editing are enabling the next generations of stem cell-based therapies that promise life-changing solutions for patients with high-risk malignancies and genetic diseases. Nanosystems represent one tantalizing solution to circumvent barriers that currently preclude the clinical translation and the broader deployment of these innovative medical interventions. For example, there is a striking unmet need for methods capable of processing stem cells quickly with high efficiency and recovery rates to enable the generation of more homogeneous and better characterized cell products for emerging gene-modified stem cell-based therapies. To address this challenge, we leverage innovations in nanoscience, microfluidics, and precise chemical functionalization to enable intracellular delivery of biomolecular cargo via 1) arrays of precisely assembled nanostructures and 2) nanorobotic strategies using magnetically guided "nanospear" probes that gently manipulate cellular membranes. In the latter case, magnetic nanospears are made of Au/Ni/Si (~5 μm in length with tip diameters <50 nm) and fabricated by nanosphere lithography and metal deposition. A magnet is used to direct their mechanical motion, enabling precise control of position and three-dimensional rotation as the spears approach target cells, mechanically penetrate their cell membranes, and deliver biomolecular payloads. In proof-of-concept studies, we demonstrate the delivery of expression plasmids for enhanced green fluorescent protein (eGFP) and a second-generation CD19-chimeric antigen receptor (CAR) to model Jurkat and K562 cells. We already achieve expression >75% while maintaining cell viabilities >90% in our preliminary work. This initial demonstration will pave the way for broader applications of these nanotechnologies, enabling new high-throughput, safe, and economical gene-modification strategies that will drive further innovation and inform how stem cell-based gene therapies are deployed clinically.

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F-2137

ENGINEERING, DIFFERENTIATION AND HARVESTING OF HUMAN ADIPOSE-DERIVED STEM CELLS MULTILAYER CELL SHEET

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Researchers are using more and more adult stem cell to cure diseases. The majority of the times, adult stem cells are directly injected into the area where they need to exert their curative effect. However, they can migrate anywhere in the body, thus the efficacy of this type of treatment is questionable. The goals of this study are 1) Engineer and harvest undifferentiated multilayer (ML)

human Adipose-Derived Stem Cells (hADSC) cell sheets 2) Engineer adipocyte, osteoblast and chondrocyte ML cell sheet, differentiated from hADSC. Ten days after seeding the hADSC, undifferentiated hADSC ML cell sheet were harvested. hADSC monolayer started to differentiate into adipocyte 3 days after the beginning of the adipocyte treatment, while the ML hADSC cell sheet started to differentiate in less than 24h, and at a higher rate. Monolayer of hADSC (undifferentiated, adipocyte and osteoblast) cell sheet could not be harvested. However, hADSC ML cell sheets (undifferentiated, differentiated adipocyte, osteoblast and chondrocyte) were successfully harvested. H&E confirmed the formation of ML cell sheets. IHC demonstrated that undifferentiated hADSC ML cell sheet preserve their stem cells markers such as CD29, CD73 and CD105. Adipocyte ML cell sheet express adipocyte markers (SREBP1, PPARg), osteogenic ML cell sheet express a specific marker osteoblast marker (Osteocalcin) and chondrocyte ML cell sheet expresses Aggrecan and SPARC. The absence of histocompatibility complex expression was maintained for all the ML cell sheets. These results indicate that hADSC confluence doesn't affect the expression of ADSC stem cell marker and additionally, increases the differentiation potential of hADSC. These results are very encouraging and offer major benefits: 1) The possibility to harvest undifferentiated hADSC cell sheet, which can be grafted directly onto any targeted tissue, 2) The possibility to differentiate a multilayer ADSC cell sheet in vitro, before harvesting and grafting.

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F-2139

HUMAN EMBRYONIC STEM CELL CULTURE ON HYDROGELS IMMOBILIZED WITH HUMAN FGF-2

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Human embryonic stem cells (hESCs) are an attractive prospect for regenerative medicine and tissue engineering because of their pluripotency to differentiate into different types of cells.1,2 In our previous study, hESCs have been successfully cultured on the o-carboxymethyl chitosan (CMC) hydrogels grafted with an oligopeptide derived from vitronectin (GCGGKGGPQVTRGDVFTMP, VN2C) (unpublished data), which is called as CMC-VN2C in the followings. Typically, the culture medium of hESCs contains growth factors such as FGF-2 and TGF-β1, which should be stored at -20 ~ -80 degree to prevent denaturation of the growth factors. This process is leading to the high cost of hESC culture medium. In this experiment, we designed hESC culture dishes immobilized with FGF-2 to reduce the usage of FGF-2 in the culture medium. CMC hydrogels were grafted with cysteine, which enables to graft the oligopeptide (VN2C) and growth factor (FGF-2) via crosslinker of PEG-SPDP. hESCs (H9) are expected to proliferate and maintain their pluripotency on CMC-VN2C surface grafted with FGF-2 (CMC-VN2C-FGF) in the medium without using FGF-2. To verify this idea, hESCs were cultured on CMC-VN2C-FGF surface in Essential 6 cell culture medium plus TGF-β1, which corresponds to Essential 8 cell culture medium minus FGF-2. We added Essential 8 medium at first day to stabilize the hESCs to attach on the surface. hESCs on CMC-VN2C-FGF surface were found to keep their pluripotency after long term cultivation (>5 passages). It should be a promising surface, which is immobilized growth factor on cell culture dishes for reducing usage of growth factor such as FGF-2 in the culture medium of hESCs as well as human induced pluripotent stem cells (hiPSCs). The next step is to immobilized the growth factors on the surface to control the hESCs differentiation into specific lineages of the cells.

F-2141

FEEDER-FREE CULTURE OF NAÏVE PLURIPOTENT STEM CELLS IN NORMOXIC CONDITIONS

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The establishment of the 'naïve' or ground state pluripotency has opened up new possibilities for the pluripotent stem cell (PSC) field. This state relates closely to the pre-implantation inner cell mass of the blastocyst, and is distinctively different from the later, post-implantation epiblast-derived stem cell. In mouse PSCs, these two stages have been thoroughly studied and defined. The ground state key characteristics are: hypomethylation, double X chromosome activation, cloning capability, contribution to chimeras, reduced doubling time and LIF-dependency. However, human PSCs have shown resistance towards reversion to the ground state. Several protocols have been described, all of them dependent on complex combinations of small molecule inhibitors, feeder cells, and hypoxic conditions. Moreover, these cells have shown dysregulated DNA methylation, with loss of parental imprints and DNA instability over time in culture. Short term culturing has been described on Matrigel, but the complex culture conditions complicate their handling. Indeed, most studies require a selection step in order to generate a defined stem cell population before analysis. We have shown that Inter-alpha inhibitor (IαI), a human serum-derived protein currently produced at clinical grade, supports human PSCs in coating-free conditions for

long-term culture without loss of pluripotency or DNA instability. Here we present successful culture of human and mouse naïve PSCs in feeder-free, coating-free conditions using the addition of Iαl to the medium. The cells show increased genomic stability, retained pluripotency features and differentiation potential. In human PSCs, reversion to the naïve state can be achieved without the required step using mouse feeder cells, and the method is compatible with normoxic conditions. We predict this new culture method will stream-line naïve human PSC culture for high-throughput and pharmaceutical applications. Moveover, Iαl is readily available at clinical grade, making this technology a step forward toward disease modelling and regenerative medicine applications using naïve human PSCs.

F-2143

DECELLULARIZED FETAL MEMBRANES: NATURALLY-DERIVED MATERIALS IMPROVE EX-VIVO EXPANSION OF MESENCHYMAL STEM CELLS AND FORM INJECTABLE THERMOGELS FOR CELL DELIVERY

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The use of mesenchymal stem cells (MSCs) in clinical therapies is limited by the inadequate number of functional MSCs that can be delivered to the injury site. Extracellular matrix (ECM) is a major component of the MSC niche and is critical for maintaining MSC functions. We produced decellularised ECM (dECM) from human fetal membranes (amnion and chorion) and after solubilising the dECM, we coated tissue culture plastic and tested if MSC functions were improved. Furthermore, we tested if the solubilised dECM (sdECM) could form a thermoreversible hydrogel that can be used as a carrier system for MSC delivery. We showed that cell culture substrates produced from amnion-derived sdECM improved several key functions of placental MSCs (pMSCs). pMSCs were more proliferative on amnion-derived sdECM, than tissue culture plastic alone, and Matrigel (~ 1.7x and 1.3x higher, respectively). pSMCs also were smaller in size and exhibited greater adipogenic differentiation capacity (~3.5x and 2.5x on sdECM compared to TCP and Matrigel). Additionally, sdECM formed injectable and thermoreversible hydrogels. Hydrogels produced from amnion-derived sdECM (8 mg/mL) improved the proliferation of pMSCs (~2x compared to Matrigel). Additionally, pMSCs cultured on these gels exhibited greater adipogenic differentiation efficiency as they contained a larger number of lipid deposits that were also larger in size. In conclusion, we produced sdECM bioactive materials and showed they are promising cell culture substrates for ex vivo expansion of pMSCs, and can act as a bioactive carrier system for the delivery of MSCs.

F-2145

LOW-INTENSITY ULTRASOUND-INDUCED PRETREATMENT FOR ACCELERATING MESENCHYMAL STEM CELL-DERIEVED EXOSOMES

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Exosomes, extracellular vesicles that are secreted in many mammalian cells, exert specific effects on their microenvironment and in intercellular communication. Mesenchymal stem cell (MSC)-derived exosomes are known as tissue regenerative regulator in a variety of diseases including cardiovascular injury, liver fibrosis, and neurodegenerative disease. Despite many therapeutic studies, the amount and yield of exosome produced in MSC culture medium is remarkably low. We focused on the physical stimulation of extracellular membrane onto MSCs. Using low-intensity ultrasound (LIUS), the physical forces have stimulated at 5-mm apart from MSCs under the following parameter; 247 kHz frequency, 300 ms sonication duration, and 50% duty cycle in the range of 100 to 500 mV. At 400 mV stimulation, MSC exosome were secreted about 8% more than normal group. Despite the physical force, tetraspanin membrane protein CD9 and CD81 were expressed in all groups and the expression of CD63, the common exosomal marker, were positive. Exosomes dock on to the surfaces of host cells, and transmit and/or transfer their signal sources. Onto MSC exosome, integrin, tetraspanin, and intercellular adhesion molecule 1 (ICAM-1) were expressed, furthermore docking molecules, Caveolin-1 and Clathrin, were specially regulated. To analyze proof-of-concept (PoC) of MSC exosome in in vivo, we examined a three dimensional structure of intact brain tissue after PKH-26- or GFP-induced exosome treatment in rat Parkinson's disease (PD) model. At 1 week after surgery, we conducted CLARITY (Clear, Lipid-exchanged, Acrylamide-hybridized Rigid, Imaging/immunostaining compatible, Tissue hYdrogel). Transplantation of exosome revealed cell survival in the striatum and substantia nigra compared to PD group. In this region, tyrosine hydroxylase positive cells were co-localized with CD9 or CD81 between the grafted and host cells. These results demonstrate the potential of LIUS that can be used to isolate efficiently and safely stem cell exosome.

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F-2147

HUMAN AND MURINE MESENCHYMAL STEM/ STROMAL CELLS ARE ENRICHED IN THE CD73+ POPULATION

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Mesenchymal stem/stromal cells (MSCs), which reside in the bone marrow (BM) and various other tissues, can self-renew and differentiate into mesenchymal lineages. Many groups have harvested MSCs from BM and have evaluated surface marker expression after long-term culture. However, MSCs gradually differentiate during expansion and exhibit altered proliferation rates, morphological features and functions in vitro. Variations in MSC isolation methods may alter the effectiveness of therapeutic applications. On the basis of CD73 expression, we prospectively isolated a population with a high colony-forming ability and multi-lineage potential from the rat BM. Moreover, successful engraftment of rat MSCs was achieved by using a fluorescence-conjugated anti-CD73 antibody. In humans and mice, MSCs were also purified by CD73, thus suggesting that CD73 may serve as a universal marker for prospective isolation of MSCs. We could isolate mammals MSCs from BM with CD73 expression. Our results may facilitate investigations of MSC properties and function. Now we are working on screening out which tissues could be effective to isolate transplantable CD73+ human MSCs.

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F-2149

AN APOPTOGENIC CELL CULTURE ENVIRONMENT ENHANCED MESENCHYMAL STEM CELLS' ABILITY TO REPAIR THE GLOMERULOPATHIC LIGHT CHAIN INDUCED MESANGIUM DAMAGE

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Glomerulopathic light-chain (G-LC) induced mesangium damage is difficult to revers. Our previous studies however have shown the potential for human mesenchymal stem cells (HMSCs) to repair and remodel the G-LC damaged mesangium especially the human mesangial Cells (HMCs).This study is to further facilitate the HMSCs ability to repair the G-LC induced mesangium damage by modifying the Cell Culture Environment. HMCs and HMSCs were cultured with medium containing either glucose or Betahydroxybutyrate under room temperature condition (5% O_2 5% CO_2 and 26^{o}C) or incubator condition (21% O_2 5% CO_2 and 37^{o}C). HMCs were first treated with G-LC and then with or without HMSCs. HMCs and/or HMSCs (GFP labeled) were video recorded by a 6-dimention live cell imaging system for up to 22 days of the treatment and the ultrastructural morphology were identified with electron transmit microscope (TEM) at the end of experiment. Culture medium in different time spot were tasted for the LDH as the cell toxicity index. LDH test showed that LDH of all groups under the room temperature condition were lower than that under the incubator temperature conditions. All groups of the medium with ketone but without glucose showed much higher LDH level than that of the ones with glucose. LDH were also higher in the D22 than that in D12. Both 6-D live cell imaging and TEM showed that the apoptogenic environment could cause the G-LC treated HMCs have more apoptosis and the extra cellular matrix (ECM) is more likely reversed with the HMSCs treatemtn. This study showed that by changing the incubation condition to create an apoptogenic cell culture environment enhances the apoptosis in the HMCs, and facilitate the repair and remodeling by HMSCs for the G-LC induced renal mesangial cell damage.

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F-2151

SINGLE-CELL RNA-SEQUENCING OF PATCH-CLAMPED HUMAN NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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The brain constitutes a complex assembly of thousands of different types of neurons, which neuroscientists have been classifying based on their morphology and functional properties since the seminal drawings of Ramón y Cajal. Recent advances in RNA-sequencing technologies have permitted the accurate analysis of single-cell transcriptomes. Bioinformatics analysis of single-cell transcriptomes can be used to discover new cell types. We have built upon the technical progress in genetics to combine classical patch-clamping electrophysiological and morphological analysis of single neurons with RNA-seq analysis. This powerful method, referred to as Patch-seq, enables a thorough multimodal profiling of single cells and permits to expose the links between functional properties, morphology and gene expression. In this poster, we explain in detail our protocol to isolate single neurons for RNA amplification and whole-transcriptome profiling. We have validated this protocol for human neurons generated from patient fibroblasts with induced pluripotent stem cell (iPSC) reprogramming technologies. However, the procedures can be applied to any kind of cell type in vitro or in tissue sections ex vivo with only slight adaptation of the cell collection protocol.

F-2153

STEMFORMATICS: A COLLABORATION PLATFORM FOR THE VISUALISATION OF STEM CELL DATA

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Stem cell research is increasingly accompanied by the generation of large, multi-omics datasets describing different molecular attributes of stem cell phenotypes. These provide the opportunity to describe molecules, processes and pathways involved in stem cell derivation, maintenance and differentiation. However, navigating the primary data can be unwieldy for the bench experimentalist. Stemformatics was developed to fast track finding and visualising relevant stem cell data. The primary ethos of Stemformatics is data curation, accessibility and reproducibility. To date, the Stemformatics platform has evaluated more than 560 stem cell and related datasets, containing over 16,000 samples. The public is able to view 368 mouse and human datasets with an additional 193 datasets held privately for collaborators prior to publication. Every dataset has passed through rigorous quality control steps, and has been manually curated by a biologist for experimental design and cell phenotypes. At present, available datasets include gene expression data from various platforms, covering the range of microarrays, RNA-Seq, ATAC-Seq, ChIP-Seq, small RNA, and proteomics. We provide interactive sample clustering and differential expression plots for a subset of transcriptome data. We have recently implemented beta-versions for processing and visualisation of single-cell data. With the collation of a large amount of curated data comes the opportunity to build over-arching insights into the behaviour of genes and cell types. Our interface is designed to assist users compare the expression of genes across different datasets. The interactive nature of this tool allows users to rapidly obtain the details of individual datasets, or compare multiple genes within one dataset. Users can log-in to save gene lists, generate plots, including heatmaps, and share information with collaborators. In-house meta-analysis of these data have identified signatures of different stem cell types, including Mesenchymal Stromal Cells. Stemformatics is an initative of "Stem Cells Australia", and hosts a number of consortia dataseries including the reprogramming series "Project Grandiose" and the Bloodwise "Leukomics" dataportal. We are freely accessible to all academic groups and have an international community of users.

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F-2155

RAPID ENGINEERING OF HUMAN EMBRYONIC STEM CELL LINES EXPRESSING ENDOGENOUS FLUORESCENT REPORTERS FOR OCT4, NANOG AND SOX2

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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 the study of three core human pluripotency genes: OCT4, SOX2 and NANOG. The establishment of live-cell reporters for these three genes has allowed us to study the distinct protein dynamics of each one of them during the pluripotency and differentiation states.

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F-2157

ADULT BOVINE PLATELET-RICH PLASMA (PRP)-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-rich plasma (PRP)-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 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§113.420), NeoSERA is finally gamma-irradiated at a dose of more than 30 kGy. Similar to blood donation, NeoSERA can repeatedly obtain from 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 serum NeoSERA profoundly enhances MSC proliferation.

F-2159

MULTI-LAYERED GOLD NANOCLUSTER FOR EFFIEICNET DIFFERENTIATION OF STEM CELLS BY LONG TERM GENE DELIVERY

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Gene therapy is a revolutionary way to treat the causes of diseases at the genetic level. In addition, it is possible to achieve long-term expression in vivo and genetic information mutated by genetic recombination can be corrected. Many studies have focused on gene delivery as a promising tool to treat many diseases at the genetic level. Genes encoding transcription factors, which regulate pathways related to cell proliferation, growth, differentiation, and death, have been the focus of great interest in this regard. Especially, internalization of genes encoding transcription factors related to stem cells differentiation has been extensively studied. Cells have been transfected with genes encoding specific transcription factors in an attempt to enhance differentiation towards the desired lineage and prevent differentiation towards other lineages. Moreover, expression patterns of delivered genes, long-term delivery or time-dependent release, are needed to be controlled for efficient differentiation. In this regard, we attempt to design and synthesize gold-based nano-cluster as a gene carrier, named gold-cluster nanoparticle

(GCNP). GCNP were produced by sequential assembly of each component several times with stable and biodegradable gold, di-hydroxylphenylalanine (DOPA) conjugated polyethylenimine (PEI), and heparin. The physical properties of GCNP were characterized by TEM, DLS, and UV-vis-spectrometer. This GCNP was applied to human mesenchymal stem cells (MSCs). GCNP deliver the pDNAs more efficiently than PEI alone. Also, comparing to the one-time fabrication of the same amount of GCNP components, expression of the delivered-gene level and how long expression of the gene could last were evaluated by RT-PCR, western blot analysis, and confocal laser microscope. To be comprehensive, this system could be a potential application to efficient differentiation of stem cell.

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F-2161

LARGE-SCALE USE OF KNOCKOUT VALIDATION TO CONFIRM ANTIBODY SPECIFICITY

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Antibodies are the most common tools in basic science and clinical research. Over the past several years there has been an ongoing discussion around antibody validation in light of poor experimental reproducibility between laboratories. This is in part due to the cross-reactivity between antibodies and off-target proteins and the variability between different antibody batches. As a result, experimental irreproducibility leads to wasted resources and compromises the advancement of science. In response to this, Abcam has introduced knockout (KO) validation to help produce target-specific antibodies. This initiative uses KO cells lines made possible through a partnership with Horizon Discovery. Target genes are mutated via CRISPR-Cas9 within a haploid cell line. This results in a frameshift and a complete loss of gene expression. These KO cell lines provide a true negative control for antibody validation as a lack of signal confirms that the antibody binds to its intended target. As a result, both antibody specificity and reproducibility on a large scale can be addressed. We have KO-validated hundreds of antibodies, including several that are relevant to immunology and immuno-oncology research. Here we present the data related to key targets validated in both flow cytometry and immunocytochemistry. The antibodies have been tested in KO and wild-type cells to confirm their specificity. By providing researchers with reliable and specific antibodies that work first time we hope to minimize wasted resources and improve reproducibility.

PLURIPOTENCY

F-2163

MAINTENANCES OF NAÏVE HPSCS USING PLASMA JET

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Human naïve PSCs may be useful to overcome the problems of heterogeneous populations, variation between cell lines, and the poorer differentiation efficiency of human PSCs compared with mouse PSCs. However, there remain many challenges in the field of naïve pluripotency. All protocols for generating and maintaining human naïve PSCs are slightly different cellular states. There are still remained many issues for maintaining naive ips and es cells. Current maintaining protocols need to develop more easy and less costs for applying Naïve pluripotency research and clinical medicine. Especially, upon transferring these cells to these current conditions, some lines adapt very well while others spontaneously differentiate or contain a mixture of differentiated cells and ips colonies although all ips lines have been generated from the same parental cell line. The problem is due to batch to batch variability of used supplements such as N2 and B27. To overcome these above fundamental limitations, we deploy reactive oxygen species (ROS) generated by plasma jet as an alternate source of novel naive hPSC culture. Over the last few years, studies have suggested that oxidative stress plays a role in the regulation of hematopoietic cell homeostasis. An optimal plasma jet treatment generates a minor amount of ROS in cells and culture medium. The generated ROS mitigates markedly dissociation-induced apoptosis of naïve hPSC and increases substantially attachment and proliferation of hPSCs. The continuous optimal ROS stimulus on naïve hPSCs facilitates fast colony formation in vitro culture. They can be differentiated into various cell types in vitro and in vivo. The optimal ROS generation protects a drop of maintain cost in culture medium without using N2 and B27 supplements and mediates safe genome stability in long-term culture of naïve hPSCs. Here, we first demonstrate a direct correlation between ROS production, long-term maintenance of naïve hPSC, and the occurrence of genomic instability. Our study will fulfill all requirements such as simple, robust, cost-efficient and safe production of naïve hPS cells by taking advantage of ROS in automated GMP facility.

F-2165

CELL-CELL CONTACT RESPONSIVE ACTIVATION OF CX30.3 IN MOUSE EMBRYONIC STEM CELLS

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Cell-cell contact and successive molecular movement between the contacted cells occur during developmental growth from the stage of embryonic single cells. In response to the entering molecules, the cells show cooperative or agonistic behavior. The molecular mechanisms occurring from cell contact to the intercellular molecular movement are still unclear. To elucidate them, we focused on GJ-mediated mechanisms and analyzed the dynamic behavior of each isoform of connexin. Here Cx30.3 in mouse embryonic stem (ES) cell has been focused because of its potential property of being able to respond to the physical contact with another ES cell. The expression level of Cx30.3 in ES cell colonies dramatically decreased immediately after the dissociation of the colonies into single-cells. In contrast, it increased markedly when single-cells were contacted with each other by hanging-drop culture method. Such an increase of Cx30.3 expression was retarded by the inhibition of E-cadherin signaling with anti-E-cadherin. The expression of Cx30.3 was speculated to be regulated by the cell contact with cells of the same cell line and by the downstream signals in E-cadherin mediated signal in ES cells. On the other, a more ubiquitously expressing Cx43 showed less response to the cell contact. Specific properties of Cx30.3 in ES cells will be discussed from the viewpoint of the control of growth and differentiation.

F-2167

KCTD16 MAINTAIN THE NAÏVE STATE OF MOUSE EMBRYONIC STEM CELLS

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Kctd16 is cytosolic proteins and is one of the auxillary subunits of the GABAB receptor. It is unknown that whether Kctd16 have function in the mouse embryonic stem cells. We found that Kctd16 is the pluripotency regulator Oct4 target gene. Kctd16 knockdown induced the transition from naïve to primed state in mouse embryonic stem, furthermore, Kctd16 knockdown affect Nodal expression. Kctd16 maintenance the naïve state of mouse embryonic stem cells is likely to be regulated Nodal signaling pathway.

F-2171

NANOSEGMENT MOLECULAR DESIGN OF CELL CULTURE BIOMATERIALS FOR HUMAN EMBRYONIC STEM CELLS PROLIFERATION

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Stem cells are an attractive prospect for regenerative medicine and tissue engineering. The dishes coated with Matrigel, Synthemax II, Laminin 511, Laminin 521, and Cellstart are typically used for the cell culture substrates for human embryonic stem cells (hESCs). These coating materials are based on extracellular matrix (ECM), which are secreted from cells. Our previous study showed that hESCs can be cultured on polyvinylalcohol-co-itaconic acid (PVA-IA) hydrogels conjugated with higher concentration of oligovitronectin (>500 mg/mL) as hESC culture substrates. In order to solve the reason of the higher concentration usage of oligovitronectin conjugated on PVA-IA hydrogels, we designed another PVA-IA hydrogels conjugated with oligovitronectin for hESCs culture substrates using a crosslinker of PEG-AEAC (poly(ethylene glycol)-2-aminoethyl ether acetic acid) and we evaluated hESC attachment and pluripotency cultured on these hydrogels. The PVA-IA hydrogels were prepared by coating aqueous PVA-IA solution on the tissue culture polystyrene (TCPS) dishes and were crosslinked with glutaraldehyde to control hydrogel stiffness. Twice activation was performed to conjugate oligovitronectin on PVA-IA hydrogels via PEG-AEAC crosslinker by the peptide bonding reaction between carboxylic group and amino group. We investigated the length effect of crosslinking agent of PEG-AEAC on the culture of hESCs on PVA-IA hydrogels grafted with oligovitronectin via PEG-AEAC having different length of PEG. After long term culture of hESCs on the PVA-IA hydrogels developed in this study (more than 10 passages), we evaluated pluripotency and differentiation ability of hESCs cultured on the hydrogels.

F-2173

LARGE-SCALE GENETIC SCREENS FOR CULTURE ADAPTATION AND TUMORIGENICITY RELATED GENES IN HUMAN PLURIPOTENT STEM CELLS

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hPSCs (human pluripotent stem cells) acquire genetic changes that may alter key cellular behaviors such as growth and differentiation. These changes can increase the cells tumorigenic potential, which is the most challenging issue of future hPSCs-based clinical treatments. Traditionally, research is focused on studying mutations that are gained and selected during prolonged culturing. Here we took an alternative approach where we introduced mutations and followed the selection forces. We generated a genome-wide screening system for genes and pathways that provide a growth advantage either in vitro or in vivo. This system consists of two large hPSCs libraries, with either gain-of-function or loss-of-function mutations. We then exposed these libraries to multiple treatments that subjected them to stress and growth competition. First, we treated the libraries with PluriSIn#1, a specific inhibitor of hPSCs growth. In the surviving colonies, we detected multiple genes from the RAS pathway being affected. Indeed, we showed that RAS activation reduces the sensitivity to PluriSIn#1 treatments. Next, we examined the selection forces during prolonged propagating of the libraries in culture. We detected specific integrations that rapidly took over the culture, accompanied by an increased growth rate. Examination of the integration sites revealed the advantageous effects of RHO-ROCK pathway inhibition. Importantly, we could show that this pathway is also altered during culturing of genetically unmodified hPSCs, emphasizing the importance of this pathway for hPSC growth. Lastly, we injected the libraries under the skin of immunodeficient mice to test selection during teraroma formation. We could demonstrate the strong clonality of the tumors, and to highlight the importance of the PI3K-AKT and HIPPO pathways for the teratoma formation process. This two pathways coordinating regulate cell proliferation, survival and organ size. Indeed, chemical inhibition of these pathways during teratoma formation significantly reduced the tumor weight. Overall, our novel study revealed the key genes and pathways relevant for the tumorigenicity and survival of hPSCs, and thus assists in understanding and confronting their tumorigenic potential.

F-2175

ANTI-APOPTOTIC MUTATIONS PROMOTE CHROMOSOME INSTABILITY IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are susceptible to genetic changes in culture, including numerical and structural chromosomal aberrations. Despite the importance of assuring genetic stability of hPSCs for clinical applications, the process leading to the generation and persistence of mutations in hPSCs remain poorly characterised. Here using live-imaging, we show that mitotic errors including lagging chromosomes or chromosomal bridges occur frequently in hPSCs. Nonetheless, the mitotic checkpoint is functional in hPSCs and drug-induced prometaphase arrest leads to rapid apoptosis in undifferentiated but not differentiated cells. HPSCs express high level of NOXA in undifferentiated state. Knocking-out NOXA by CRISPR or upregulation of BCL-XL significantly reduced mitotic cell death, lead to significant larger teratoma formation and allowed survival of aneuploid cells. Transcriptome profiling revealed that NOXA knock-out or BCL-XL overexpression promoted cell survival by inhibiting apoptosis and immune response pathway genes. Moreover, during stress conditions, the mutation rate increased significantly in these cells compared with that in normal cells. Our results demonstrated that the low threshold of apoptosis safeguards hPSCs' genome integrity by clearing out cells undergoing abnormal division or genome stress, and a particularly frequent mutation in hPSCs, the amplification of BCL2L1 on chromosome 20q, although not directly oncogenic, allows cells to develop larger teratoma and possibly chromosome abnormalities.

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PLURIPOTENT STEM CELL DIFFERENTIATION

F-3001

DIFFERENTIATION OF HIPSC TOWARDS HEPATIC LINEAGE IN 3D CULTURE YIELDS MIXED POPULATION OF DUCTAL AND HEPATOCYTE-LIKE CELLS

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The liver is a complex and highly organized tissue with important metabolic functions. To perform multi-step processing of xenobiotics, secretory (i. e. ductal and biliary tissue) and hepatocytes cross-talk is extremely important. iPS-derived cell technologies towards large scale drug development/screening and disease modeling must comply with tissue physiology. Most currently available differentiation protocol attempts to isolate/purify hepatocytes from cholangiocytes and vice-versa, which requires expansive and long protocols. Here we report a simple and relatively low-cost method of hepatic differentiation from hiPSC that yields a mixed population of ductal and hepatocyte-like cells in 3D culture. hiPSC are harvested and cultured in low-binding plates and kept in orbital agitation (95 RPM) for 24 hours. Agitation in low-binding plates is kept thought-out the entire protocol. Embryonic bodies are then differentiated towards definitive endoderm for three days in presence of Activin A (100ng/mL) and Wnt3a (25 ng/mL). Hepatic lineage commitment is achieved by culture of spheroids in presence of knock-out serum replacement (20%) for six days. For final differentiation spheroids are cultured with Oncostatin A (20ng/mL) and HGF (100ng/mL). After 18 days, mixed population of ductal-like hollowed spheroids and rounded cell aggregates are visible. Characterization of cell spheroids and ductal-like spheroids are CK7+/EpCAM+ and non-ductal structures are ALB+/CK18+/A1AT+. RT-qPCR shows expression of important metabolic enzymes of phase I, II and III. Spheroids have CYP450 iso-enzymes (1A2, 2B6 and 3A4) activity and are able to store glycogen. Furthermore, secretion of albumin, alpha1-anti-trypsin, APOB100 and alfa-fetoprotein confirmed enhanced hepatic function of the 3D culture. Proteomics and metabolic profiling will be performed next. In conclusion, 3D culture and differentiation of hiPSC towards hepatic lineage is capable of generating most important liver parenchyma cells and can be easily scaled-up.

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F-3003

CHARACTERISTICS OF HUMAN CRANIAL MESENCHYMAL STEM CELLS DERIVED FROM SPHENOIDAL, TEMPORAL AND OCCIPITAL BONE

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Human mesenchymal stem cells (MSCs) have been considered as one of the candidates for cell transplantation therapy. Most human bone marrow-derived MSCs have been harvested from iliac bone. We previously reported the isolation and establishment of MSCs from human cranial bone (cMSCs), which had a greater tendency to differentiate into neuron-like cells. The facial bone, skull base, frontal bone, and temporal bone are derived from the neural crest, but occipital bone is derived from the mesodermal germ layer. Therefore, the characteristics of MSCs harvested from cranial bone marrow may be different depending on the sampling site. In the present study, we compared the differences in characteristics of cMSCs harvested from the temporal or sphenoidal bone and occipital bone. Cranial bone marrow waste was collected from the temporal, sphenoidal, and occipital bones during craniotomy or craniectomy in the operative procedure. The samples were seeded onto culture dishes and cells were established. Adherent cells were collected at confluency and counted at every passage until six passages to evaluate proliferation potential. We then analyzed the gene expression associated with neural crest markers (snail, slug, p75) and neurotrophic factors. Adherent cells were observed at 7-10 days after seeding. We successfully established cMSCs from all sampling sites. There were no specific differences in proliferation potential or expression of neural crest markers and neurotrophic factors between cMSCs harvested from the temporal or sphenoidal bone and occipital bone. We are now planning to investigate their differentiation potential, but the sampling sites for cranial bone marrow are restricted by surgical procedure. In the present study, cMSCs derived from different sites of cranial bone

exhibited similar characteristics related to expressions of neural crest markers. Further evaluation is needed to disclose functional recovery after cell transplantation into model animals of CNS disease.

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F-3005

HAIR FOLLICLE REGENERATION BY DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS ENGINEERED INTO MICROSPHERES

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iPSCs have been directed to various cell fates, however, the derivation of Dermal Papilla (DP) cells had not been reported before. DP plays a dominant role during hair follicle morphogenesis and is critical in defining hair thickness, length, and life cycle. Our proposed study attempted to explore for the first time the derivation of functional DP cells from iPSCs to induce de novo formation of hair follicle after transplantation, to overcome the limitations currently present in this field. For this purpose, we developed an alternative approach to generate unlimited numbers of hair-inducing DP cells from iPSCs using Neural Crest (NC) cells as intermediate. We investigated the effect of growth factors, such as Wnt, FGF, BMP and R-spondin on the differentiation of iPSC-derived NC cells into DP cells. We observed that the activation of Wnt pathway enhances the differentiation process, as demonstrated by the expression of classical DP cell markers (Versican and Alkaline Phosphatase) and the up-regulation of relevant DP genes (Nexin, Corin, LEF1, SDC1, HEY1, EGR3). Moreover, we used FACS methodologies to enrich DP cells using Syndecan-1 and Integrin-α9β1 as surface markers. Based on the evidence of the importance of an appropriate 3D environment for DP cells to maintain their hair follicle-inducing properties, we cultured the iPSC-DP cells in spheres using the hanging drop method. iPSC-DP spheres were then transplanted on the back skin of Athymic nu/nu in combination with mouse embryonic keratinocytes. After 2 weeks, we observed formation of ectopic hair follicle expressing Versican, P-cadherin, Sox9, and keratins 5 and 8. No hair follicle growth was observed when keratinocytes alone were transplanted. In conclusion, the turning point of this work is the derivation of functional iPSC-DP cells in a well-defined environment, capable of stimulating a robust hair growth, providing an unlimited source of cells for transplantation. The use of iPSC might allow us to induce the neo-genesis of human hair follicle from autologous adult cells in vitro, contributing to create a highly customized, patient-focused approach to stem cell treatment for hair loss disorder. This will eventually help people to reacquire their sense of beauty and self-esteem in their social life.

F-3009

INVESTIGATION OF MARKER GENES PREDICTING OSTEOGENIC DIFFERENTIATION POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS

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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, we aimed to search for genes that are predictable markers of osteogenic differentiation potential of hMSC before induction of bone differentiation. We collected hMSC strains with different bone differentiation potential and performed comprehensive gene expression analysis between those hMSC strains. 13 lots of hMSC derived from bone marrow of different donors were induced to osteogenic differentiate in two kinds of methods and the each degree of bone differentiation of those hMSCs was determined by measurement of calcium deposits by Alizarin Red staining. 13 lots of hMSC were divided into 3 groups by bone differentiation potential. mRNA expressions before and after induction of bone differentiation into each hMSC was comprehensively analyzed. Then, we extracted some genes whose mRNA expression levels before induction correlated with the bone differentiation potential of hMSC. hMSCs were divided into three groups, potentially having high, middle, and low bone differentiation (5, 3 and 5 lots, respectively). There were 52 genes whose hMSC mRNA expression level before induction of bone differentiation was significantly higher depending on its osteogenic differentiation capacity, and 35 genes which were significantly lower. Depending on some bio-functions of the gene, we narrowed down from 87 to 33 genes, and found multiple genes such as growth factors and genes involved in cytokine receptors as marker candidate genes that can predict the osteogenic potential of hMSC. It was confirmed that some of those

genes expressions were influenced by induction of osteogenic differentiation. Several genes also showed a correlation between their expression levels and lowering of differentiation potential due to passage of cells.

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F-3011

INTRODUCTION OF CRISPR/CAS9 IN STEM CELLS FOR NOVEL MEGAKARYOCYTE-ERYTHROID PROGENITOR CELL LINE DEVELOPMENT

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Recent advances in genome editing techniques including CRISPR/Cas9 enabled us to design novel strategies to study mechanisms of cell development and cell therapy. Many researchers have shown that Cas9 and single guide RNA (sgRNA) could indeed target the gene of interest and successfully conduct knockout of the gene. However, the physiological outcome after CRISPR/Cas9-induced knockout during megakaryopoiesis have not been estimated. Previously, we reported a strategy that inducible suppression of Gata1 by short hairpin RNA (shRNA) in murine ES cells could expand megakaryocyte-erythroid progenitors (MEP) termed G1ME2 and then upon GATA1 restoration, the cells terminally maturated to generate functional platelets. The time and cost for platelet differentiation in vitro were reduced significantly using the G1ME2 cell line. Not only GATA1, a variety of transcription factors such as FLI1 and TAL1 are also involved in hematopoiesis, suggesting that G1ME2 system can be improved by targeting more genes. Here, we introduced Cas9 under the tetracycline response element (TRE) using ZX1 mESCs that can be modified by various sgRNAs. Assessment of the knockout will be functionally analyzed by generation of proliferative MEPs. We are also trying to modify human hematopoietic stem cells (HSCs) in the future. Therefore, we designed sgRNAs to target human GATA1 and screen them on human erythroleukemia cell line (K562) that expresses GATA1 protein. This study will provide a novel strategy for development of stem cell-derived platelet transfusion therapy.

F-3013

DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO CARDIOMYOCYTES CULTURED ON BIOMATERIALS IMMOBILIZED NANOSEGMENTS IN OPTIMAL CULTURE MEDIUM

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Patients suffer from loss and damage of organs from accidents, birth defects, and diseases every year. Stem cells, such as human embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells, are attractive prospects for regenerative medicine. However, it is difficult to induce human ES cells to differentiate into particular cell types, which we desired. Currently, it has not yet investigated the effect of cell culture biomaterials immobilized with extracellular matrices (ECMs) or nanosegments derived from ECMs and the effect of cul-ture medium to induce hPSCs differentiation into cardiomyocytes. We developed nanosegment-grafted biomaterials having different elasticity and de-veloped optimal differentiation medium to induce the differentiation of hES cells into cardi-omyocytes. We developed several biomaterials having different elasticity for hPSCs differentiation into cardiomyocytes. We prepared (1) ECM-coated dishes, (2) PVA-IA (polyvinylalcohol-co-itaconic acid) hydrogel dishes having different elasticity that are grafted with several ECMs, and (3) PVA-IA hydrogel dishes having different elasticity that are grafted with cell-adhesion oligopeptide. We also investigated the differentiation efficiency using different induction medium: (a) Commercial cardiomyocyte induction medium, (b) RPMI 1640 medium supplemented with B27 and bovine serum albumin, (c) CDM3 medium developed by literature.2 On day 0, we replaced the expansion medium into cardio-myocytes differentiation medium containing the GSK3B inhibitor.3 On days 1-2, we ob-served that 30%~40% of the cells in the medium shown in (a) and 50~60% of the cells in the medium (b) of the cells were died and detached from the surface. However, the center of the colony of living cells were getting thicker and became compact. These cells were differenti-ated into cardiomyocytes on days 5-6. On day 8-10, we successively observed the contract-ing colonies on the surface. We evaluated the optimal elasticity of biomaterials, preferable nanosegments immobi-lized

on the biomaterials and medium components for the differentiation of hPSCs into car-diomyocytes. The results for cardiomyocyte induction of hPSCs will be used in clinical ap-plication and in the investigation of molecular mechanism of specification and maturation of cardiomyocytes.

F-3015

A COMBINATORIAL APPROACH TO GENERATE MATURE RED BLOOD CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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The manufacture of therapeutic blood cell types from a potentially limitless source such as human pluripotent stem cells (hPSC) in a controlled and reproducible manner is a critical challenge in regenerative medicine. It has proven particularly challenging to produce fully functional adult-like hematopoietic cell types. The failure to generate mature cell types from hPSCs could be related to embryonic-like properties of the hPSC-derived haematopoietic progenitor cells (HPCs). Here we combined two innovative technologies, a combinatorial bead-based screening platform (CombiCult®) and lineage specific reporter cell lines to identify novel and effective protocols for generation of adult-like HPCs that are capable of differentiating into mature red blood cells (RBCs). We used 2 hPSC lines that carry fluorescent reporters in genes involved in HPC development in vivo. The RUNX1C-GFP reporter hESC line mimics endogenous expression of the haematopoietic transcription factor, RUNX1, and monitors production of HPCs from hemogenic endothelium. KLF1-mCherry reporter iPSCs line marks CD235a+ expressing cells, representing the primitive wave of erythropoiesis at the early stage of the differentiation process. We had carried out two CombiCult® screens that systematically tested tens of thousands of protocols to identify combinations of small molecules, cytokines and growth factors that promote differentiation of hPSCs into adult-like HPCs that are marked by the positive expression of RUNX1c-GFP and negative expression of KLF1-mCherry reporter genes. Following 4-step combinatorial screening, new protocols that generate HPCs with maximum expression of RUNX1-GFP and the hematopoietic stem cell marker, CD34, together with minimum expression of KLF1-Cherry were identified and ranked using Plasticell's bioinformatic software, Ariadne™. 15 top-ranking protocols were selected for further validation and for functional analyses including CFU-C assays. The proportion of CombiCult® beads containing HPCs were further exposed to media that promote differentiation into the erythroid lineage. Beads carrying cells positive for erythrocyte markers and negative for expression of embryonic hemoglobin-epsilon were selected for tag analysis to discover continuous protocols for hPSC-> HPC ->RBC differentiation.

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F-3017

FULLY DEFINED DIFFERENTIATION OF MEDIUM SPINY NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Recent advances in disease modeling with directed differentiation of induced pluripotent stem cells have offered alternatives to mouse models and given us the ability to understand disease on a cellular level. Familial Dyskinesia with Facial Myokymia (FDFM) is an autosomal dominant movement disorder. Characterized by involuntary twitches or tremors, FDFM has been causatively linked to several mutations in the adenylate cyclase 5 (ADCY5) gene, which is highly expressed in the striatum. Medium spiny neurons (MSNs), most well known for their role in Huntington's disease, are the principal projection neurons of the striatum and the hypothesized affected cell type in FDFM. Previous MSN differentiation protocols reported enriched populations containing between 5-50% MSNs. Here we report an improved and fully defined method for MSN differentiation through stimulation of the TGFb signaling pathway by Activin A. We have successfully direct human induced pluripotent stem cells (iPSCs) to enriched populations of 54% DARPP32+/CTIP2+ MSNs, with a 50-fold and 200-fold increase respectively in gene expression levels compared to the no treatment control. Our findings allow for more efficient generation of MSNs from IPSCs for stem-cell based drug discovery and therapies.

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F-3019

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

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Donor-derived red blood cells (RBCs) are the most common form of cellular therapy. However the source of cells dependent on donor availability with a potential risk of alloimmunization and blood borne diseases. In vitro derivation of RBCs allow their thorough characterization therefore provides access to better matched product and by using iPSCs, an immortal source would avoid donor-dependency. The in vitro derivation of iPSC-RBC has proven to be possible, however the reproducibility, yield, purity and enucleation potential require further improvement. We have established a tightly controlled and reproducible single cell-derived iPSC monolayer differentiation protocol that, upon hematopoietic specification using three defined growth factor supplementation steps yielded erythroblasts (EBL). This method resulted in 2-6*10^5 EBLs/iPSC with 100% purity (CD71+/CD235+) within 21 days using non-adherence G-rex system, which was reproducible using different iPSC lines. Maturation of iPSC-EBLs yielded CD71low/CD235+ orthochromatic normoblasts, which were a mixed population concerning its Hb content: fetal Hb (50,1%), adult Hb (<5%) and embryonic Hb (primitive 15%). These matured erythroid cultures had 30-40% enucleation rate based on their nuclei count, however the resulting reticulocytes appeared to be instable. RNA-seq analysis comparing iPSC-erythroid cells to human fetal liver-, cord blood- and peripheral blood-derived erythroid cells during differentiation revealed significant differences and identified specific genes that may be used to further augment iPSC-erythroid terminal differentiation to erythrocytes. In conclusion, we showed that our monolayer differentiation approach is simple, robust and produces 100% pure erythroid cells in a reproducible manner and is compatible with upscaling. The iPSC-derived erythroid cells have relatively high enucleation potential however the reticulocytes are unstable which may be caused by the presence of different erythroid waves linked to a distinct erythroid program. Our system is a combination of virus-, integration- (episomal) and feeder-free iPSC derivation with differentiation using GMP-media (Cellquin, Home-made), which facilitates the implementation to GMP-grade conditions moving toward clinical application.

F-3021

STUDY OF YAP1 DURING EARLY CARDIAC DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Cellular specification is precisely orchestrated during heart development to assure proper organ functionality and regenerative capacity during the life of the organism. This control includes regulation of cell-type-specific gene expression on the single cell level as well as intercellular signaling and cell-matrix interaction in the whole-organ perspective. Important role in the heart development plays Hippo signaling pathway. In our work we focused at YES Associated Protein 1 (YAP1) that was originally described as a downstream effector of Hippo pathway. It controls cell- and organ-size and 3D organization. Together with its paralog TAZ1, YAP1 acts in the nucleus as transcriptional co-activators, by binding to hundreds of stage- and cell-specific transcription factors and activating given genetic programs. As YAP1 does not possess any ability to bind DNA per se, we analyzed the transcription factor binding motifs known to target the sites identified by ChIP-Seq. Annotation of the main molecular functions among gene targets revealed that YAP1 is involved in regulation of various cellular processes such as morphogenesis and development, cell growth, proliferation, differentiation and pluripotency and several others. Among exponentially raising evidences of particular YAP1 functions, understanding of the interplay among them is becoming unclear and laborious. Based on the results of RNA sequencing (RNA-seq) arising exponentially during the last decade and revealing that 90-95% of genes in humans undergo alternative splicing and thanks to the recent annotation of YAP1 transcriptional variants in human cells we further decided to check for the role of YAP1 splicing variants in human heart and during cardiomyocyte differentiation from iPS cells in vitro. Our results suggest that expression of YAP1 transcriptional variant differ among individual cell types of the heart. We also measured changes in the

expression of total YAP1 and individual isoforms during early steps of cardiac differentiation. On the model of IPS derived cardiomyocytes we further show that over-expression of those YAP1 variants that are typical for adult CMs, influences several cardiac markers. All together our result suggest that YAP1 has important and transcription-variant-specific regulatory role in cardiac tissue.

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F-3023

DIFFERENTIATION OF MSCS FROM HUMAN IPSCS RESULTS IN DOWNREGULATION OF C-MYC & DNA REPLICATION PATHWAYS WITH IMMUNOMODULATION TOWARD CD4 & CD8 CELLS

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Multilineage tissue-source mesenchymal stem cells (MSCs) possess strong immunomodulatory properties and are excellent therapeutic agents, but require constant isolation from donors. Differentiation of human induced pluripotent stem cells (iPSCs) into MSCs offers a renewable source of MSCs; however, reports on their immunomodulatory capacity are discrepant. Using MSCs differentiated from iPSCs reprogrammed using diverse cell types and protocols, and in comparison to human embryonic stem cell (ESC)-MSCs and bone marrow (BM)-MSCs, we performed transcriptome analyses and assessed for immunomodulatory properties. Differentiation of MSCs from iPSCs results in downregulation of c-Myc and DNA replication pathways. Similar to ESC-MSCs and BM-MSCs, all 4 lines of iPSC-MSCs can significantly suppress activated human T cell proliferation, strongly modulating both effector CD4 and CD8 T cell populations—including IFNγ+- and IL-17+-secreting populations—toward more immunomodulatory populations such as regulatory CD4 T cells in vitro and in vivo in a mouse model of induced inflammation. These findings support that iPSC-MSCs possess low oncogenicity and strong immunomodulatory properties regardless of cell-of-origin or reprogramming method, and are good potential candidates for therapeutic use.

F-3025

GENERATION AND CHARACTERIZATION OF RETINAL PHOTORECEPTORS FROM MUTANT AND CRISPR/CAS-EDITED USHER SYNDROME PCDH15 IPSCS

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Usher Syndrome (USH) is the leading cause of inherited deaf-blindness and one of the most devastating sensory impairments. The PCDH15 gene at the USH1F locus, encodes a 1,955 amino acid, is a member of the cadherin superfamily and plays an essential role in maintenance of normal retinal and cochlear function. Patients with the Arg245XTer variant in exon 8 of the PCDH15 gene typically have congenital deafness and develop retinal degeneration in teenage years. As a prelude to therapy development, we have combined patient-specific induced pluripotent stem cells (iPSCs) technology with CRISPR/Cas-editing to generate a robust in vitro model of this disease. We reprogrammed fibroblasts from a patient's homozygotic for the PCDH15 Arg245Ter disease causing variant and virtual karyotyping confirmed the absence of chromosomal aberrations. Given that homology modelling of the PCDH15 protein lacking exon 8 (exon skipped) suggests no gross alterations to tertiary structure, we designed two guide RNAs targeted to intron 7 and 8, such that the simultaneous DNA cleavage in both introns by CRISPR/Cas9 system and NHEJ DNA repair remove exon 8 (exon skipped). After screening 480 clones, Eend-point PCR across the genomic locus revealed the desired 1146bp deletion and Sanger sequencing confirmed that exon 8 had been deleted in both chromosomes in 1 clones. Prior to proceeding to photoreceptor cell differentiation, additional embryoid body assays confirmed the pluripotent state of exon skipped iPSCs. Isogenic iPSCs were cultured to ~80% confluency, then induced with chemically defined medium to generate self-forming neuroretinal structures in a feeder-free condition.

Structures were isolated as a floating culture to enable photoreceptor differentiation. We characterized derived cells by the presence of specific photoreceptor markers with immunohistochemistry and RT-PCR at week 25. For all experiments, isogenic exon-skipped H9 hESC cell lines were generated and analysed in an identical manner. Ongoing characterisation is being undertaken to functionally investigate the consequences of exon 8 skipping in the PCDH15 gene, and it is envisaged this could establish the utility of CRISPR/Cas-based genome editing as a potential therapy for genomic-based rescue in this form of Usher Syndrome.

F-3027

OVER-EXPRESSION OF BCOR-SHORT INHIBITED THE OSTEOGENIC DIFFERENTIATION POTENTIAL OF STEM CELLS FROM APICAL PAPILLA

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To investigate the effect of BCOR-short on the osteogenic differentiation potential of stem cells from apical papilla (SCAPs). Retroviral HA-BCOR-short was used to over-express the BCOR-short in SCAPs. Western Blot was used to detect the expression of HA-BCOR-short. Alkaline phosphatase (ALP) activity assay was used to detect the early marker of osteogenic differentiation-ALP activity, Alizarin-red staining and quantitative analysis of calcium were used to investigate the mineralization potential of SCAPs in vitro. Real time RT-PCR was used to detect the osteogenesis related genes expressions bone sialoprotein (BSP), osteocalcin (OCN) and AP2A. Western Blot result showed that HA-BCOR-short could over-express in SCAPs. The over-expression of HA-BCOR-short inhibited ALP activity, mineralization, and the expressions of BSP and OCN in SCAPs. The AP2A expression was repressed in HA-BCOR-short over-expressed SCAPs compared with control group. Our results indicated that over-expression of HA-BCOR-short repressed osteogenic differentiation potential of SCAPs and the expression of AP2A.

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F-3029

GENERATION OF CLINICALLY SAFE AND EFFICACIOUS CARDIOVASCULAR PROGENITORS IN A CHEMICALLY DEFINED AND XENO-FREE LAMININ-221 BASED SYSTEM

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The human heart has limited regeneration capacity after birth and methods for safe and reproducible generation of stem cell-derived therapy for use in patients have not been successful. These include culturing of the stem cells or its products in undefined conditions and and on xenogenic materials like MatrigelTM that render the cells unsuitable for human therapy and extensive variations in the differentiation protocols. In order to replace the xenogenic MatrigelTM and the use of ROCK inhibitor for a fully defined protocol, we have explored the extracellular matrix (ECM) components in the heart and found that a cardiomyocyte laminin protein is a highly biologically culture matrix. Here, we show based on deep RNA sequencing human heart muscle, that laminin 221 (LN-221) is the most abundantly expressed laminin in the human heart. We synthesized LN-221 as a recombinant human protein and found it to drive pluripotent human embryonic stem cells (hESCs) to the cardiovascular lineage under fully defined human conditions. LN-221 induces specific biological effects in hESCs by downregulating genes involved in pluripotency and teratoma development, while upregulating genes for cardiac development. We have also identified a highly reproducible expression signatures during differentiation of two separate hESCs to cardiovascular progenitors (CVPs) that become beating cardiomyocytes (CMs). Cardiac transplantation of CVPs into ischemic reperfusion heart infarction region in mice resulted in the formation of human muscle bundles. These bundles were formed from single CVPs which later organized itself in vivo into well-organized CMs with normal sarcomeres and gap junctions. Transplanted hearts also showed improved cardiac function by echocardiogram. Moving towards a clinically safe therapy, we investigated the safety of these CVPs using a teratoma assay and in vivo imaging. We propose that LN-221-mediated differentiation of hESCs to CVPs may be developed as a new and fully human methodology for regenerative cardiology.

F-3031

REGULATION OF MESENDODERM DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS BY TATA-BOX-BINDING PROTEIN-RELATED FACTOR 3

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Transcriptional activation is a major step in the regulation of gene expression during development. Organogenesis requires a cascade of transcriptional events to control the spatial and temporal expression of tissue-specific genes. General transcription factors (GTFs) can bind to core promoter region to initiate gene transcription. In generally, tissue-specific gene expression is primarily attributed to the interaction between tissue-specific transcription factors and its target genome sequences, while the role of GTFs and core promoter recognition in directing tissue-specific gene expression remains largely unknown. TATA-box-binding protein-related factor 3 (TRF3) is a vertebrate-specific member of TBP family with a conserved carboxy-terminal region and a highly different N-terminal region to TBP. As a general TF, TRF3 expresses at varying levels in human and mouse tissues. It mediates early embryo development of frog, zebrafish and mouse. However, due to the highly variable N-terminal among different species, the function of TRF3 in human early development is unknown and cannot be speculated from animal models. Here we reported the important role of TRF3 in regulating mesendoderm differentiation of human embryonic stem cells (hESCs). During mesendoderm differentiation, the abundance of TRF3 transcripts was upregulated, while the mesendoderm differentiation was significantly hindered in TRF3-knockout hESCs. With ChIP-RTPCR analysis, we found that TRF3 directly bound to the promoter region of mesendoderm genes. Further, we revealed that the transcription co-factors governed the germ layer specific transcription ability of TRF3. Our results demonstrate the "specificity" of transcriptional regulation by a "general" transcription factor in hESCs early fate decision, and provide new knowledge for the regulation of mesendoderm differentiation of hESCs.

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F-3033

NEW GENERATION LAMININS FOR PARAXIAL MESODERM CELL DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (hiPSCs) derived paraxial mesoderm cell lineages would differentiate to bone, cartilage and skeletal muscle, making them promising models of organ development and cell therapy. However, the full potential of clinical application has remained unrealized, due to the poorly defined animal-derived matrix in which they are cultured. Here we describe a Xeno-free 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 attachment domain of perlecan. Comparing with Engelbreth-Holm-Swarm mouse tumor derived hydrogel (Matrigel), NGL dramatically increased the marker genes expression and the cell population of primitive streak and paraxial mesoderm lineage. This paraxial mesoderm lineage enabled the generation of human skeletal muscle progenitors, myocytes, myotubes and muscle stem cells. The unique effect of NGL in paraxial mesoderm differentiation was dependent on the heparan sulfate (HS) though FGFR signaling pathway. Using this Xeno-free matrix, we established a highly efficient differentiation system for paraxial mesoderm differentiation, that may be useful for disease modeling and regenerative medicine.

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F-3035

APPLICATION OF ATMOSPHERIC PRESSURE AND HYPOXIA DURING CULTURE PROMOTES NEURAL INDUCTION OF IPSCS AND SUBSEQUENT DIFFERENTIATION INTO MOTOR AND CNS-TYPE NEURONS

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Induced pluripotent stem cells (iPSCs) can be used for autologous regenerative medicine to treat a multitude of health conditions, including spinal cord injuries and neuropathology-associated genetic disease. iPSCs can successfully differentiate to neural progenitor cells (NPCs), a multipotent stem cell population that can give rise to all lineages of adult neuronal cells. However, a confounding limitation of differentiated neurons from iPSC-derived NPCs is that they are not genetically and functionally equivalent to adult neurons in vivo, thus rendering them sub-optimal for studying pharmaceutical or environmental responses in the dish. Recent studies highlight the significance of micro-environmental factors such as hypoxia and mechanical force / pressure on stem cell maintenance and directed-differentiation to specific cell lineages, yet none have evaluated the combined contribution of these factors towards differentiation of iPSCs to NPCs or on the differentiation of NPCs into adult neurons. Here we demonstrate the biological impact of oxygen and atmospheric pressure on differentiation of human iPSCs to NPCs as well as on differentiation of both motor and central nervous system-type neurons using the AVATAR™ cell control system. For this study, we demonstrate that combinatorial oxygen and pressure are significant drivers of NPC differentiation from human iPSCs. We compared either 5% oxygen versus normoxia and increased pressure (+2 PSI) versus atmospheric pressure during neural induction of human iPSCs to NPCs and show an increase in PAX6+ cells relative to standard incubator culture conditions. We further show that low oxygen and increased pressure

promotes the differentiation and survival of central nervous system-type neurons and motor neurons as determined by the enhanced and pro-longed expression of mature neuronal markers using immunofluorescence microscopy. Our findings suggest that physiologically relevant oxygen and pressure are important drivers of neural differentiation from human iPSCs, and that these micro-environmental factors have the potential to induce maturation of neurons such that they are better suited for translational studies in vitro and in the clinic.

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PLURIPOTENT STEM CELL: DISEASE MODELING

F-3037

SYSTEMS GENETICS IMPLICATES RNA-PROCESSING IN PERIVENTRICULAR HETEROTOPIA

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Periventricular nodular heterotopia (PH) is a malformation in development whereby populations of neurons fail to migrate to the neuronal layers of the cerebral cortex and instead adopt heterotopic positions along their sites of origin - adjacent to the lateral ventricles. Although seven loci are currently implicated in its causation these genes explain only 25% of sporadic instances. To gain further insight into this condition we recently contributed to the exomic analysis of 202 individuals with PH and their unaffected parents. Although representing the largest study of its kind for this condition and despite a significant excess of deleterious variants detected (n=35/219), no novel gene could be causally associated given the extreme genetic heterogeneity observed. In this study, using the interactive tool brain-coX we integrate human brain transcriptomic data and show that known PH loci significantly co-express to form a defined molecular signature indicative of potential etiology. Mapping candidate PH loci mutated in the 202 patients onto these co-expression modules highlighted 34 genes with significant overlapping expression signatures and that were also enriched for factors involved in mRNA splicing via the spliceosome. For at least one of these factors, in vitro functional assays identify disrupted splicing in cells harboring the mutation. The candidate mutant protein also binds a differential set of proteins significantly involved in mRNA stability and its introduction into the developing mouse cortex induces heterotopic cell positioning below the neuronal layers of the cortex. Collectively, our data highlight the utility of integrative system genetic analyses for the organization of candidate genetic loci potentially involved in PH.

F-3039

INSIGHT INTO PATHOGENIC MECHANISM OF TRMT10A DEFICIENCY USING PATIENT iPSC-DERIVED β-CELLS: tRNA HYPOMETHYLATION AND FRAGMENTATION MEDIATE HUMAN β-CELL DEMISE

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Loss-of-function mutations in TRMT10A, a putative tRNA methyltransferase, cause young-onset diabetes and microcephaly. Intronic variants in tRNA methylthiotransferase CDKAL1 are associated with impaired insulin secretion and type 2 diabetes. These findings suggest that tRNA hypomodification causes pancreatic β-cell failure. Here we aimed to elucidate TRMT10A function and identify mechanisms of β-cell demise induced by TRMT10A deficiency. Human iPSCs from controls and TRMT10A-deficient patients were differentiated into pancreatic β-cells using a 7-stage protocol. The TRMT10A-deficient cells showed similar differentiation capacity as control cells, as monitored by stage-specific mRNA and protein expression. To study their functionality in vivo, iPSC-β-cells were transplanted under the kidney capsule of NOD/SCID mice. Control iPSC-β-cells showed glucose-stimulated insulin secretion, and maintained normoglycemia upon

selective mouse β-cell ablation with streptozotocin, demonstrating iPSC-β-cell graft function. In contrast, streptozotocin induced marked hyperglycemia in mice transplanted with TRMT10A diabetic patients' iPSC-β-cells, suggesting reduced β-cell maturation, function and/or enhanced cell death. tRNA methylation, assessed by qPCR, was reduced in tRNAGln, tRNAIniMeth, tRNAArg and tRNAPro in TRMT10A-deficient iPSC-β-cells, identifying these tRNAs as TRMT10A targets. Patient iPSC-β-cells showed enhanced sensitivity to apoptosis. In TRMT10A-deficient cells, tRNAGln hypomethylation led to tRNAGln fragmentation, assessed by qPCR. tRNAGln fragments were identified as key mediators of β-cell death via induction of the pro-apoptotic Bcl-2 protein Bim. In conclusion, we confirm that TRMT10A is a tRNA methyltransferase in man and identify its tRNA targets in β-cells. TRMT10A-deficiency leads to tRNA hypomethylation and fragmentation; this represents an entirely novel mechanism of β-cell demise in diabetes. Patient iPSC-derived β-cells constitute a novel and powerful model to study the pathophysiology of diabetes.

F-3041

MODELING ENDOTHELIAL DYSFUNCTION IN LMNA-RELATED DILATED CARDIOMYOPATHY

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Mutations in the gene that encodes the nuclear envelope proteins lamin A and C (LMNA) accounts for 6% of all cases of Dilated Cardiomyopathy (DCM). However, the molecular mechanisms that underlie "cardiolaminopathy" remain elusive, and it is unknown why mutations in this ubiquitously expressed gene have such a disproportionate effect on the heart. Hypothesis: Despite the fact that LMNA is abundantly expressed in endothelial cells (ECs) and mutations in LMNA are known to induce EC dysfunction, little is known about the EC-specific phenotype of LMNA-related DCM. As EC dysfunction has been known to contribute to DCM, we hypothesize that EC dysfunction due to LMNA mutation has a significant impact on the pathogenesis and disease progression of DCM. Results: Intriguingly, our preliminary data showed that induced pluripotent stem cell derived ECs (iPSC-ECs) from patients (n=5) harboring the LMNA-mutation exhibit decrease functionality as seen by impaired angiogenesis and decreased NO production (control iPSC-ECs vs LMNA iPSC-ECs; $p<0.05$). Similarly, genome editing of isogenic iPSC lines enabled us to recapitulate the EC disease phenotype further allowing us to dissect the effects of LMNA mutations on EC function. LMNA-corrected iPSC-ECs (via use of CRISPR/Cas9 genome editing tool) showed restoration of EC function. Whole genome RNA-sequencing identified Krüppel-like Factor 2 (KLF2) as a potential transcript responsible for EC dysfunction in LMNA-mutated patients, which was further confirmed by loss-of-function studies using shRNA towards KLF2. Importantly, treatment of LMNA-mutated ECs with KLF2 agonists showed an improvement in endothelial function. Furthermore, iPSC-derived cardiomyocytes (iPSC-CMs) from LMNA-mutated patients that exhibited DCM phenotype, showed improvement in CM physiology when co-cultured with iPSC-ECs treated with KLF2 agonists. Conclusion: This study is a first step towards understanding the molecular mechanisms of cardiolaminopathy by modeling endothelial dysfunction using patient-specific iPSCs. Moreover, our results suggest that improving EC function in cardiolaminopathy patients could have a significant impact on the pathogenesis of DCM. Results from this work could potentially lead to new strategies that could improve the management of DCM patients.

F-3043

NONSENSE MEDIATED MRNA DECAY FACTORS UPF3A AND UPF3B HAVE OPPOSING ROLES IN CELL CYCLE REGULATION IN HUMAN EMBRYONIC STEM CELLS

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Nonsense mediated mRNA decay (NMD) pathway targets and destroys transcripts with 'NMD inducing features', and is known to regulate ~10% of the entire transcriptome. NMD is highly active in stem cells and regulates proliferation, self-renewal and cell fate decisions, whilst its loss is embryonic lethal in multiple animal systems. UPF3A and UPF3B are gene paralogs that facilitate the execution of NMD. Whilst UPF3B is known as a strong NMD activator, the role of UPF3A is less clear, shown to have only weak NMD activity, and more recently, even reported to act as an NMD inhibitor. Our aim was study the role of NMD, UPF3B and UPF3A in particular in human embryonic stem cells (hESCs). We used CRISPR-Cas9 genome editing technology to generate three independent knockout hESCs lines each of UPF3A and UPF3B using unique gRNAs to cater for off-target effects. In UPF3B knockout clones, UPF3A protein was elevated, identifying existence of a compensatory NMD mechanism previously described in

other cells. In contrast, UPF3B protein levels remained unchanged in UPF3A knockout clones. Although UPF3A protein was elevated in UPF3B knockout clones, we observed increased mRNA levels of bonafide canonical NMD targeted genes consistent with impaired NMD activity. We observed no such changes in the same target genes in UPF3A knockout clones. Thus UPF3A has only weak or non-canonical NMD activity in hESCs. Loss of either UPF3A or UPF3B had no overt effect on hESCs morphology, or the expression of a suite of pluripotency marker genes. Cell cycle analysis however revealed that UPF3B knockout clones progressed slower through the G1 phase and faster through G2/M phase, whilst in opposite, UPF3A knockout clones progressed faster through the G1 phase, and slower through the G2/M phase. The expression of NMD targeted cell cycle regulatory gene CNK2A was highly upregulated only in UPF3B knockout clones, and may in part explain the cell cycle defect. These data demonstrate that whilst UPF3A may be dispensable for canonical NMD in hESCs, it functions in a non-redundant, opposing manner to UPF3B to control hESCs cell cycling. Our results demonstrate that the persistence of this only example of NMD gene paralogs maybe due to their different roles in cells.

F-3045

IPSC AND CRISPR/CAS9 TECHNOLOGIES ENABLE PRECISE AND CONTROLLED PHYSIOLOGICALLY RELEVANT DISEASE MODELING FOR BASIC AND APPLIED RESEARCH

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Human-induced pluripotent stem cell (hiPSC) technology has provided unique ways to understand and potentially treat human diseases using cells from individual patients. When combined with genome editing techniques such as the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas system, it is now possible to establish highly-controlled experimental models via correction of disease-causing mutations in patient lines or introduction of the same genetic defects into healthy iPSCs. Typically, isogenic cell pairs that differ in a single genetic change are further differentiated into target cell types that are relevant to the disease and then used to interrogate disease phenotypes or to screen novel therapeutic agents in high-throughput format. Here, we illustrate this process by showing the generation of hiPSCs from healthy donors and patient populations using fibroblasts and PBMCs as the somatic cell source. Using CRISPR/Cas9 or our proprietary TARGATT™ technology, customized gene-edited hiPSC lines can be efficiently generated, including lines with monoallelic or biallelic gene mutations, large-fragment knock-in and conditional/inducible expression models. Finally, differentiation of hiPSCs towards neural lineages is presented as an example of an experimental platform with potential for drug screening and neurotoxicity assays.

F-3047

USING HUMAN INDUCED PLURIPOTENT STEM CELLS AND CRISPR/CAS9 GENOME-EDITING TO MODEL AUTISM SPECTRUM DISORDER

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Autism spectrum disorders (ASD) affect 1 in 68 children in the US and involves social difficulties, seizures, and intellectual disability. Within the past decade the research community identified several ASD-associated mutations, but there is still no medical test available for diagnosis. Recent studies indicate that ASD patients have decreased numbers of neurons and glutamatergic synapses and increased levels of inflammatory markers in their cerebral spinal fluid and serum. However, the driving forces behind these anomalies are unknown. Therefore, we hypothesize that loss-of-function mutations in FMR1, NHE6, SHANK3, and TSC2 cause synaptic and network dysregulation and abnormal inflammatory responses that lead to neuronal and glial cell injury. In this study, we used a CRISPR/Cas9 vector containing a green fluorescent protein (GFP) marker with sgRNAs targeting FMR1, NHE6, SHANK3, and TSC2 to generate isogenic hiPSC lines of four forms of monogenic ASD. Post-transfection, we used two fluorescently activated cell sorting instruments to select GFP+ hiPSCs: The BD FACSAria, and the

NanoCellect's WOLF Microfluidic Cell Sorter. We then plated the selected hiPSCs, picked and expanded the isogenic colonies, and genotyped each clone. Overall, we generated homozygous knockout (KO) hiPSC lines for FMR1, NHE6, and TSC2 and a heterozygous KO line for SHANK3, all of which maintained pluripotency and normal karyotypes. We found that ~35% of the FACSAria-sorted colonies maintained normal karyotypes, whereas ~85% of the WOLF ones maintained normal karyotypes, which facilitated the characterization process. Each ASD hiPSC line differentiated into neural progenitor cells (NPCs) that stained positive for PAX6, OTX2, and the live NPC marker, NeuroFluor-CDr3, and formed neurospheres when in suspension. In conclusion, we established four monogenic ASD hiPSC KO lines that will serve as valuable tools to investigate the underlying causes of ASD. These KO ASD lines are now being differentiated into cortical neurons, astrocytes, and microglia that will be analyzed using RNA-seq to identify cell type-specific RNA signatures in order to shed light on molecular pathways driving ASD. Ultimately, this work will provide new avenues for future ASD studies to discover prospective drug targets and novel diagnostic approaches.

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F-3049

MECHANISMS UNDERLYING HYPEREXCITABILITY OF HIPPOCAMPAL NEURONS DERIVED FROM BIPOLAR DISORDER PATIENTS

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Bipolar disorder (BD) affects 2-3% of the world population. People with BD experience disabling episodes of mania and depression, often associated with cognitive changes, physical morbidity and some of the highest risk of suicide. Using induced pluripotent stem cells technology we derived two types of hippocampal neurons: Dentate gyrus granule neurons and CA3 pyramidal neurons. Using whole cell patch clamp we found that while bipolar disorder neurons were hyperexcitable compared to controls as DG neurons, as CA3 pyramidal neurons only those that were derived from Lithium responsive (LR) patients were hyperexcitable compared to controls. We found a strong correlation between bipolar disorder neuronal hyperexcitability and the amplitude and kinetics of fast potassium currents. qPCR experiments showed an over-expression of a few types of fast potassium channels. Blocking these channels rescued the hyperexcitability phenotype. A computational model for both DG and CA3 bipolar disorder neurons recapitulated the experimental phenotype of hyperexcitability, by increasing the conductance of the overexpressed channels, further supporting this mechanism of hyperexcitability. In both DG and CA3, continuous lithium treatment reduced neuronal hyperexcitability in neurons derived from bipolar disorder patients. In addition, all types of neurons and neural progenitor cells that we measured that were derived from bipolar disorder patients were consistently larger than control neurons. Finally, when differentiating the cells as motor neurons, there was no significant difference in bipolar disorder excitability compared to controls.

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F-3051

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

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Non-alcoholic fatty liver disease (NAFLD) is a prominent cause of chronic liver disease and a major indication for liver transplant in both children and adults. Genome-wide association studies (GWAS) have found a single nucleotide polymorphism (SNP) variant in the gene PNPLA3 that 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 (hIPSC) of known genetic background with the variant (VAR) and wildtype (WT) homozygous alleles of PNPLA3. We induced differentiation of WT and VAR hIPSCs to generate hepatocyte like cells (HLC) and measured phenotypes relevant to the onset and progression of NAFLD. To mimic environmental stressors that are relevant in NAFLD pathogenesis, we used palmitic acid (PA), a toxic fatty acid that is present in saturated fats. Because steatosis may be the disease-initiating event in NAFLD, we measured triglycerides in PNPLA3 WT and VAR HLCs and found that the VAR HLCS accumulate lipid droplets

upon exposure to PA. Expression of PNPLA3 is reduced in VAR HLCs and PA-mediated induction of PNPLA3 is blunted in VAR HLCs as compared to WT. Expression of other genes involved in lipolysis is induced, suggesting an adaptive mechanism to PNPLA3 loss of function. Furthermore, VAR HLCs have increased expression of cytokines relevant to the development of inflammation in NAFLD, such as IL1B, IL1A and IL6. With this knowledge, we designed a miniaturized assay for triglyceride quantification to identify compounds that can ameliorate lipid accumulation and inflammation in PNPLA3 VAR HLCs. Our results suggest that certain PPAR-α agonists may decrease triglyceride accumulation and rescue inflammatory phenotypes in PNPLA3 VAR HLCs through induction of PNPLA2 expression. We are currently validating positive hits from our primary screening and testing additional compounds that are predicted to enhance lipolysis in PNPLA3 VAR HLCs. Our work will open the door to a new range of experimentation in elucidating the mechanism underlying the association between PNPLA3 and NAFLD, predictive diagnostics and therapeutic discovery.

F-3053

GORLIN SYNDROME IPS-DERIVED CELLS AS A MODEL FOR HUMAN MEDULLOBLASTOMA AND ALLOWING FOR NOVEL TARGET IDENTIFICATION

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Medulloblastoma (MB) is one of the most common brain cancers in children. Aberrant Sonic Hedgehog (SHH) signaling, such as inactivating mutations of PTCH1, contributes to the Sonic Hedgehog (SHH)-subtype of MB, which constitutes about of 30% of total MB cases. Here we describe a MB model using iPS cell-derived human neuroepithelial stem (NES) cells generated from Gorlin syndrome patients carrying germline mutations in the Sonic hedgehog (SHH) receptor PTCH1. We found that Gorlin NES cells formed tumors in mouse cerebellum resembling human MB. Re-transplantation of isolated tumor cells resulted in accelerated tumor formation, cells with reduced growth factor dependency, enhanced neurosphere formation in vitro, and increased sensitivity to the SMOOTHENED (SMO) inhibitor, Vismodegib. The pro-metastatic gene LGALS1, which has been correlated with poor prognosis in many cancer types, was upregulated in both Gorlin tumor cells and SHH-subgroup MB patients. Treatment with a GALECTIN-1 inhibitor reduced viability of Gorlin cells, but not control cells, suggesting GALECTIN-1 as a potential target for treating SHH-MB. Taken together, we demonstrate that NES cells generated from reprogrammed non-cancerous somatic cells carrying a PTCH1 germ line mutation give rise to MB when injected into mouse cerebellum, creating a novel model for MB to study disease onset and progression as well as identifying and testing novel therapeutic targets.

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F-3055

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS HARBORING LEUKEMIA-SPECIFIC FUSION GENE ETV6-RUNX1

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The treatment outcome for patients with childhood leukemia has been improved remarkably by the development of efficacious treatment protocols. Nevertheless, questions regarding the molecular mechanisms of the pathogenesis and relapse of leukemia as well as the origin of leukemia stem cells remain unanswered. It is well known that induced pluripotent stem (iPS) cells, which are generated from the patient's own tissues, are powerful tools for understanding the detailed pathology of human diseases. However, the reprograming of human primary cancer cells is still technically challenging, and leukemia-specific human iPS cells have yet to be generated from tissues of patients with acute leukemia. In this study, we generated human iPS cells that are specific to childhood leukemia by introducing leukemia-specific fusion gene ETV6-RUNX1, which is the most common genetic aberration found in childhood leukemia, into human iPS cells. After the integration of the fusion gene with a doxycycline-inducible gene expression system into the Jump-In vector, the constructed plasmid was lipofected into singly dispersed human iPS cells. After culturing with antibiotics, several clones were isolated.

The frequency of the resistant clones was in the range of 10-6 or less. We confirmed that the isolated clones exhibited the properties of human iPS cells because the proliferative properties of the isolated clones did not change; the clones expressed human pluripotent stem cell markers (TRA-1-60, TRA-1-81, and SSEA-4), were competent in forming teratomas, and retained a normal karyotype. Furthermore, genetic analysis suggested that the isolated clones contained the fusion gene and other components in their genomes; however, the gene expression system was silenced in almost all the clones. On the other hand, in the clones that escaped silencing, the fusion gene was expressed in a doxycycline-dependent manner even after multiple passages. We then induced the stepwise differentiation of the human iPS cells that harbored the fusion gene into mesodermal, hematopoietic stem or progenitor, and matured hematopoietic cells, and evaluated the influence of the fusion gene in hematopoietic cell differentiations. Overall, we suggest that human iPS cells harboring ETV6-RUNX1 fusion gene would be useful as a model for childhood leukemia.

F-3059

IN VITRO MODELING OF MOTOR NEURON DISORDERS USING IPSCS SUGGESTS UNIQUE PROPERTY OF PRIMARY LATERAL SCLEROSIS

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Motor neuron disorders such as Amyotrophic Lateral Sclerosis (ALS) and primary lateral sclerosis (PLS) involve different types of neurons. While ALS affects both upper and lower motor neurons, PLS only affects upper motor neurons. It has been reported that there is difference in metabolism could differentiate fibroblasts from ALS and PLS. Nevertheless, it is still difficult to differentiate the disorders at early stage. To study the pathogenesis of motor neuron disorders, we derived iPSCs from patient blood samples and subsequently generated a variety of in vitro neuronal cell cultures including neuronal stem cells and 3D brain organoids to study their neural differentiation capabilities as well as metabolism status. We found it was difficult to compare the metabolism of neural stem cells derived from patients and normal donors due to the lack of consistence between cell passages which may also arise with inconsistent culture conditions. However, 3D brain organoids cultures showed that while iPSCs from both motor disorders resulted in delayed neuronal differentiation, the markers for peripheral motor neurons surprisingly increased in some of the PLS derived cultures. These findings indicated a unique property of PLS compared to ALS in 3D cultures which may potentially be useful to differentiate PLS from ALS at early disease stage.

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F-3061

UPREGULATION OF ENDOPLASMIC RETICULUM STRESS (ER STRESS) IN HUMAN IPSC DERIVED CORNEAL ENDOTHELIAL CELLS FROM PATIENTS WITH FUCHS ENDOTHELIAL CORNEAL DYSTROPHY

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Fuchs endothelial corneal dystrophy (FECD) is a common corneal dystrophy and a major indication of corneal transplantation. Although the detailed pathophysiology of this disease is still not understood, the involvement of endoplasmic reticulum stress (ER stress) and oxidative stress are suggested. Genetic linkages recently revealed in genome-wide association studies include single nucleotide polymorphisms (SNPs). We newly established human induced pluripotent stem cells (iPSCs) from six FECD patients for the investigation of this disease. Sendai virus vector was used to generate iPSCs from human blood cells. The advantage of Sendai virus vector is the high reprogramming efficiency and safety. We examined the SNPs reported in previous publications, and 3 different SNPs were found in the TCF4 gene from one patient. Next, we derived corneal endothelial cells (CEC) from iPSCs using a protocol reported previously . We compared C-CEC (control normal iPSCs derived CEC) and F-CEC (FECD iPSCs derived CEC). Multi array real time PCR (QIAGEN® PROFILER PCR ARRAY) was performed to pick up candidates from multiple ER stress markers. Several major ER stress markers (CHOP, ERN1, PERK) were upregulated in F-CEC compared with C-CEC. Preliminary results show that ER stress is involved in the pathogenesis of FECD.

F-3063

AN ISOGENIC WISKOTT-ALDRICH SYNDROME IPSC MODEL REVEALS NOVEL ROLES OF WASP IN CANCER PREDISPOSITION

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The Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disease that caused by the genetic defect in WAS protein (WASP). The classical syndrome is characterized by thrombocytopenia, immunodeficiency, atopy, autoimmunity, and malignancy, leading to premature death in severe patients. Studies have shown that 13% to 22% of patients develop cancer with the only average onset of 9.5 years; however, the molecular mechanism underlying the early onset and high morbidity of cancer in WAS is still an unsolved issue. As the founding member of actin nucleation-promoting factors, WASP plays a critical role in mediating actin polymerization and remodeling the cytoskeleton. Nevertheless, this function fails to explain malignancy development. Here, by using induced pluripotent stem cells (iPSCs) generated from WAS patient, we found that WAS mutant cells have an abnormal cell cycle. The cell cycle-related genes/pathways are significantly deregulated. We show that WASP interacts with SRSF2 is essential for nuclear body organization. WASP deficiency leads to of splicing regulation and epigenetic regulation involved in cell cycle. Besides, the binding of WASP to EZH2 effects the cell cycle process by targeting CDKN2A (aka p16). We confirmed our findings in 4 independent patients with different mutations using macrophages, T, B and dendritic cells. Interestingly, WASP's multifaceted function in the nucleus results in synergistic disruption of cell cycle regulation when it is mutated. Taken together, our finding identified a new function of WASP in the cell cycle, which offers a potential target for predicting early cancer in WAS patient as well as a new therapy.

REPROGRAMMING

F-3067

UNIQUE MOLECULAR EVENTS DURING REPROGRAMMING OF HUMAN SOMATIC CELLS TO INDUCED PLURIPOTENT STEM CELLS (IPSCS) AT NAÏVE STATE

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Derivation of human naïve cells in the ground state of pluripotency provides promising avenues for developmental biology studies and therapeutic manipulations. However, the molecular mechanisms involved in the establishment and maintenance of human naïve pluripotency remain poorly understood. Using the human inducible reprogramming system together with the 5iLAF naïve induction strategy, integrative analysis of transcriptional and epigenetic dynamics across the transition from human fibroblasts to naïve iPSCs revealed ordered waves of gene network activation sharing signatures with those found during embryonic development from late embryogenesis to pre-implantation stages. More importantly, Transcriptional analysis showed a significant transient reactivation of transcripts with 8-cell-stage-like characteristics in the late stage of reprogramming, suggesting transient activation of gene network with human zygotic genome activation (ZGA)-like signatures during the establishment of naïve pluripotency. Together, Dissecting the naïve reprogramming dynamics by integrative analysis improves the understanding of the molecular features involved in the generation of naïve pluripotency directly from somatic cells

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F-3069

GENERATION OF CHONDROCYTES THROUGH TRANSCRIPTION FACTOR-MEDIATED REPROGRAMMING PREDICTED BY THE COMPUTATIONAL FRAMEWORK MOGRIFY

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With an aging population, the promise of regenerative medicine has drawn more and more attention. However the availability to develop protocols to produce desired cell types by differentiation of direct reprogramming if often tedious, time consuming and depends on expert knowledge on the different factors that control the target cell identity. Therefore, it is crucial to devise robust and streamlined methods to source cells with therapeutical potential from diverse available starting material. We have previously shown that the algorithm Mogrify can predict necessary and sufficient transcription factors to rewire the transcriptional program of one cell type into another. Building up on this predictive power, we have established a platform to successfully generate novel cell conversions with regenerative medicine potential. First, a set of transcription factors necessary to reprogram a cell type into a target cell of interest is identified by the Mogrify algorithm. Second, this set of transcription factors is introduced into primary cells by lentiviral-mediated delivery. Finally, transduced cells are assessed for the expression of cellular and molecular markers of interest. As an example, we show how we can successfully apply this framework to the generation of chondrocytes from human dermal fibroblasts.

F-3071

OPTIMISATION OF DIRECT CELLULAR REPROGRAMMING TO MODEL HUNTINGTON'S DISEASE

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The study of neurodegenerative disorders such as Huntington's disease (HD) has been impaired by limited access to live human disease-affected neurons. Cellular reprogramming of patient-derived somatic cells now offers an opportunity to generate live human neurons for the study of disease pathology and mechanisms. We have developed a highly efficient protocol for direct reprogramming of adult human fibroblasts (HDFs) to induced neural precursor cells (iNPs) by co-transfection of chemically-modified mRNA encoding the pro-neural transcription factors SOX2 and PAX6 in a defined reprogramming medium. Based on our direct-to-iNP technology, the current study aimed to optimise the generation of DARPP32+ striatal neurons and demonstrate the capability to successfully reprogram HD patient-derived HDFs. We observed that directly reprogrammed iNPs expressed the neural transcription factors OTX2, FOXG1, and SIX3, and the general neural stem cell marker NESTIN. Importantly, directly reprogrammed iNPs expressed the neural transcription factors GSX2, ASCL1, DLX2, FOXP1, and MEIS2, required for the development of striatal neurons. A series of optimisation studies were performed in which a range of small molecules and growth factors, including Forskolin and Activin A, were investigated to enhance the differentiation of DARPP32+ neurons from iNPs. These studies confirmed the expression of the calcium and sodium channel genes CACNA1C and SCN8A, and the synaptic genes SNAP25, SAP97, and PSD95. After 30 days of differentiation, high yields of neurons were generated (>30% TUJ1+/DAPI+), with >90% of TUJ1+ cells expressing the striatal neuron marker DARPP32. This level of striatal differentiation was maintained out to 56 days with a yield of >70% DARPP32+/TUJ1+ neurons. Electrophysiological activity was detected after day 42 of differentiation. Most importantly, we confirmed our optimised protocol was able to efficiently reprogram HD patient-derived HDFs (nCAG 41-57) and generate yields of >70% DARPP32+/TUJ1+, providing an effective model for future research into HD pathology and mechanisms.

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F-3073

AN IMPROVED MOGRIFY ALGORITHM FOR CELL REPROGRAMMING

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The discovery of sets of transcription factors that can induce cell reprogramming can be a slow and expensive process. To ameliorate this, a number of computational methods have been developed to identify factors for cell reprogramming. One such approach is Mogrify, which incorporates the extensive FANTOM5 gene expression atlas and biological interaction networks to predict non-redundant sets of transcription factors for reprogramming between any two cell types. Here, we present further improvements to Mogrify (Mogrify.v2), targeted at accelerating experimental reprogramming efforts as follows: Firstly, Mogrify.v2 improves reprogramming predictions by considering the effect of transcription factor combinations rather than individually. For each combination a regulatory influence is calculated and serves as an indicator for comparing the potential reprogramming efficiency between different transcription factor combinations. This new algorithm also incorporates a much broader spectrum of regulatory network information in order to better model the underlying cellular biology. Secondly, Mogrify.v2 can measure the molecular similarity between two cell populations by comparing their core regulatory networks. These comparisons are useful to assess the fidelity of a reprogramming experiment by comparing the reprogrammed cells with the target cell

population. Also, a set of cell-specific marker genes can be determined from these core regulatory networks, which can in turn be used for determining cell identity. Overall, these improvements seek to minimize trial and error in the selection of transcription factors and assess the biocompatibility of reprogrammed cells.

F-3075

GENERATION OF DOPAMINERGIC NEURONS FOR CELL-BASED REPLACEMENT THERAPY: REPROGRAMMING OF ADULT HUMAN FIBROBLASTS FROM HEALTHY INDIVIDUALS AND PARKINSON´S DISEASE PATIENTS

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Parkinson's disease (PD) is a neurodegenerative disease resulting from the progressive loss of dopaminergic cells within the nigrostriatal system. Previous work has shown that it is possible to generate dopaminergic (DA) neurons from fetal tissue and embryonic stem cells and, when transplanted into animal models of PD, these cells can integrate and provide improvement in motor symptoms. However, in order to translate these into clinical therapies there are a number of logistical and ethical concerns with using cells derived from embryonic sources. With new reprogramming technologies we can now generate DA neurons from somatic cell sources, which also allows for the possibility to use patient specific cells or matched donors. The main purpose of this project is to develop robust protocols for the generation of DA neurons from adult human fibroblasts, from both healthy individuals and PD patients. We will reprogram cells directly into induced neurons (iNs) and also via a pluripotent stem cell (iPSC) intermediate and investigate if there are any differences in gene expression, morphology or phenotype between the DA neurons generated from healthy, genetic PD, and sporadic PD donors. The results will provide a more robust protocol for the generation of reprogrammed DA neurons from adult donors and will help to pave the way for future research assessing their potential for brain repair and disease modelling.

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F-3077

GENERATION OF INTEGRATION-FREE HUMAN NEURAL PROGENITORS FROM URINE-DERIVED CELLS BY USING SYNTHETIC MRNA

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Neurological disease is difficult to recover to a normal stage. Neural progenitors can differentiate to neuronal and glial cells, which could be employed as a powerful tool for management of neurological diseases. However, it has been challenging to isolate neural progenitors from human brain tissues, thus leading to induction of neural progenitors by various methods in vitro. Induced neural progenitors (iNPs) are mainly generated using viral vectors containing required genes, which are associated with the risk of genetic mutations due to integration of exogenous genes into host genome. In this study, we generated human iNPs from urine-derived cells through temporal expression of exogenes, based on synthetic mRNA. The iNPs expressed neural progenitor markers including SOX1, SOX2, NESTIN, PAX6 and PLZF, while no pluripotency marker such as OCT4 and NANOG was observed. The iNPs showed differentiation potential to astrocytes, oligodendrocytes, and functional neurons in vitro. Under chemically defined culture condition, the iNPs could maintain their proliferation over 20 passages as well as a normal karyotype. Taken together, the integration-free iNPs could be considered as safe, efficient and patient-specific cell source for clinical applications.

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F-3079

ESTABLISHMENT OF A ROBUST REPROGRAMMING SYSTEM TO GENERATE MOUSE FUNCTIONAL CARDIOMYOCYTES WITH THE AID OF CHEMICAL COMPOUNDS

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The direct conversion of cardiac fibroblasts into cardiomyocytes (iCMs) in vitro and in vivo by forced expression of Gata4, Mef2c, Tbx5 (GMT) represents a novel and promising approach for cardiomyocyte regeneration. However, the low reprogramming efficiency to obtain functional cardiomyocytes is a major problem which hampers its further application. Here, by chemical screen for about 2,000 selected small molecules and further validation, we identified a combination of two chemical compounds (2C) that greatly boost mouse cardiac reprogramming induced with GMT, and thereby

established a robust reprogramming system to generate functional cardiomyocytes from mouse postnatal fibroblasts. Contractile cardiomyocyte-like cells emerge as early as two weeks post transfection. Within 4 weeks, more than 70% of the iCMs co-express cardiomyocyte sarcomere protein cTnT and α-actinin, with well-organized sarcomere structure. Importantly, more than 50% cells are contractile and have spontaneous Ca2+ waves, while the recently reported 2 efficient chemical boosters (SB431542 plus XAV939) only induce less than 1% beating cardiomyocyte-like cells at the same time point. Furthermore, most of these iCMs induced with 2C express Myl2v, a marker for ventricular cardiomyocytes. GMT+ 2C also efficiently reprograms mouse cardiac fibroblast into spontaneous beating iCMs within 2 weeks, when no beating cell can be found with GMT in the absence of 2C. With the aid of 2C, Mef2c and Tbx5 (MT) can also efficiently induce spontaneous beating iCMs within 2 weeks. Moreover, adult cardiac myofibroblasts isolated from day 7 myocardial infarction mice can be reprogrammed by an efficiency nearly 70-fold higher, as indicated by cTnT expression. Besides, 2C can be substituted by two clinical drugs targeting the same pathways with 2C, demonstrating a potentially safer and more promising applications in vivo. In summary, a robust cardiac reprogramming system was developed by combining transgenic and chemical strategy, which provides a promising candidate cocktail for further study of cardiac reprogramming and heart regeneration in situ. Moreover, the chemical boosters identified in this study provide new opportunities to further study the mechanisms of cell fate reprogramming and the establishment of cardiomyocyte identity.

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F-3081

BAK/BAX-MEDIATED APOPTOSIS IS A ROADBLOCK TO REPROGRAMMING

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Considerable progress has been made in optimizing procedures for reprogramming somatic cells to induced pluripotent stem cells (iPSCs) through the expression of defined transcription factors. It remains, however, a slow, stochastic, and inefficient process that generates considerable cellular stress. Senescence and apoptosis, two common responses to cellular stress, have both been proposed as barriers that limit the efficiency of reprogramming. The significance of senescence as an obstacle to reprogramming has been well established, but the role of apoptosis is less clear. To resolve this question we measured reprogramming in mouse embryonic fibroblasts (MEFs) lacking the essential mediators of mitochondrial apoptosis, BAX and BAK. We found that MEFs unable to perform mitochondrial apoptosis reprogrammed significantly more efficiently and produced iPSC of high quality as measured by the integrity of their genome and their capacity for differentiation. Our data suggests that blocking apoptosis during reprogramming may enhance iPSC derivation for research and therapeutic purposes.

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F-3083

LINEAGE CONVERSION WITHOUT TRANSIENT ACQUISITION OF PLURIPOTENCY

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Substitution of a single transcription factor in the reprogramming cocktail for induction of pluripotent stem cells (iPSCs) could convert somatic cells directly into a distinct lineage. We previously reported that Brn4 in cooperation with Klf4, Sox2, and c-Myc (BKSM) induces a transdifferentiation of fibroblasts into induced neural stem cells (iNSCs). A recent lineage tracing study proposed that such a transdifferentiation involves a transient iPSC stage even when neuro-specific Brn4 is used instead of pluripotency-specific Oct4. Here we employed genetic lineage tracing and show that BKSM transduced by individual vectors could directly convert somatic cells into iNSCs without generating Oct4-positive intermediate cells. We revealed that a fusion protein of Brn4 and Klf4 generated by an incomplete cleavage within the polycistronic cassette gains the ability to reprogram towards pluripotency to a level similar to Oct4. Furthermore, non-cleavable POU-KSM polycistronic constructs in which Brn4 was replaced with other neuro-specific POU factors, such as Oct6 and Brn2, were also capable of iPSC generation. Our study

demonstrates that BKSM-mediated generation of iNSC can be a direct process that does not involve a transient acquisition of pluripotency. Therefore, iNSC technology can potentially provide a safer alternative to iPSCs for clinical applications.

F-3085

KDM2B REGULATES SOMATIC REPROGRAMMING THROUGH VARIANT PRC1 COMPLEX-DEPENDENT FUNCTION

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Polycomb repressive complex 1 (PRC1) plays essential roles in cell-fate determination. Recent studies have found that the composition of mammalian PRC1 is particularly varied and complex; however, little is known about the functional consequences of these variant PRC1 complexes on cell-fate deter- mination. Here, we show that Kdm2b promotes Oct4-induced somatic reprogramming through recruitment of a variant PRC1 complex (PRC1.1) to CpG islands (CGIs). Furthermore, we find that bone morphogenetic protein (BMP) represses Oct4/ Kdm2b-induced somatic reprogramming selectively. Mechanistically, BMP-SMAD pathway attenuates PRC1.1 occupation and H2AK119 ubiquitination at genes linked to development, resulting in the expres- sion of mesendodermal factors such as Sox17 and a consequent suppression of somatic reprogramming. These observations reveal that PRC1.1 participates in the establishment of pluripotency and identify BMP4 signaling as a modulator of PRC1.1 function.

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F-3087

CANCER REPROGRAMMING OF HUMAN GASTRIC CANCER CELLS INHIBITS TUMORIGENICITY INDUCED BY HOXA13-LNCRNA HOTTIP AXIS

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Reprogramming of cancer cells into induced pluripotent stem cells (iPSCs) is a compelling idea for inhibiting oncogenesis, especially the role of homeobox proteins on stemness and cancer features. We examined the role of various long noncoding RNAs (lncRNAs)-homeobox protein HoxA13 axis on the switching of the oncogenic function of bone morphogenetic protein 7 (BMP7), which is significantly lost in the gastric cancer cell derived iPSC-like cells (iPSLCs). BMP promoter activation occurred through the co-recruitment of HoxA13, mixed-lineage leukemia 1 lysine N-methyltransferase (MLL1), WD repeat-containing protein 5, and lncRNA HoxA transcript at the distal tip (HOTTIP) to commit the epigenetic changes to the trimethylation of lysine 4 on histone H3 in cancer cells. By contrast, HoxA13 inhibited BMP7 expression in iPSLCs via the co-recruitment of HoxA13, Enhancer of zeste homolog 2, Jumonji and AT rich interactive domain 2, and lncRNA HoxA transcript antisense RNA (HOTAIR) to various *cis*-element of the BMP7 promoter. Knockdown experiments demonstrated that HOTTIP contributed positively, but HOTAIR regulated negatively to HoxA13-mediated BMP7 expression in cancer cells and iPSLCs, respectively. These findings indicate that the recruitment of HoxA13-HOTTIP and HoxA13-HOTAIR to different sites in the BMP7 promoter is critical for the oncogenic fate of human gastric cells. Reprogramming with OCT4 and JDP2 can inhibit tumorigenesis by switching off BMP7. This OCT4-JDP2-HOXA13-lncRNA-BMP7 cascade was also observed in the case of Helicobacter pylori infected gastric cancer derived iPSCs.

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LATE BREAKING ABSTRACTS

F-4001

TREATMENT OF COLLAGEN-INDUCED ARTHRITIS USING IMMUNE MODULATORY PROPERTIES OF HUMAN MESENCHYMAL STEM CELLS-DERIVED SECRETOMES

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Secretomes derived from mesenchymal stem cells (MSCs) contribute to improved recovery against tissue injury. However, the therapeutic effects of secretomes are not fully understood. We investigated the potential therapeutic effects of secretomes in an experimental animal model of rheumatoid arthritis (RA), and explored the mechanism underlying immune modulation by secretomes. We evaluated the therapeutic effect of adipose derived MSCs-derived secretomes in DBA/1 mice with collagen-induced arthritis (CIA). CIA mice were injected intraperitoneally with 200 μg of secretomes. 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. Mice treated with secretomes showed significant improvement in clinical joint score, comparable to MTX-treated mice. Histologic examination showed greatly reduced joint inflammation and damage in secretomes-treated mice compared with untreated mice. Secretomes 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 secretomes compared to untreated or MTX-treated mice. MSCs-derived secretomes 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 secretomes could be an effective therapeutic approach for RA.

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F-4003

THE DEVELOPMENT OF A STATE-OF THE ART, HIGH SENSITIVITY STOOL DNA TEST TO IMPROVE SCREENING FOR SERRATED POLYPS AND EARLY LESIONS

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The serrated or alternate pathway to colorectal cancer (CRC), accounts for 25% of all CRC. Serrated polyps frequently have sessile (flat) morphology, are much less likely to bleed and therefore be detected by faecal immunochemical testing (FIT), and are less conspicuous at colonoscopy. As such, patients with these early cancers often present or are diagnosed later, a major impediment to successful removal and treatment. Not surprisingly, this group contains treatment-resistant and worst-prognosis subtypes as well as being over-represented in interval polyps and cancers. Serrated CRC and the development and detection of these lesions are the focus of this project. We model serrated CRC through combining recent advances in stem cell biology, genome editing and small animal colonoscopy. We incorporate serrated specific genetic changes into primary colon organoids, orthotopically inject the resulting 'serratoid' lines into recipient mouse colon and follow tumour formation and progression in vivo. The beauty of our approach using genome editing is that, unlike traditional transgenic approaches, we can rapidly incorporate multiple and previously untested genetic alterations associated with serrated CRC. Preclinical models have also yet to be effectively used to develop new strategies for CRC screening. To this end, we are testing a new, non-invasive, highly sensitive method for detecting early genetic changes, such as BrafV600E, found within these hard-to-detect serrated lesions. We utilise the most sensitive digital PCR technology on the market (Raindrop) to analyse stool DNA samples from our preclinical models for serrated specific genetic changes. We envisage this could one day lead to a test that will complement the current National Bowel Cancer Screening Program, to improve the detection of these hidden polyps and cancers.

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F-4005

THE PATHOPHYSIOLOGICAL ROLE OF MICRORNAS IN DIABETIC CARDIAC STEM CELLS

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Cardiac stem cells (CSCs) have been implicated as the most suitable source of stem cells in regenerating the diseased heart. However, diabetes is known to cause a progressive loss in the functional efficacy of CSCs. The mechanism behind this loss is unclear. MiRs are small, non-coding RNA molecules that regulate genes at the post-transcriptional level. In this study we aimed to investigate if dysregulation of miRs could be the underlying cause for the functional deterioration of diabetic CSCs and whether genetic manipulation of miRs can improve their function. Sca-1+ CSCs were isolated from type-2 diabetic (n=5) and non-diabetic (n=5) db/db mice. Among 598 miRs evaluated by an n-counter miR expression assay, the expression profiles of 16 miRs were significantly altered in the diabetic condition. Among the 16 miRs, miR-329 (3.4 3 0.6 (P=0.02)), miR-376c (3.9 3 0.7 (P=0.01)) and miR-495 (2.3 3 0.4 (P=0.02)) were significantly upregulated while miR-30c (0.4 3 0.1 (P=0.02)) was significantly downregulated in diabetic CSCs. These miRs are involved in cell proliferation and survival. Mass spectrometry analysis predicted pro-apoptotic VDAC1 as a direct target for miR-30c and cell cycle regulator CDK6 as the direct target for miR-329, -376c and -495. Western blot analyses confirmed a marked increase in VDAC1 expression (3.9 3 0.9 (P=0.01)) and a significant decrease in CDK6 expression (0.76 3 0.1 (P=0.04)) in the diabetic CSCs. Moreover, luciferase assays confirmed VDAC1 as the direct target for miR-30c. An overexpression of miR-30c in diabetic CSCs showed a marked reduction in VDAC1 (0.7 3 0.1 (P=0.03)) and cleaved caspase-3 (0.6 3 0.1 (P=0.04)) protein expression, as well as reduced caspase-3/7 activity, suggesting that genetic manipulation of miRs could be beneficial in promoting CSC survival and function. Altogether, our results suggest that altered miR-30c expression may contribute to the reduced functional efficacy of CSCs in the diabetic heart by regulating apoptosis.

F-4007

AKT/MTOR PATHWAY CROSSTALKS WITH HETEROCHROMATIN REMODELING DURING MSC SENESCENCE

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Cellular senescence is a process that cells experience the gradual loss of proliferation and function, which can be induced by telomere shortening, DNA damage, reactive oxygen species or activation of oncogenes. Mesenchymal stem cells are multi-potent stromal cells which can differentiate into osteoblasts, chondrocytes, and adipocytes. The decline in self-renewal and function of senescent MSCs contributes to geriatric diseases such as osteoporosis. Recent studies suggest that long-term activation of Akt/mTOR pathway is associated with aging and a diminished pool of self-renewing stem cell population. On the other hand, it has become evident that chromatin-based epigenetic mechanisms underlie important aspects of cellular senescence. However, although Akt/mTOR pathway and chromatin reorganization have both been causally implicated in the establishment of cellular senescence, their relationship remains largely undefined. In this study, we successfully established acute MSC senescence model using Doxorubicin and H2O2, which represent genotoxic- and ROS-induced senescence, respectively. Consistently, we demonstrate that MSC senescence is accompanied by activation of Akt/mTOR/S6K1 pathway and dynamic heterochromatin reorganization. Rapamycin treatment as well as knockdown of mTOR using siRNAs suppress DOX- and ROS-induced senescence, and promote heterochromatin remodeling process. By contrast, activation of Akt/mTOR pathway through MHY1485 or insulin treatment suppress the heterochromatin formation and aggravate cellular senescence. These data suggest that Akt/mTOR pathway crosstalks with heterochromatin remodeling during MSC senescence.

F-4009

TARGETING EAE-INDUCED DEMYELINATION AND AXONAL PATHOLOGY BY TRANSPLANTING HAEMATOPOIETIC STEM CELLS THAT OVEREXPRESS NGR(310)ECTO-FC FUSION PROTEIN

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Haematopoietic stem cell (HSC) transplantation is currently in clinical trials to treat multiple sclerosis (MS) as a means of modulating autoimmune-mediated inflammation and neurological disability. As MS is an immune-mediated neurodegenerative disorder, immunomodulation may not be effective for progressive neurodegeneration. Nogo receptor 1 (NgR1) is a high affinity receptor for myelin-associated inhibitory factors (MAIFs) that blocks for neurite outgrowth and may potentiate axonal degeneration in an animal model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE). As MS and EAE exhibit large numbers of inflammatory cell infiltrates within central nervous system (CNS) lesions, we utilised transplantable HSCs as a cellular delivery method of the NgR(310)ecto-Fc fusion protein. There were no immunomodulatory effects of the transplanted, modified HSCs with LV-NgR(310)ecto-Fc on immune cell lineages in the EAE-induced recipient female mice. We exclusively identified macrophages that were positive for the myc-tag (NgR-Fc-positive) occupying significant areas of inflammation and demyelination (2.0 x 10^3 3 0.5 x 10^3 cells/mm^2, p<0.0001), signifying the engulfment of NgR-Fc protein-MAIF complex by activated macrophages, which may increase the phagocytic activity of these populations and enhance repair. Importantly, three animals transplanted with LV-NgR(310)ecto-Fc-overexpressing HSCs, were rescued from symptoms associated with EAE, and exhibited axonal regeneration as well as remyelination in the white matter tracts of spinal cord. These results suggest that HSCs can be utilised as carriers of the therapeutic protein for specific delivery into EAE lesions and can potentiate neurological recovery.

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F-4011

DATA SHARING AND ITS REGULATIONS FOR TRANSLATIONAL HUMAN PLURIPOTENT STEM CELL RESEARCH

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Translation of human pluripotent stem cells (hPSC) research requires more human-derived data than traditional cell culture work. Disease modelling studies with hPSC are most informative when the cell lines can be characterised with genotypic and phenotypic profiles of the original sample donor. Therapeutic application of hPSC-derived cells raises the question of how to interpret genetic variability in the cells to be

transplanted. One option is to utilise existing registries genomic variants known to be associated with human cancers (and potentially selected other serious pathologies as well) to screen hPSC and derived cells for harmful mutations. Much human-derived genomic and phenotypic information counts as personal data under current data protection laws. The revised Act on the Protection of Personal Information (APPI) of 2015 in Japan and the forthcoming European General Data Protection Regulation (2018) both explicitly apply protections to genetic information derived from living persons. Complying with data protection law therefore becomes an important consideration for translation of hPSC and especially for the growing number of cell repositories set up to facilitate translational research around the world. Systems for managed access to human genomic data have been the subject of considerable policy development from governments and funding agencies since the completion of the Human Genome Project and provide a template for how hPSC research and biobanking can ensure access to cell-line associated human-derived genomic and phenotypic data whilst complying with data protection regulations. This paper will outline the requirements of data protection legislation in Europe (focusing on the GDPR) and Japan and review genomic data sharing polices produced by research funders such as the Research Councils UK, the Wellcome Trust, and the Agency for Medical Research and Development (AMED) in Japan to identify key lessons for hPSC research and biobanking.

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F-4013

GENERATION AND VALIDATION OF A TARGETED PITX2 HUMAN INDUCED PLURIPOTENT STEM CELL REPORTER LINE ALLOWS TO ISOLATE PERIOCULAR MESENCHYMAL CELLS

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In the eye development, cranial neural crest cells (NCCs) migrate from the neural tube and form periocular mesenchymal cells (POMs). POMs differentiate into diverse cells in several ocular tissues such as corneal endothelial cells, corneal stromal cells, mesenchymal cells of the ocular glands, trabecular meshwork cells, ciliary stromal cells, iris stromal cells, and scleral cells, eventually. Paired-like homeodomain transcription factor 2 (PITX2) is expressed and plays important roles in the development of POMs during normal embryogenesis. In this study, we established a human induced pluripotent stem (hiPS) cell line which expresses fluorescent reporter gene under the control of PITX2 gene using TALEN technology. First, in order to obtain better expression of enhanced green fluorescent protein (EGFP) at the downstream of PITX2 gene variant 3, we evaluated the efficiency of both bicistronic sequences (IRES or 2A) and polyadenylation (polyA) signals (BGH, TK, SV40, PITX2 3'UTR or b-actin 3'UTR) using the forced expression system in 293T cells. IRES sequences and SV40-polyA signal were decided as better candidates of bicistronic sequence and polyA signal, respectively. Secondly, the DNA double strand at the downstream of the stop codon in the exon 8 of PITX2 was cut using TALEN technology, then, PITX2-IRES-EGFP-SV40 PolyA sequence was heterozygously knocked in by homologous recombination. Next, POMs were directly induced from the hiPS cell reporter line according to the reference. EGFP fluorescence signals were confirmed by fluorescence microscopy. EGFP positive cells were collected by cell sorting, and gene expression and protein expression were assessed by qRT-PCR and immunofluorescence staining, respectively. The expression levels of EGFP and PITX2 corresponded to the fluorescence intensity of EGFP. In conclusion, we successfully established and validated a PITX2-EGFP hiPS cell reporter line. This cell line can be utilized for detailed analysis of PITX2 expression during induction of POMs from hiPS cells. In addition, this is useful for the purification of POMs for further analysis of the development of POMs-originated ocular tissues for regenerative medicine.

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F-4015

INVESTIGATING NOVEL REGULATORS OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Haematopoiesis is the process by which cell lineages undergo hierarchical development to produce mature cell types that constitute the blood. At the foundation of the haematopoietic system are the haematopoietic stem cells (HSCs), which possess multipotency and the ability to self-renew. These cells are subject to stringent regulatory processes to ensure adequate supply of blood cells without the risk of HSC exhaustion or the development of malignancies. Since the discovery that

HSCs can protect an organism from otherwise lethal irradiation, they have proven to be of great clinical value, providing treatment for many blood related diseases. Advancements in functional assays, purification techniques, and signal cell technologies (barcoding and single cell RNA-seq) has led to a richer understanding of HSC biology. Perhaps most excitingly, these studies have indicated that capacity for long-term self-renewal (which was previously thought to be unique to the stem cell) is retained by the multipotent progenitor populations. This has challenged the dogma of how life-long blood production is sustained. Improving our understanding on the intrinsic and extrinsic mechanisms that regulate the stem and progenitor compartments, as well as methods for in vitro and in vivo stem cell manipulation, will be essential for informing new therapeutics in regenerative medicine. During embryonic development, HSCs emerge from specific precursor cells (pre-HSCs) formed in the aorta-gonad mesonephros region (AGM). This project has focused on genes up-regulated during the maturation of pre-HSCs into HSCs. A knockout strategy has been applied to identify genes essential for HSC biology and assist in the development of better protocols for regenerative medicine. The top 10 differentially expressed transcription factors between the pre-HSC of the (AGM) and the fetal liver HSC were identified. A knockout mouse line for one of these candidates was generated, and subjected to phenotypic analysis. Preliminary analyses indicate an important role for this gene in regulating the size of the MPP populations during fetal development. Further characterization of the role of this gene will help identify mechanisms regulating HSC/MPP biology.

F-4017

FUNCTIONAL ANALYSIS OF HUMAN POLYMORPHISMS OF NICOTINIC RECEPTOR GENES IN VIVO USING IPS CELLS

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Schizophrenia is one of the top ten causes of long-term disability worldwide with a high economic cost associated. Like most psychiatric disorders, it is very genetically complex. A recent Genome Wide Association Study (GWAS) which identified 108 loci associated with schizophrenia found a strong link with the CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster on chromosome 15. Moreover, a recent study of the chromosomal interactions of those genes has shown that this cluster could have a regulatory role during development. We therefore want to delineate the role of genetic variation in nicotinic receptor genes for schizophrenia-relevant phenotypes. We hypothesise that these variations may contribute to early brain network formation and function, thereby shaping mental disease phenotypes. To study the impact of such genetic variations, we use neuronal precursors derived from human iPS cells injected into the frontal cortex of newborn mouse pups. We are then able to follow and study their maturation, morphology, function and network integration after transplantation into the rodent brain.The neurons integrate efficiently into the host brain, and can be studied functionally over months. We have characterised the different cell types deriving from these precursors as the cells differentiate in the host brain, using immunohistochemistry. In parallel, we monitor the activity of the injected neurons in the host brain network by opening cranial windows in mice injected with GCaMP6f-expressing neuronal precursors. Recordings can be made of the calcium transients caused by neuronal activity, and analysed by chronic long-term two-photon imaging in the anaesthetised and wake behaving mouse. This model of the human cortical development can then be used to compare the development of neuronal precursor cells, where specific mutations have been introduced using CRISPR technology, from healthy subjects and patients.

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F-4019

OVERCOMING THYROID HORMONE RESISTANCE IN HUMAN OLIGODENDROGLIAL PRECURSOR CELLS PROMOTES MYELINATION

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Cell membrane thyroid hormone (TH) transport can be facilitated by the monocarboxylate transporter 8 (MCT8), encoded by the solute carrier family 16 member 2 (SLC16A2) gene. Human mutations of the gene, SLC16A2, result in the X-linked-inherited psychomotor retardation and hypomyelination disorder, Allan-Herndon-Dudley syndrome (AHDS). We posited that abrogating MCT8-dependent TH transport limits oligodendrogenesis and myelination. We show that human oligodendrocytes (OL), derived from the NKX2.1-GFP human embryonic stem cell (hESC) reporter line, express MCT8. Moreover, treatment of these cultures with DITPA (an MCT8-independent TH analog), up-regulates OL differentiation transcription factors and myelin gene expression. DITPA promotes hESC-derived OL myelination of retinal ganglion axons in co-culture. Pharmacological and genetic blockade of MCT8 induces significant OL apoptosis, impairing myelination. DITPA treatment limits OL apoptosis mediated by SLC16A2 down-regulation primarily signalling through AKT phosphorylation, driving myelination. Our results

highlight the potential role of MCT8 in TH transport for human OL development and may implicate DITPA as a promising treatment for developmentally-regulated myelination in AHDS.

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F-4021

CAN XENOTRANSPLANTATION OF IPSC-DERIVED HEMATOPOIETIC PROGENITORS BE USED TO MODEL HUMAN MICROGLIA AND THEIR RESPONSES TO INJURY AND PATHOLOGY?

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Studies of human microglia have been hindered by the difficulty in obtaining these cells and the need to examine them either in vitro or within post-mortem specimens. This problem was further highlighted by recent findings that isolated human microglia undergo rapid transcriptional changes with in vitro culture. Given these data, there is pressing need to develop new approaches to directly observe and examine human microglial function within the CNS. To address this challenge, we hypothesized that transplantation of human iPSC-derived hematopoietic progenitor cells (HPCs) into the early postnatal mouse brain would result in differentiation of functional microglia that more accurately recapitulate in vivo human microglia than current models. GFP-expressing iPSC-derived HPCs were transplanted into P1 immunodeficient MITRG mice that express humanized versions of key cytokines that facilitate long-term survival and differentiation of human myeloid cells. Transplanted mice were aged and then exposed to either intrinsic or extrinsic insults including peripheral LPS, laser cell ablation, or transgenic-mediated production of beta-amyloid plaques. Brains were then collected for analysis by immunohistochemistry and recovery of human microglia by FACs sorting for GFP expression. Robust engraftment of human cells that morphologically closely resemble microglia was observed throughout the brain and these cells stained for multiple microglia markers including P2RY12, Iba1, CD11b, Pu.1, and TMEM119. Furthermore, exposure to insults resulted in human microglial activation, proliferation, and/or migration to injury sites and pathology. In addition RNA sequencing of FACS-sorted human microglia recovered from the mouse brain revealed numerous changes in the microglial transcriptome in response to LPS and the adoption of a more 'in vivo-like' transcriptional profile following transplantation. Both histological and functional analyses show that transplantation of human HPCs into the early postnatal mouse brain results in robust engraftment of microglia which actively monitor the CNS and rapidly respond to insults or injury. Taken together these data suggest that this model can be used to advance our understanding of human microglial biology in the context of normal brain development, aging, and disease.

F-4023

TRANSIENT FOXO1 INHIBITION ACTIVATES WNT SIGNALING IN THE DIFFERENTIATION STEP FROM PDX1+/ NKX6.1+ PROGENITORS TO NGN3+ ENDOCRINE PRECURSOR CELLS

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For the generation of pancreatic organoids from human pluripotent stem cells, previous researchers have improved the protocol at each differentiation step. Especially, generation of PDX1+/NKX6.1+ trunk cells, the origin of endocrine cells, was shown to be pivotal for the efficient endocrine cell production. On the other hand, induction of NGN3+ endocrine progenitors, the next differentiation step, has been remained a challenge since previous developmental biology provided a limited information in the regulatory machinery of NGN3 induction except for NOTCH inhibition. We found that the transient inhibition of FOXO1 activity significantly elevated generation of the NGN3+ progenitors resulting in the more endocrine cell production. Here we present the mechanistic bases for this observation that the transient FOXO1 inactivation activates Wnt signaling in PDX1+/NKX6.1+ trunk progenitors and stimulates their proliferation without altering the HES1 expression. Our findings indicate the previously unknown roles of Wnt signaling in this specific differentiation step of pancreatic endocrine lineage.

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F-4025

NOVEL HUMAN IPSC-DERIVED HEPATOCYTES WITH ADVANCED FUNCTIONALITY AND LONG-TERM 2D CULTURES OF HUMAN PRIMARY HEPATOCYTES FOR METABOLIC DISEASE STUDIES

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The liver plays an essential role in metabolic disorders and progression of multiple diseases. Therefore, a physiologically relevant in vitro hepatocyte model with low batch-to-batch variation, conserved genetic inter-individual variations and of unlimited supply is needed. Human pluripotent stem (hPS) cell-derived hepatocytes have a great potential to become a future model for numerous hepatocyte applications if they possess relevant functionality and usage window. Our new version of highly homogenous cryopreserved hPS cell-derived hepatocytes with a novel maintenance medium shows multiple mature liver features. They secrete albumin and urea during a 2-weeks window and show mRNA expression levels of albumin and urea cycle enzymes that are comparable to those of human primary hepatocytes (hphep). In addition, genes involved in both glucose and lipid metabolism are expressed on the same levels as in hphep. The hPS cell-derived hepatocytes respond to insulin by phosphorylation of AKT and show capacity to take up low-density lipoproteins and become steatotic if incubated with fatty acids. Moreover, activity of cytochrome P450 enzymes and expression of genes essential for the drug metabolizing machinery are stably detected during a 2-weeks window and on similar levels as in hphep. The novel maintenance medium developed for hPS cell-derived hepatocytes was tested, with minor modifications, on hphep in order to see if similar improvements in long-term viability and functionality could be achieved. Surprisingly, the novel culture medium allowed maintained morphology, viability and functionality of hphep for 4 weeks post-thawing and, thus, could prevent the typically observed rapid loss in functionality and cell viability of hphep in conventional 2D cultures. This is in sharp contrast to existing hepatocyte maintenance media. Both the novel generation of hPS cell-derived hepatocytes with mature hepatocyte functions and the new maintenance medium for hphep will empower the usage of hepatocytes in the area of metabolic diseases.

F-4027

LIN28 DEFICIENCY IN MOUSE PLURIPOTENT STEM CELLS INFLUENCE TWO-CELL LIKE STATE GENE EXPRESSION

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Embryonic stem (ES) cells derive from the inner cell mass of the blastocyst of mouse early embryos and can differentiate into three germ layers and germ cells, but can not differentiate into trophectoderm and extraembryonic tissues, and therefore, are pluripotent but not totipotent. At the two-cell stage, embryonic blastomeres are totipotent cells and have the potential to differentiate into all cell types of embryonic and extraembryonic tissues. The two-cell state is characterized by activation of endogenous retrovirus or ERV genes which often overlap with regulatory regions of early developmental genes, and thereby influence their expression and early embryo development. In cultured mouse ES cells, there is a rare and transient cell population with activated two-cell marker genes including ERVs. How are ERVs activated and inactivated during the two-cell state, and how do these events influence the whole embryonic genome activation and early embryo development are important questions to be answered. We found that the pluripotency factor Lin28 is sharply decreased upon entry into the two-cell state, and gets re-induced during the exit of two-cell state, and maintains expression until the blastocyst state. Lose-of-function Lin28 PS cells show altered expression of two-cell state genes, whereas gain-of-function let-7 PS cells recapitulate Lin28-deficient cell phenotypes. These findings suggest a novel role for Lin28 in embryonic genome activation during mouse early embryo development.

F-4029

COMPARISON OF THE CONTRACTILE PROPERTIES OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES DIFFERENTIATED IN 2D- AND 3D- CULTURE SYSTEMS

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been expected to provide new tools for regenerative medicine, drug discovery and safety assessment. These applications require characteristic information of hiPSC-CMs, such as how these respond to a compound, and this has been widely investigated. hiPSC-CMs can be generated through three-dimensional (3D) embryoid body formation or two-dimensional (2D) monolayer methods. Although 3D embryoid protocols generate larger hiPSC-CM yields, use of costly growth factor supplements (Activin A, BMP4, Dkk-1, VEGF etc.) are necessary, which can cause variations in differentiation efficiency according to inconsistencies in the supplements used. The 2D method is made possible through the use of chemical compounds (CHIR99021, IWP2 etc.), but generates hiPSC-CMs typically at low yields. Both 2D and 3D methods feature positive and negative aspects, and may present further complications where hiPSC lines may demonstrate differential yields in 2D and 3D systems. Thus, scientists examine and determine optimal conditions for their cells. Differences in the

iPS-CMs characteristics between 2D and 3D, however, are not fully understood. We have developed a media formulation that can be applied to both 2D and 3D culture systems using chemical compounds, resulting in a versatile, cost-effective hiPSC-CMs production system. Here, we investigate the iPS-CMs differentiated in 2D- and 3D- culture systems with high-speed video microscopy for characterizing contractile properties, which method is label-free and non-invasive, and based on analyzing motion vectors.

F-4031

INDUCTION OF FUNCTIONAL HUMAN MESENCHYMAL STROMAL CELLS FROM IPSCS IN XENO-FREE CONDITION

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Mesenchymal stromal cells (MSCs) are adult multipotent stem cells that can differentiate into various cell types such as osteocytes, chondrocytes, adipocytes. Because of their multipotency, MSCs are expected to be applied to regenerative medicine. The aim of this study is to induce MSCs from pluripotent stem cells for cell therapy. To this end, we tried to induce MSCs from iPSCs through neural crest cell (NCC) lineage in xeno-free condition, and evaluate their in vivo functions. First, we modified the previous MSCs induction method (Fukuta et al., PLOS ONE, 2014) to xeno-free condition. Bovine serum albumin contained NCC differentiation medium was replaced by StemFit AK03N (Ajinomoto) based medium and fetal bovine serum was removed in MSC differentiation medium. By using modified induction method, iPSCs differentiated into MSCs with high efficiency. We named the MSCs induced by newly method as XF-iMSCs (Xeno-Free inducing MSCs from NCCs). Next, to evaluate their in vivo function, we transplanted the XF-iMSCs into model mice for bone regeneration and skeletal muscle regeneration, and confirmed regenerative potency. Notably, transplanted XF-iMSCs contributed regeneration of surrounding host cells, suggesting that XF-iMSCs secreted some soluble factors or exosomes into affected part. In summary, we succeeded to induce functional XF-iMSCs from human iPSCs what would be beneficial for future application of regenerative medicine.

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F-4033

HPSC-DERIVED MICROGLIA IN AN IN VITRO TRI-CULTURE PLATFORM TO ELUCIDATE MECHANISMS OF ALZHEIMER'S DISEASE

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Microglia derived from human pluripotent stem cells are a powerful tool that can be used to explore non-cell autonomous interactions between human neurons and microglia when modeling human neurodegenerative diseases. In this study, we have derived microglia from hPSCs with a novel, step-wise developmental paradigm that closely resembles in vivo development of microglia. We use them in both co-culture (2 cell types) and tri-culture (3 cell types) with hPSC-derived neurons and astrocytes to build a more complex in vitro model to study neural disease pathogenesis in which non-neuronal cells play a central role. Particularly, we apply our tri-culture platform to study Alzheimer's disease, which is defined by not only a neuronal phenotype but also a potentially pathogenic microglial and astrocytic component. We cultured APPSwe mutant hPSC-derived neurons, a genetic model for Alzheimer's disease exhibiting increased amyloid-beta burden and tau pathology in vitro, with wildtype hPSC-derived microglia and astrocytes, and compared to cultures containing wildtype hPSC-derived neurons. These co-culture and tri-culture comparisons yielded three phenotypes that appear to be modulated in a non-cell autonomous manner: 1) cultures containing wildtype microglia showed decreased amyloid-beta burden in a selective manner, 2) cultures containing wildtype microglia showed reduced tau pathology within the Alzheimer's neurons, and 3) tri-cultures containing APPSwe neurons showed increased complement component 3 (C3) levels, indicating increased reactivity in the disease condition. Our future studies will include culture of Trem2 mutant microglia within the tri-culture disease condition to assess for a reversal of the protective phenotypes seen in 1) and 2). Additionally, we will use C3 knockout microglia or astrocytes to discriminate which cell type primarily releases C3 within the disease vs. wildtype condition.

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F-4035

THE RARE GENETIC DISORDER OF ARGINASE-1 DEFICIENCY STUDIED IN INDUCED PLURIPOTENT STEM CELLS

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Arginase-1 deficiency (ARG1D) is a rare genetic disorder of the urea cycle that leads to severe neurological impairment. Arginase-1 (ARG1) catalyzes the conversion of arginine to ornithine and urea in the liver to detoxify ammonia. An inducible mouse ARG1 KnockOut (KO) model, whereby exons 7 and 8 were deleted by Cre-mediated recombination, along with induced pluripotent stem cells (iPSCs) derived from these mice, were used to study ARG1D. Successful gene editing repair of the mouse Arg1 gene in iPSCs, with subsequent differentiation to form hepatocyte-like cells (HLCs) and macrophages took place. To complement this work, we used a human iPSC (hiPSC) line (wild type; WT), along with two isogenic ARG1 KO hiPSC lines that were generated by the CRISPR/Cas9 system with dual gRNAs targeting exon 2 (clone E2 with a 1-bp deletion; E2 line and clone G1 with a 1 bp insertion; G1 line). The hiPSC lines differentiated to HLCs in a 21-day hepatic differentiation protocol and expressed the hepatocyte genes ALB, HNF4A, ASGR1, CYP3A4 and SERPINA1, albeit at significantly lower levels compared to primary human hepatocytes, as well as AFP, which was absent in the primary cells. ARG1 expression and the ability to form urea was minimal compared to mature hepatocytes, which highlights the need to develop more robust protocols for mature hepatocyte specification. The three hiPSC lines formed uniform and typical embryoid bodies and expressed the markers of neural precursors (nestin, PAX6 and SOX1) and upon further neuronal lineage differentiation resulted in βIII tubulin/MAP2-positive neurons. The formation of cerebral organoids was also evaluated using the STEMdiffTM Cerebral Organoid differentiation protocol. Organoids derived from the hiPSC lines expressed PAX6 at day 20. ARG1 was expressed at low levels in the WT hiPSC day (d)20 organoids but not in d30 or d40 organoids. Expression of ARG1 was abolished in the ARG1 KO E1 line at all three timepoints. RNAseq analysis of gene expression in d20 organoids has been carried out to assess gene network alterations in the absence of ARG1 expression. We hope to gain further insight surrounding ARG1D by using these or other similarly gene-edited hiPSC KO lines to investigate whether the loss of hepatic ARG1 or loss of brain-expressed ARG1 contributes to the defective neurological phenotype.

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F-4037

COMPARISON OF CHARACTERISTICS IN INSULIN PRODUCING CELLS DERIVED FROM HUMAN ADIPOSE AND UMBILICAL CORD MESENCHYMAL STEM CELLS

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Pancreatic beta cells dysfunction causes diabetes and hyperglycemia, leading to serious diabetes complications. Therefore, Islet transplantation has been used as therapy for persistent hyperglycemia diabetes patients. But islet transplantation has obstacles that are limitation number of donor and poor yield of islet isolation. So, stem cell therapy for diabetes has been consider as replacement of transplanted human islets. Stem cell therapy studies for replacement of human islets include differentiation of insulin producing cells(IPCs), functional beta cells, through reprogramming adult stem cells into functional beta cells. Because Human umbilical cord mesenchymal stem cells (hUC-MSC) are extracted from relatively young donor, hUC-MSC have recently emerged as a promising solution for cell therapy. But limited donors will not allow conclusion on potential alternative in islet transplantation. In this study, we investigate that characterization of isolated mesenchymal stem cells from different human tissue and comparison of reprogramming inulin producing cells in human adipose mesenchymal stem cells (hAd-MSC) and human umbilical cord mesenchymal stem cells (hUC-MSC). We first examined that hAd-MSC and hUC-MSC are defined in mesenchymal stem cell markers. And then, we compared the yield of the IPCs that are derived from the hAd-MSC and hUC-MSC using direct reprogramming method. IPCs derived from the hAd-MSC and hUC-MSC not only are exhibited changed morphology like cell cluster, but also increased in the mRNA expression of the beta cell specific markers. Moreover, when IPCs derived from hAd-MSC and hUC-MSC are exposed in high glucose condition, all IPCs are enhanced Glucose stimulated insulin secretion (GSIS) and C-Peptide levels. These results suggest that reprogramming human IPCs can differentiate not only into insulin producing cells but also into functional beta cells. Moreover, hAd-MSC and hUC-MSC are no significant difference in production and function of IPCs. hAd-MSC can easily obtain because adipose tissue has been discarded as a medical waste. Therefore, our study can provide an effective in vitro differentiation method as compared different human mesenchymal stem cells and serve hAd-MSC as new potential source for islet transplantation.

F-4039

SAFETY AND TOLERANCE OF MAGNETIC BACTERIA BASED MRI CELL TRACKING CONTRAST AGENTS

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An emerging new technology utilizes intact magnetotatic bacteria labeling agents for eukaryotic cells in magnetic resonance imaging (MRI) based cell-tracking. Magnelle® cell tracking agent or MEs, which function as MRI-contrast agents, have successfully been incorporated into a range of stem and progenitor cells, allowing for non-invasive MRI tracking of transplanted cells, in vivo. Although non-pathogenic, because MEs are derived from Gram-negative bacteria, a detailed understanding of safety and processing are paramount prior to their translational use. Gram-negative bacteria, which have been used in medical settings for decades, require unique consideration as they contain liposaccharide (LPS), also known as endotoxin. LPS is highly pyrogenic, and exposure to LPS can lead to a range of adverse effects. However, the level of pyrogenicity can vary depending on the molecular structure of the lipid A component of the LPS present. The rabbit pyrogen test (RPT) is the standard in vivo model to evaluate pyrogenicity. Different cohorts of naïve New Zealand white rabbits were injected with approximately 2 x 10e5 hMSCs that were either labeled with MEs or unlabeled. Under USP <151> RPT standards, the presence of pyrogens is indicated by an observed body temperature rise of 0.5°C or more over a steady-state background body temperature within three hours of exposure. No temperature change greater that 0.2°C was observed in either ME-labeled or unlabeled cohorts consistent with the presence of a non-pyrogenic LPS in MEs. Tolerance of MEs was further assessed in female nude BALB/c mice introduced IV, IM or IT with 10e9 to 10e11 MEs/ml. Test limits for weight loss and mortality were not exceeded for these doses demonstrating that MEs are well tolerated. Furthermore, safety analysis of ME-labeled hMSCs injected into the rat gastrocnemius muscle suggested no evidence of test-related tissue injury. These results combined, strongly to support the conclusion that MEs and ME-labeled cells are safe for a variety of cell types and in differing animal models and sites of injection.

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F-4041

MAPPING THE STRUCTURE AND BIOLOGICAL FUNCTIONS OF HUMAN MESENCHYMAL STEM CELL SPHEROIDS USING MICROFLUIDICS

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The culture of human mesenchymal stem cells (hMSC) as three-dimensional spheroids enhances their secretion of immune-regulatory molecules. However, neither the spatial organization of productive cells within the spheroids nor the molecular mechanisms that lead to this increase are understood. Here we map the spatial distribution of immune-regulatory cells within spheroids of umbilical cord derived hMSCs (UC-hMSCs), by using an integrated droplet microfluidic platform for high-density culture and quantitative imaging. The analysis of single-cells, in situ within thousands of spheroids, reveals spatial heterogeneities in the cell arrangements that correlate with their level of production of immune-regulators. Indeed the UC-hMSCs located near the edge of the spheroids display more functional N-cadherin junctions linked to the polymerized actin, and produce a higher level of COX-2, which is an intra-cellular marker for immune-regulatory secretions. In contrast, the cells in the core of the spheroids display weak interactions that correlate with a lower level of COX-2 expression and an ensuing caspase-3 activation. Moreover, inhibiting the functional junctions also affects the distribution of COX-2 expression in the spheroids. Altogether these results indicate that the structural organization in hMSC spheroids dictates differential activation of specific molecular signaling (NF-κB and actin polymerization), in turn leading to the regional patterning of the cells' biological functions. This new intra-spheroid understanding can guide further development of the clinical uses of hMSC spheroids.

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F-4043

A NEW SYNTHETIC READY-TO-USE FN1 MOTIFS SURFACE FOR HUMAN INDUCED PLURIPOTENT STEM CELL EXPANSION IN AN ANIMAL-COMPONENT-FREE CULTURE SYSTEM

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Thanks to their extensive in vitro self-renewal property and their large differentiation potential, human pluripotent stem cells (hPSCs), constitute an exciting tool in research fields as regenerative medicine, pharmaceutical applications as well as disease modeling. hPSC expansion conditions including growth surface and culture medium have progressively evolved in the last decade from the traditional mouse embryonic fibroblast (MEF) feeder layer-based culture system towards more defined feeder-free cell culture systems. Optimal hPSC culture systems must combine a completely defined and animal-component-free composition and high consistency while ensuring robust pluripotent stem cell proliferation, routinely characterized by cell morphology, key pluripotency marker expression, genetic stability and differentiation potential maintenance. Based on a proprietary coating technology, a new ready-to-use surface, made up of synthetic fibronectin-derived motifs, was specifically designed to mimic the cell attachment site of native extracellular matrix proteins. Used in combination with well-defined culture medium and dissociation solution, the fully synthetic Eppendorf CCCadvanced™ FN1 motifs surface represents an effective animal- and human-component-free alternative to the conventional feeder layer-based culture system and to other biological coating-dependent hPSC culture systems. The present experimental work demonstrates the suitability of this ready-to-use surface for the long-term expansion of undifferentiated human induced pluripotent stem cells (hiPSCs). Throughout 20 successive passages cultured on this surface, hiPSCs maintain their typical cell morphology, a stable doubling time and karyotype as well as pluripotent marker expression. The in vitro trilineage differentiation capacity confirms finally the maintenance of their functional pluripotency. With its unique properties, this surface combines convenience with reliable high-quality hPSC performances.

F-4045

GENERATION OF HUMAN LIVER TISSUE WITH FUNCTIONAL BILIARY STRUCTURE

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Human induced pluripotent stem cells (iPSCs) have been used as an alternative cell source for production of human hepatocytes. Although there are many studies reporting hepatic differentiation of human iPSCs, iPSC-derived hepatocytes displayed immature phenotypes. To improve their functional maturation, three-dimensional (3D) culture of hepatocytes has been used. However, as they synthesize and excrete cytotoxic bile acid, it is difficult to maintain the 3D culture of hepatocytes for a long term. In this study, to overcome these challenges, we isolated the Dlk1(+) hepatoblasts and Epcam(+) biliary epithelial cells from the mouse liver, and established a 3D co-culture system with a functional biliary structure. Immunocytochemistry demonstrated that bile duct-like structures were formed in the liver spheroids. In addition, we found that the expression levels of hepatocyte marker genes such as Alb, Cyp3a11 and Abcb11 were induced in the 3D co-culture system. Based on these experiments in mouse liver cells, we also established the 3D co-culture system using human iPSC-derived hepatoblasts and biliary epithelial cells isolated from the liver of tdTomato transgenic mice. After 14 days of culture, tdTomato-positive biliary epithelial cells formed cysts with the luminal structure in the liver spheroids. Then, we assessed the liver function of this co-culture model using Cholyl-Lysyl-Fluorescein (CLF), which is a fluorescent bile acid. After incubation of liver spheroids with CLF, fluorescence was accumulated inside the cysts. Thus, all of these results indicated that the 3D co-culture model is useful for generating functional liver tissue in vitro. We are currently investigating whether this 3D co-culture model could be maintained for a long term.

F-4047

INJECTABLE THERMOSENSITIVE HYDROGEL BASED NANOPARTICLES-LOADED SYSTEM FOR LOCAL DELIVERY OF VANCOMYCIN FOR THE TREATMENT OF OSTEOMYELITIS

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The successful treatment of antimicrobial therapy have not yet achieved in the bone infections due to the physiological characteristics of bone. Adequate concentrations of anti-infective agents and adequate retention time in local areas are two essential conditions for the treatment of osteomyelitis. Vancomycin (VCM), a water solubility antibiotic, had been used to therapy osteomyelitis for anti-infection. A local VCM delivery system is recognized as the most effective therapeutic approach for the treatment of osteomyelitis. However the studies of VCM at different nanocarrier, such as nanoparticles were shown low drug loading. For enhance the drug loading, we utilized the positive charge quaternary ammonium chitosan (QAC) and negative charge of carboxylated chitosan (CC) to encapsulate VCM, respectively, and then prepared the new nanoparticles (VCM/CC-QAC-NPs). To prolong anti-infection in the osteomyelitis site, we further put the nanoparticles entrapped in hydrogel to achieve sustained

drug release. And for effective filling the irregular shape tissue, we select chitosan (CS) and glycerol phosphate disodium salt (GP) to form thermosensitive hydrogel and inject into marrow cavity. In the present study, we constructed VCM/CC-QAC-NPs/CS-Gel drug delivery systems, which the VCM/CC-QAC-NPs were entrapped in CS/α-GP/β-GP thermosensitive hydrogel, to realize prolong retention in the damage area. The VCM/CC-QAC-NPs were prepared by a self assembly method and the characterized of NPs with small particles size (178.4 3 5.0 nm) and higher drug loading efficiency (15.95 3 1.0%), compared with drug loading efficiency of VCM/PLGA NPs (8.8 3 0.1%) and VCM/PCL NPs (12.6 3 2.1%). And further to investigate the formulation of VCM/CC-QAC-NPs/CS-Gel delivery system, the gelation time of the best formulation was 5 min at 37 ℃. The research also obtained an excellent result of the efficiency of antimicrobial activity and osteoblast proliferation in vitro. In vivo, the VCM/CC-QAC-NPs/CS-Gel treat rabbit tibia osteomyelitis model had shown significant anti-infection and bone repair capability. These results indicated that the VCM/CC-QAC-NPs/CS-Gel drug delivery system is a potential formulation against osteomyelitis and would be appropriate for further study in the clinical of osteomyelitis disease.

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F-4049

IMMUNOMODULATORY EFFECTS OF THE INTRATRACHEAL INSTILLATION OF HUMAN MESENCHYMAL STEM CELLS IN BALB/C MICE SUBMITTED TO THE ALLERGIC ASTHMA EXPERIMENTAL MODEL

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Asthma is a chronic disease, characterized by inflammation and airway remodeling, with immune response of TH2 type. Mesenchymal stem cells (MSCs) has demonstrated its potential in modulating of lung inflammatory process and repair damage tissue. The aim of this study was to investigate the immunomodulatory effects of MSCs from human bone marrow in Balb/c mice submitted to the allergic asthma experimental model. Ninety male Balb/c mice were distributed into eight groups. Five groups were sensitized (intraperitoneal injections) and provoked (intratracheal instillations) with ovalbumin, and three were used as control group. On day after the last allergen provocation, two groups were treated with 1x106 MSCs (intratracheal injection). Two groups were euthanized on day after the last allergen provocation (to validate the asthma model), three on 7th day and three on 14th day after treatment. Bronchoalveolar lavage (BAL) fluid was collected for quatifications: total protein, hydrogen peroxide (H2O2), total and differential cytological, proinflammatory and anti-inflammatory interleukins (IL). The lungs were removed for histopathological evaluation of inflammation severity. On day 0, asthma control group showed a significant increase in eosinophils percentage, proteins, IL-13 and IL-17 in BAL fluid. On 7th day after transplantation, the group that received MSCs demonstrated significant reduction in eosinophils, proteins, H2O2, IL-5, IL-13 and IL-17, and increase in IL-10. On 14th day after treatment, the group that received MSCs presented significant reduction in total cells, proteins, IL-13 and IL-17 in BAL fluid, both compared to control group. Treatment with MSCs reversed alterations in lung remodeling in groups that received MSCs, with statistical difference in most of criteria: inflammatory infiltrate, collagen deposition, epithelial thickening, epithelium desquamation and muscle thickening (there was no significant difference just between 14 days groups, $p>0.05$). There was no significant difference in mucus production between groups that received MSCs or not ($p>0.05$). MSCs showed immunomodulatory effects in Balb/c mice submitted to the allergic asthma experimental model, through the inflammatory process control and lung remodeling, suggesting its potential use on regenerative medicine.

Funding Source: This study was supported by Araucaria Foundation - No. 22701 (Curitiba, Paraná, Brazil).

F-4051

HYPOXIA- MEDIATED EPIGENETIC REGULATION OF BRAIN TUMOR STEM CELLS

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Shivani Arora Mittal - *University of Delhi South Campus, New Delhi, India*
Jonita Chongtham - *University of Delhi South Campus, New Delhi, India*
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Malignant glioblastomas (GBMs) are highly invasive and heterogeneous tumors with dismal prognosis. The hypoxic core of such solid tumors is enriched with continually self-renewing CSCs, which significantly contribute to chemo- and radioresistance. Hence, investigating the molecular machinery that regulates these has relevance in effectively targeting such cancers. In this study, we provide novel insights into hypoxia-mediated regulation of CSCs in GBM. We have studied epigenetic mechanisms controlling the overexpression of two key pluripotency-associated factors, OCT4 and NANOG, in hypoxic microenvironment. we have demonstrated DNA demethylation and chromatin modifications at Oct4 and Nanog regulatory regions as key mediators of their induction in hypoxic glioma cells. We observed global hypomethylation accompanied with reduction in transcript levels of DNA methyl-transferases (Dnmt1, Dnmt3a, and Dnmt3b) in the hypoxic microenvironment. Site-specific analysis at three of Oct4 and one Nanog promoter region exhibited simultaneous enrichment of 5-hmC along with reduction in 5-mC levels in hypoxia. Concurrently, their repressive histone mark, H3K27me3, was also reduced. Our work has demonstrated involvement of epigenetic modifications mediated by TET in regulating master transcription factors that maintain pluripotency in GBM hypoxia. we propose a model in which hypoxia-induced TET1/TET3 and 5-hmC clusters at regulatory regions of core pluripotency genes activate the transcriptional program to promote pluripotency by differentiated glioma cells. Our study thus implies the pivotal role of hypoxia in maintenance of pluripotency in cancer cells, and further elaborates on the epigenetic mechanism activated in gliomas.

POSTER SESSION III-EVEN 19:00 – 20:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

F-1002

RATE AS A POTENTIAL DETERMINANT OF THE DISTRIBUTION OF HUMAN MESENCHYMAL STEM CELLS INJECTED VIA THE LATERAL VENTRICLE OF THE MOUSE BRAIN

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Emerging studies have reported on the promising role of stem cell therapy as a treatment option for varying diseases. In order to improve the effectiveness of stem cell treatment, many factors must be considered. Recently, we have reported that when human mesenchymal stem cell (MSCs) are injected at lower concentrations into the lateral ventricle of wild-type mice, not only was the viability of the MSCs enhanced but MSCs were also distributed farther from the injection site. After performing this study, we carried out an additional study where we observed changes in the distribution of MSCs when injected at fixed rates but varying concentrations (high, middle, and low). According to magnetic resonance (MR) images, immunohistochemistry (IHC) stains, and real time quantitative PCR results, keeping the rate constant did not significantly increase the widespread distribution of MSCs in the mouse brain. For the high group, the highest number of remaining cells was identified at the injection site while for the middle and low groups, more cells were detected posterior to the injection site (about 2mm from the injection site). While these results were observed 1 day following the injection, it is possible that greater distribution could have been observed if sacrificed at a later time point. The results of this study stress that when administering MSCs into the lateral ventricle, cell concentration, injection rate, and sacrifice time point must be addressed.

Funding Source: This study was supported by the the Basic Science Research Program through the National Research Foundation of Korea; (NRF) funded by the Ministry of Education, Republic of Korea (NRF-2017R1D1A1B03033979).

F-1004

CSRP2BP IS A NEW NOVEL MARKER IN TROPHOBLAST DEVELOPMENT PREECLAMPSIA

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Preeclampsia(PE) is the leading cause of maternal and perinatal mortality and morbidity. The underlying mechanism is still not completely elucidated. Epigenetic abnormalities might be closely related to the pathogenesis of PE. Cysteine-Rich Protein 2-Binding Protein (CSRP2BP) is a newly defined histone acetyltransferase, however, there the research of its function in human cells is very limited. It will be interesting to explore the potential function of CSRP2BP in the process of early trophoblast development and the pathogenesis of preeclampsia(PE). We examined the expression of CSRP2BP in placental tissues. CSRP2BP was enriched in the nucleus of trophoblast cells from early pregnant stage. It was significantly decreased in placental tissues of PE patients compared with that in control group by qPCR, western blotting and immunohistochemistry. Meanwhile, we generated CSRP2BP loss and gain of function models in HTR8 cells by using siRNA or lentivirus systems combined with Crispr/case9 technology. Transwell assays, scratch-wound assays, EDU and plate clone formation assays, cell apoptosis assays, cell cycle assays were used to examine the function of CSRP2BP in HTR8 cells model. We demonstrated that knockdown of CSRP2BP in HTR8 dramatically decreased the cell proliferation, migration and invasion. It also showed the significantly lower plate clone formation rate in CSRP2BP knockdown -HTR8 cells compare with the WT HTR8 cells. RNA-seq was used to examine the gene expression panel which might be regulated by CSRP2BP. More studies will be performed to explore its underline mechanism. This study was the first time to explore the role of CSRP2BP in PE. It will help us to better understand the pathogenesis of PE, which might be helpful in future application of novel therapeutic targets in PE.

Funding Source: International Science & Technology Cooperation Program of China [2014DFA30180 to Y.M]; Hainan Provincial Department of Science and Technology [ZDKJ2017007]; National Science Foundation of China [81660433].

F-1006

USING HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS AS A MODEL FOR INTRAUTERINE EFFECTS OF GESTATIONAL DIABETES ON OFFSPRING TISSUES

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Gestational Diabetes Mellitus (GDM) is one of the most common pregnancy complications in the world. The prevalence of GDM is 20% of live births in Singapore. Epidemiologic studies have also highlighted associations between maternal hyperglycemia and significantly increased offspring risks of obesity, heart diseases and diabetes. Human umbilical vein endothelial cells' (HUVECs) functional and metabolic properties have been shown to be affected by high maternal blood glucose levels. Using HUVECs harvested from the umbilical cords of children born to either normal or GDM mothers, we aimed to study cellular and developmental differences between these two cohorts. We found that the level of anti-apoptotic protein Bcl-xL is much lower in HUVECs harvested from GDM pregnancies compared with those from normal pregnancies. This suggests an inferior protection against apoptosis in endothelial cells from offspring from GDM mothers. If this persists, it might explain the dysfunctional phenotypes observed in GDM endothelial cells and an increased risk of heart diseases later in life. Microarray and RNA-sequencing data comparing normal and GDM HUVEC samples also show differential regulation at the transcriptome level. Overlapping gene number between these two assays is low, showing that GDM only affects a small subset of genes. However, many of these genes - such as CRYAB, APCDDL, DCN, etc. - are associated with Type 2 diabetes and pancreatic beta cell functions. We plan to investigate the differential regulation of these genes by GDM and how they might impact pancreatic beta cell development and functions. The findings will give us some clue on how maternal hyperglycemia leads to an increased risk of insulin resistance later in the offspring's life.

Funding Source: The research is supported by the Singapore National Research Foundation and administered by the Singapore Ministry of Health's National Medical Research Council, Singapore. Investigators are supported through A*STAR funding.

F-1008

MESENCHYMAL STEM CELL CULTURE SUPERNATANT PREVENTS LUMINAL STRICTURE FORMATION AFTER LARGE ENDOSCOPIC SUBMUCOSAL DISSECTION IN THE RECTUM OF PIGS

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Takeshi Mizushima - *Hokkaido University, Sapporo, Japan*
Marin Ishikawa - *Hokkaido University, Sapporo, Japan*
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Endoscopic submucosal dissection (ESD) for colorectal tumors has been widely accepted in the last decades as a minimally invasive treatment option with higher en bloc resections and a lower recurrence rate. However, ESD often causes postoperative stricture when widely dissected. Mesenchymal stem cells (MSCs) are a valuable cell source in regenerative medicine, and MSC culture supernatant (MSC-CS) has reportedly inhibited inflammation and fibrosis. The aim of this study was to examine the effect of MSC-CS enema on post-ESD stricture. We performed circumferential ESD with 5 cm of long axis in the rectum of 20 kg pigs. We prepared MSC-CS gel by mixing MSC-CS obtained from human amnion with 2% carboxymethyl cellulose and administered into the rectum immediately after ESD from day 1 through day 4. We applied standard medium gel as a control group. We euthanized the pigs on day 22, measured the luminal stricture rate, and performed histological analysis. We also euthanized the pigs on day 8 to observe the acute healing phase. Rectal stricture rate was significantly lower in the MSC-CS group compared with control group on day 22 (14.7 0.6% versus 63.9 27.4%, P<0.05, n=3 in each group). Furthermore, MSC-CS significantly attenuated the numbers of infiltrated neutrophils and activated myofibroblasts as well as fiber accumulation on day 22. As for acute phase, the degree of stricture was significantly lower in the MSC-CS group compared with control group on day 8 (31.2 5.5% versus 56.5 3.4%, P < 0.01, n = 3 in each group). In addition, MSC-CS also significantly reduced the numbers of infiltrated neutrophils, macrophages and activated myofibroblasts as well as fiber accumulation on day 8. MSC-CS enema prevented the stricture formation after circumferential ESD in the rectum by suppressing the infiltration of neutrophils and following myofibroblast activation and fibrosis. MSC-CS enema would be a promising treatment to prevent post-ESD stricture.

Funding Source: This study was funded by a Grant-in-Aid (C) from the Japan Society for the Promotion of Science (JSPS, 16K09300) and by the Translational Research Network Programme of the Japan Agency for Medical Research and Development (AMED).

F-1010

STUDY OF ACID SPHINGOMYELINASE INHIBITOR EFFECTING ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS FROM RADIOATIVE

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Yawen Chen - *Department of Oral Maxillofacial Surgery, Capital Medical University School of Stomatology, Beijing, China*

Radiotherapy is one way for the treatment of head-neck malignant tumors. It leads to the irradiation injury to the salivary glands. Studying how to protect salivary gland from radiation that induce acinar cells, endothelial cell apoptosis and reduce microvascular injury is one way. Acid sphingomyelinase (ASMase) play a critical role in endothelial cell apoptosis to which led by radiation, but there is seldom report about ASMase inhibitor. We will study the role of ASMase inhibitor such as sphingosine-1-phosphate (S1P), G-CSF and 3-acetyl-11-keto-beta-boswellic acids (AKBA) in human umbilical vein endothelial cells (HUVEC) on prevent endothelial cell from radiation.Three kinds of ASMase inhibitors are added to HUVEC cell in concentration range from 0ng/ml, 50ng/ml, 75ng/ml to 100ng/ml. Cell cycle and apoptosis were assayed with flow cytometry. The inhibitors show to promoting proliferation on cells of HUVEC with irradiation or not, and the higher the concentration, the more obvious. We have to, besides prophylactic use of S1P can enhance the ability of promoting proliferation, especially in 2 hours after irradiation. Different concentration of AKBA has significant proliferation effect on HUVEC cell with irradiation, and it is a correlation of concentration. G-CSF shows a role in promoting the proliferation of HUVEC cells with irradiation. The S1P has an effect on HUVEC cell of inhibiting apoptosis in 24 hours, meanwhile when the concentration is 100ng/ml the inhibition effect is evident. The higher the concentration of S1P, the more obvious the inhibition in 2 hours after irradiation, and the apoptosis rate was (4.79730.497) %. The impact of AKBA on inhibiting apoptosis is not stable. S1P is an effect on cells of HUVEC with irradiation or not, which can promote its proliferation and inhibit apoptosis, otherwise with increase the concentration, the impact becomes more significant.

ADIPOSE AND CONNECTIVE TISSUE

F-1012

ADSC OF METABOLICALLY HEALTHY VS TYPE 2 DIABETES MELLITUS OBESE PATIENTS: INFLAMMATION, PROLIFERATIVE STATUS, CELL CYCLE DISTRIBUTION AND ADIPOGENIC DIFFERENTIATION

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Obesity, remaining a topical issue of modern medicine, leads to development of latent inflammation and insulin resistance in adipose tissue with subsequent development of diabetes mellitus type 2 (DM2T). Among patients with obesity, cases of diabetes mellitus type 2 (DM2T), i.e. metabolic unhealthy obesity (MuHO), are often encountered, differing from metabolic healthy obesity (MHO). In our study we addressed the question, if the adipose tissue derived mesenchymal stromal cells (ADSC) of these patients differ in their features and properties. We isolated ADSC from samples of subcutaneous (sADSC) and visceral (vADSC) adipose tissue, which were obtained during bariatric surgery from patients with morbid obesity (BMI>35) and presence/absence DM2T (4/6 patients). ADSCs were analysed for proliferative activity by MTT-test and by propidium iodide-based cell cycle assay. We have tested adipogenic differentiation capacity of isolated ADSC by OilRedO staining and real time PCR using GLUT4, PPARg, FABP4 as markers. To evaluate the inflammatory state of adipose tissue, we examined level of CD68+-cells in cryosections of subcutaneous and visceral adipose tissue samples taken from patients. We have shown that sADSC and vADSC from MHO patients had significantly higher proliferative activity in comparison with those derived from MuHO subjects. This difference persists on 24, 48, 72 and 96 hour of assay, with slower cell growth dynamics in MuHO compared to MHO patients. Cell cycle analysis confirmed data of MTT assay, showing that ADSC of MHO subjects have more G2/M and S phase cells compared to MuHO patients. Adipocytes matured from ADSC of patients with MuHO have fewer amounts of fat droplets and decreasing expression of mRNA of adipogenic markers compared to patients with MHO, which have adipocytes with many little fat droplets and higher expression of adipogenic markers. Immunohistochemistry showed that patients with MuHO have much more infiltration of CD68+-cells in subcutaneous and visceral adipose tissue. We assume that the findings will help to understand the causes of diabetes in obese patients. At this stage, it can be assumed that a violation of the proliferative potential of ADSC leads to braking of the formation of new fat stores, hypertrophy of adipocytes and the development of inflammation in adipose tissue.

Funding Source: This study was supported by Russian Scientific Foundation grant #17-15-01435.

F-1014

FIRST EVIDENCE OF TELOCYTES IN ADIPOSE TISSUE: HISTOLOGICAL AND FUNCTIONAL STUDY

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Neural crest descendants are retained in the adult tissues as multipotent progenitor cells, which could be involved in the regeneration. These cells express nestin, which is used by researchers as a marker protein allowing tracking neural crest descendants. The aim of this study was to analyze the localization and functions of nestin-expressing cells in adipose tissue in vivo and to investigate their possible role in regeneration. We used mice with expression the green fluorescent protein (GFP) driven by the regulatory elements of nestin promoter (nesGFP+). Using confocal microscopy we found groups, chains and single nesGFP+ cells within whole-mount adipose tissue fragments as well as thick sections of adipose tissue. These cells had small-sized bodies and very long and thin processes with synapse-like endings. Using immunofluorescent staining of adipose tissue samples we found that nesGFP+ cells localized in a close proximity to CD31-positive endothelium of medium-size blood vessels and within adipose tissue stroma between mature adipocytes. Part of these nesGFP+ cells also expressed NG2, alphaSMA and PDGFRbeta. To further characterize nesGFP+

cells we isolated them from adipose tissue within a population of mesenchymal stem/stromal cells (MSCs) according to a standard protocol. The proportion of nesGFP+ cells in total MSCs population was 5-10% at the passage 0 and only slightly declined to 2-3 passages. We analyzed the expression of markers characteristic for murine mesenchymal stromal and stem cells (CD34, PDGFRα, PDGFRβ, Sca-1, CD106, CD105, CD73, CD29, CD44, CD90, c-kit, Oct4, Nanog, SOX2, SSEA1) as well as CXCR4, NG2, alphaSMA and PPARgamma using immunofluorescence and flow cytometry. These cells were negative for leukocyte markers CD11b and CD45. Sorted nesGFP+ cells were able to give clones and differentiate in adipogenic, osteo- and chondrogenic directions. Thus, nestin-expressing cells in adipose tissue have the characteristics of stem cells. NesGFP+ cells are also capable of migrating in «scratch assay». Together morphology, tissue distribution and immunophenotype suggest that neural crest derived nestin-expressing cells in adipose tissue represent telocytes, which were previously found in various tissues as mediators of intercellular signaling process.

Funding Source: This study was supported by RSF grant 14-15-00439 . This study was conducted using the biomaterial collected and archived with the support of the Russian Science Foundation (grant 14-50-00029

F-1016

NOTCH SIGNALING UPMODULATES GLYCOLYSIS BY REGULATING HIF-1ALPHA TO MAINTAIN STEMNESS IN HUMAN ADIPOSE TISSUE-DERIVED STEM/STROMAL CELLS

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Human adipose tissue-derived stem/stromal cells (hASC) are attractive for regenerative medicine, but their limited lifespan in vitro culture systems hinders their therapeutic application. Recent data demonstrate that hypoxia may exhibit a high level of glycolytic metabolism. However, the physiological role of glycolytic activation and its regulatory mechanisms are still incompletely understood. Previously, we have demonstrated that 5% O2 dramatically increases the glycolysis rate, improves the proliferation efficiency, prevents senescence, and maintains the multipotency of hASC. These effects were mediated by Notch signaling, which was activated in 5% O2 condition. Five percent O2 significantly increased glucose consumption and lactate production of hASC. In addition, our data have shown that Notch signaling is involved in induction of PGK1, SLC2A1, and PFKFB3 in addition to reduction of SCO2 and TIGAR expression through p53 inactivation. Activated Notch1 enhanced nuclear p65 levels, resulting in the upregulation of glycolytic factor. These data have suggested that the Notch-HES1 signal enhanced the glycolytic pathway through p53 and NF-κB. In this study, we found that activated Notch signaling under hypoxic conditions enhanced the transcriptional activity of HIF, resulting in the upmodulation of glycolysis. Overexpression of intracellular domain of Notch1 (NICD1) significantly increased HRE-luc activity, whereas treatment of DAPT, an inhibitor of Notch signaling, resulted in a significant reduction in HRE-luc activity. Knockdown of HIF-1α attenuated the expression of PGK1, SLC2A1, and PFKFB3. We also found that glucose consumption and lactate production were significantly reduced by knockdown of HIF-1α. Furthermore, the NICD1-upregulated glycolytic genes were attenuated by the knockdown of HIF-1α under both normoxic and hypoxic conditions, indicating that Notch signaling upregulates glycolytic genes by enhancing the transcriptional activity of HIF-1α. Overall, our observations provide new regulatory mechanisms for the glycolysis by Notch signaling to maintain the stemness of hASC. In addition, our study sheds new light on the regulation of replicative senescence, which might have an impact for quality control of ASCs preparations used for therapeutic applications.

F-1018

REPARATIVE POTENTIAL OF SCF-EXPRESSING ADIPOSE TISSUE-DERIVED MESENCHYMAL STROMAL CELLS

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Stem cell factor (SCF) promotes survival, proliferation and mobilization of hematopoietic stem cells and c-kit+ progenitors through its cognate receptor -c-Kit. Adipose tissue-derived mesenchymal stromal cells (ASC), the potential effector of progenitor cell maturation, can produce this factor, but in a small amount. In order to increase SCF expression rat ASC were infected with adeno-associated viral vector (AAV) or lentiviral vector (LV). The average concentration of the transgene in media for LV was 72-75 ng/ml, and for AAV - 15-17 ng/ml. Genetic modification of ASC did not affect their ability to differentiate in adipogenic and osteogenic directions and did not significantly affect the adhesion to matrix proteins. However, the rate of increase in the number (MTT-test) of modified ASCs was significantly lower than in control cells, especially for LV-transduced ASC, so we did not use LV for further experiments. We also found that the medium from ASC transduced with SCF had a significant stimulating effect on the viability of rat c-kit + cardiac cells, comparable in strength to the

effect of fetal serum and exceeding the effect of IGF-1 at a concentration of 50 ng/ml. We addressed the question whether the effect of conditioned media from SCF-transduced ASC is mediated by extracellular vesicles (EV). At first, we optimized EV isolation protocol for performing series of sequential centrifugations. Most of the vesicles isolated by ultracentrifugation were 80-160 nm in diameter, had the typical cup-shaped form and were successfully captured by cells. Proteomic and RNA analysis identified a wide spectrum of proteins and microRNAs in EV studied. We found some difference in protein content between EV isolated from unmodified and modified cells. Specifically, modified ACS contained SCF, as well as MCP-3 chemokine and pentraxin-3, which were not present in unmodified ASC. However, EV did not influenced proliferation and migration of c-kit+ cells. Thus we can conclude that the effect of conditioned medium from SCF- transduced ASC is mostly mediated by some secreted soluble factors. In general, our results indicate an increase in the regenerative properties of ASCs when they are transduced with SCF, which justifies their successive use for transplantation into a damaged myocardium in order to stimulate its repair.

Funding Source: The study was supported by a Russian Science Foundation grant #16-15-00181 (26.01.2016); This work was supported by RFBR grant № 16-15-00181

MUSCULOSKELETAL TISSUE

F-1020

IS ALL CARTILAGE CREATED EQUAL? DIFFERENCES IN BONE MARROW AND PLACENTAL MESENCHYMAL STEM CELL CHONDROGENESIS

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Mesenchymal stem cells (MSCs) are a well-established cell source for chondrogenesis, but significant challenges remain as chemical differentiation with growth factors often result in chondrocte hypertrophy. It is also well established that chondrogenic potential varies significantly with MSC source (e.g. bone marrow, iPS, fat) and donor age. Embryonic stem cells are more chondrogenic than adult MSC populations, highlighting the advantage of fetal MSC in understanding early chondrogenicc pathways, but the underlying mechanisms of differentiation are still poorly understood. In this study, we aimed to compare the chondrogenic potential of first-trimester placental MSCs with that of term MSC and bone-marrow MSC (BM-MSC). MSCs were isolated from first-trimester and term placentae. BM-MSCs were obtained from StemPro. Chondrogenesis was induced by pellet culture in commercial chondrogenic media for 7, 14, 21 or 28 days. Pellets were processed for histology, RNA extraction, or DNA extraction and glycosaminoglycans content. Gene expression was profiled using Qiagen RT2 human MSC arrays. Cartilage formation was morphologically evident in all alcian blue stained pellets with characteristic spindle-like cells in the outer layer and heterogenous blue staining within the pellet core. Placental MSC-derived pellets were more heterogenous than those from BM-MSC. Placental MSCs produced 5-fold more glycosaminoglycans than BM-MSC over 28 days of chondrogenic culture. Placental MSCs upregulated different sets of chondrogenesis-associated genes than BM-MSC. First-trimester MSC showed a significantly greater linear increase in the cartilage-associated genes ACBC1, BMP2, BMP4, KAT2B and TGFB1 with chondrogenic culture than term MSC. Taken together, these data suggest that placental MSC appear a promising cell source for cartilage generation. The early embryonic nature of first-trimester MSC makes them a unique in vitro model to understand the developmental pathways involved in generating articular cartilage.

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F-1022

THERAPEUTIC EFFECT OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS ACTIVATED BY UMBILICAL CORD EXTRACTS ON POSTMENOPAUSAL OSTEOPOROSIS MODEL RATS

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Bone marrow-derived mesenchymal stem cells (BM-MSCs) are the most valuable source of autologous cells for transplantation and tissue regeneration for treating osteoporosis. Although BM-MSCs are the primary cells responsible for maintaining bone metabolism and homeostasis, their regenerative ability may be attenuated in postmenopausal osteoporosis patients. The purpose of this study was to investigate the functional abnormality of BM-MSCs derived from an ovariectomized osteoporosis model and verify whether the novel BM-MSC activator, Wharton's jelly extract supernatant (WJS) from human umbilical cord extracts, restores the functional recovery of abnormal BM-MSCs and their therapeutic effects on OVX rat. First, we revealed potential abnormalities of BM-MSCs in an estrogen-deficient rat model constructed by ovariectomy (OVX-MSCs). Cell proliferation, mobilisation and regulation of osteoclasts were downregulated in OVX-MSCs. The expressions of osteoprotegerin (Opg) and transforming growth factor β1 (TGF-β1), which regulate the activity

of osteoclasts, were also downregulated in OVX-MSCs. Moreover, therapeutic effects of OVX-MSCs decreased in OVX rats. Accordingly, we developed a new activator using WJS, which improved cell proliferation, mobilisation and suppressive effects on activated osteoclasts in OVX-MSCs. The addition of WJS improved the secretion of OPG and expression of TGF-β1 in OVX-MSCs. Bone volume, RANK and TRACP expression of osteoclasts and proinflammatory cytokine expression in bone tissues were ameliorated by OVX-MSCs activated with WJS (OVX-MSCs-WJ) in OVX rats. Fusion and bone resorption activity of osteoclasts were suppressed in macrophage-induced and primary mouse bone marrow cell-induced osteoclasts via suppression of osteoclast specific genes, Nfatc1, Clcn7, Atp6i and Dc-stamp, by co-culture with OVX-MSCs-WJ in vitro. In this study, we developed a novel method to activate abnormal BM-MSCs using WJS, and demonstrated the morphological and functional improvement of OVX-MSCs in vitro and their therapeutic effect on OVX rats in vivo. This method may provide great benefits towards the autologous transplantation of BM-MSCs in osteoporosis patients not only in the postmenopausal period, but also for bone diseases caused by other pathological mechanisms.

F-1024

MESO-ENDOTHELIAL BIPOTENT PROGENITORS FROM HUMAN PLACENTA DISPLAY DISTINCT MOLECULAR AND CELLULAR IDENTITY

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The existence of bipotential precursors for both mesenchymal and endothelial stem/progenitor cells in human postnatal life is debated. Here, we hypothesized that such progenitors are present within the human term placenta. From a heterogeneous placental single cell suspension, directly flow-sorted CD45-/CD34+/CD144+/UEA-I-/CD31Lo population uniquely differentiated into both endothelial and mesenchymal colonies in limiting dilution culture assays. Of interest, systematic analysis of placenta tissue revealed that these bipotent cells, termed as Meso-Endothelial bipotent progenitors, were in vessel walls but not in contact with the circulation. RNA sequencing and functional analysis demonstrated that Notch signalling was a key driver for endothelial and bipotential progenitor function. In contrast, the formation of mesenchymal cells from the bipotential population was not affected by TGFbeta receptor inhibition, a classical pathway for Endothelial-Mesenchymal Transition. This study reveals a bipotent progenitor phenotype in the human placenta at the cellular and molecular level, giving rise to endothelial and mesenchymal cells in vivo, which potentially can be used for musculoskeletal and cardiac tissues regeneration.

Funding Source: National Health and Medical Research Council.

F-1026

LONG NON-CODING RNA H19 PROMOTE CHONDROGENESIS OF MESENCHYMAL STEM CELLS BY COMPETITIVELY REGULATE EZH2/H3K27ME3 AXIS WITH HOTAIR

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Long noncoding RNAs (lncRNAs) are important regulators in cell differentiation and tissue regeneration. Human fetus derived mesenchymal stem cells (fMSCs) are good models to study MSCs differentiation. Comparing to adult MSCs (aMSCs), fMSCs have superior cell proliferation and differentiation properties. To identity OA-related lncRNAs which may have differentially expressed in aMSCs and fMSCs, we screened candidate lncRNAs may associate with OA development. Under chondrogenic condition, expression of H19 and HOTAIR were significantly different, H19 was significantly higher whereas HOTAIR was significantly reduced in the fMSCs compared to aMSCs. H19 was also significantly reduced in the OA patients and rat model cartilage tissues. Overexpressing H19 in fMSC and rat MSCs significantly promoted their chondrogenesis through promoting collagen type II (Col2) and suppressing collagen type X (Col10) expression, and enhancing their extracellular matrix production. For mechanistic studies, we screened histone modification markers and found H3K27me3 was significantly increased in the OA chondrocytes, and associated with the degree of cartilage lesion. Meanwhile, EZH2, the specific methyltransferase of H3K27me3, were highly expressed in the same tissue areas. RNA immunoprecipitation and chromatin immunoprecipitation results showed that both H19 and HOTAIR competitively bind to EZH2. Overexpression of H19 directly reduced the level of H3K27me3 around Col2 promoter and increased H3K27me3 level at Col10 promoter. Luciferase assay showed that H19 inhibited the expression of HOTAIR, a lncRNA could also bind to EZH2. In this study, we found a novel epigenetic regulatory mechanism that two lncRNAs, H19 and HOTAIR competitively bind with EZH2, lead to the shift of H3K27me3 modification pattern in MSCs and thereby regulate chondrogenesis. H19 may be a new diagnostic and therapeutic target for cartilage degeneration and repair.

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F-1028

FUNCTIONAL AND HIERARCHYCAL ANALYSIS OF LEPR+ MOUSE BONE MARROW MESENCHYMAL STROMAL CELLS

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Bone marrow mesenchymal stromal cells (BMSCs) that are the major source of osteoblasts and adipocytes in adult bone marrow are marked by leptin receptor (LepR) expression. We found that conditional deletion of Lepr using Prx1-Cre led to *Lepr* deletion in bone marrow BMSCs in limb bones but not in the axial skeleton or hypothalamic neurons. Prx1-Cre; $Lepr^{fl/fl}$ mice exhibited normal body mass and normal hematopoiesis. However, limb bones from Prx1-Cre; $Lepr^{fl/fl}$ mice exhibited significantly increased osteogenesis, decreased adipogenesis, and accelerated fracture healing. Leptin increased adipogenesis and reduced osteogenesis by BMSCs by activating Jak2/Stat3 signaling. A high fat diet increased adipogenesis and reduced osteogenesis in limb bones from wild-type but not Prx1-Cre; $Lepr^{fl/fl}$ mice. The effects of a high fat diet on osteogenesis reflected local effects of LepR on bone marrow BMSCs and systemic effects on bone resorption. Leptin/LepR signaling promotes adipogenesis and reduces osteogenesis by BMSCs in adult bone marrow in response to nutritional status. We also discovered that BMSCs, as well as osteoblasts, synthesize a secreted C-type lectin domain superfamily member, Clec11a, which promotes osteogenesis. Clec11a-deficient mice were born in normal numbers, appeared developmentally normal, and had normal hematopoiesis but exhibited significantly reduced limb and vertebral bone. Adult Clec11a-deficient mice exhibited significantly reduced trabecular bone formation, reduced bone strength, and delayed fracture healing. Bone marrow stromal cells from Clec11a-deficient mice showed impaired osteogenic differentiation, but normal adipogenic and chondrogenic differentiation, in culture. Recombinant Clec11a promoted osteogenesis by bone marrow stromal cells in culture and increased bone formation in vivo. Administration of recombinant Clec11a prevented osteoporosis in ovariectomized or dexamethasone-treated mice. Therefore, Clec11a appears to act within the bone marrow to promote osteogenesis and to maintain the adult skeleton. We are now investigating the mechanisms by which Clec11a promotes bone formation, and trying to dissect the hierarchy of adult BMSCs in order to improve its clinical application.

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CARDIAC TISSUE AND DISEASE

F-1030

SYSTEMIC HIGH-MOBILITY GROUP BOX 1 ADMINISTRATION MAY PROMOTE CARDIAC REGENERATION BY MOBILIZING BONE MARROW MESENCHYMAL STEM CELLS TO INFARCTION AREA IN A RAT INFARCTION MODEL

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High-mobility group box 1 (HMGB1), a necrotic cell-derived damage-associated molecular-pattern protein molecule, reportedly enhances bone marrow mesenchymal stem cell (BM-MSC) recruitment to damaged tissue for tissue regeneration. We hypothesized that systemic HMGB1 administration might promote tissue repair in a rat myocardial infarction (MI) model. HMGB1 (3 mg/kg) or PBS (control; 3 ml/kg) was administered for 4 days to 26 MI model rats via the femoral vein. Cardiac performance was evaluated by ultrasonography; antifibrotic action and angiogenesis, by immunostaining. We then checked MSC recruitment to damaged tissue in green fluorescent protein bone marrow (GFP-BM) chimera rat by immunostaining. Four weeks after each injection, the LVEF was significantly improved in the HMGB1 group than in the control (4933 vs. 3434%, respectively; $p<0.01$). LV remodeling exhibiting extracellular collagen deposition (fibrotic area; HMGB1 vs Control, 1234 vs. 2737%; $p<0.01$) and cardiomyocyte hypertrophy (2032 vs. 2631μm; $p<0.01$) were significantly attenuated in the HMGB1 group unlike in the control. Neovascularization was significantly noted in the HMGB1 group compared with the control (capillary density, 159324 vs. 85310; $p<0.01$). In the peri-infarct area, QT-PCR analysis demonstrated that VEGF (1.230.1 vs. 0.930.1%; $p<0.01$)

and IL-10 mRNA level (1.630.6 vs. 1.130.2; p<0.01) were significantly higher and TGFβ mRNA level (1.130.2 vs. 1.930.8%; p<0.01) was significantly lower in the HMGB1 group than in the control. In the GFP-BM chimera rat, confocal-laser microscopy image of the peri-infarct area revealed that there were more GFP+/PDGFR+ cells in the HMGB1 group than in the control (131335 vs 20315; p<0.01). The confocal microscopy image showed VEGF expression around the BM-MSCs in the border zone. In addition, some GFP+/ PDGFR+ cells were stained by isolectin-B4 or NG2 antibody, and these GFP+ cells were present at the vessel. These findings suggest that some BM-derived MSCs may differentiate into the vessel constituent cells at the border zone. Systemic HMGB1 administration induced angiogenesis and reduced fibrosis by recruiting BM-MSCs, suggesting that HMGB1 administration might be a new approach of heart failure.

F-1032

THERAPEUTIC POTENTIAL OF ACTIVATED MESENCHYMAL STEM CELLS IN CHRONIC MYOCARDIAL INFARCTION -AS NOVEL STRATEGIC CELL THERAPY

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Cell therapy is promising for cardiac diseases and lots of trials have been challenged. Many reported showed their efficacy in the investigational trials, but few showed dropout in the large pivotal ones. One of the issues has supposed to be the loss of quality of mesenchymal stem cells (MSCs) due to the cryopreservation. Here, we supposed that MSCs-activation could overcome them. In this study, we propose the novel strategy to identify the bio-active protein which could activate MSCs, and show the activated MSCs could improve the cardiac dysfunction. Human adipose tissue-derived multi-lineage progenitor cells **(**hADMPCs), as the representatives of MSCs, were isolated from subcutaneous adipose tissues of healthy volunteers and expanded. hADMPCs were challenged to 160 kinds of bio-actives, and the cells were applied for DNA microarray analysis. Principal component analysis showed 7 bio-actives could prime hADMPCs. IL-1β was selected as the activating reagent for them, because IL-1β-activated ones could show augmented probes, which are known to be cardio-protective, -activating, or vascular network constructive, but less augmented with harm. To confirm the activated hADMPCs could improve cardiac dysfunction, the cells were applied for the chronic myocardial infarction swine models. The models were introduced by 2 step occlusion-reperfusion of the diagonal and the left ascending coronary artery. Four weeks later, the animals were injected with hADMPCs, IL-1β-activated ones ($3x10^5$ cells/kg body) or placebo via the coronary artery. Cardiac function was assessed by MRI at 0 and12-week post-injection. IL-1β-activated hADMPC-administration showed significant improvement than those of hADMPCs and controls (ΔEF% of controls were -1.0%, hADMPCs, 4.5% and IL-1β-activated hADMPCs 9.0%, respectively). Histological analysis showed significant reduction of fibrotic area of the cardiac tissues, the number of cardiomyocytes were increased, and the vascular network has been reconstructed in IL-1β-activated ones-treated animals. We showed the novel strategy to identify IL-1β as MSCs-activating reagents, and show the IL-1β-activated MSCs could be applicable for therapeutics to chronic myocardial infarction

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F-1034

POPULATION AND SINGLE-CELL ANALYSIS OF HUMAN CARDIOGENESIS REVEALS UNIQUE LGR5 CONO-VENTRICULAR PROGENITORS IN EMBRYONIC OUTFLOW TRACT

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The human heart is composed of highly diverse cell types, including cardiomyocytes, pacemaker and conductive cells, and vascular cells. The morphogenetic process of mammalian cardiac development is complex and highly regulated spatiotemporally by multipotent cardiac stem/progenitor cells (CPCs). CPCs have attracted much interest, due to the notion that CPCs are more capable of engrafting to host myocardium by their strong proliferative potential but also their ability to generate multiple cardiac derivatives. Mouse genetic fate-mapping studies have provided many insights for understanding mammalian cardiogenesis, however, similar information has been poorly established in humans. Thus it is still undefined what are specific markers of the CPCs and their progenies and what are the specific molecular cues driving the CPCs into the committed intermediates and mature cardiac cells

in humans. Here we report a comprehensive gene expression resource, characterizing the transcriptional dynamics of human cardiac lineage specification and identifying novel markers of developing cardiac derivatives from multipotent CPCs to differentiated cardiac cells, through population and single-cell RNA-seq using human embryonic stem cell- and embryonic/fetal heart-derived cardiac cells. Importantly, we discover the human unique subset of cono-ventricular region-specific CPCs, marked by a Wnt signal activator LGR5. We found that after induction of the $MESP1^+$ mesodermal lineage (4-5 weeks of gestational stage), the $LGR5^+$ population emerges specifically in cardiac outflow tract with co-expression of a well-known CPC marker ISL1, thereafter promoting cardiac differentiation and development through expansion of $ISL1^+TNNT2^+$ intermediates. Further studies have revealed the human-specific cardiogenic program driven by a sequential transcriptional network with a MESP1-LGR5-ISL1 axis at the early embryonic stages, which likely promotes cono-ventriculogenesis in humans. Collectively, we chart the developmental landscape of human cardiac formation at the cellular and molecular basis, contributing to our deeper understanding of human cardiogenesis. This may also uncover the putative origins of certain human congenital cardiac malformations and potentially advance cardiac regenerative medicine.

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F-1036

EXOSOME COMMUNICATION IN A HUMAN IPSC-DERIVED CARDIOMYOCYTE MODEL OF HYPERTROPHIC CARDIOMYOPATHY

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Hypertrophic cardiomyopathy (HCM) is a thickening of the ventricles that can lead to devastating conditions such as heart failure and sudden cardiac death. HCM is linked to mutations in genes encoding cardiac sarcomeric proteins, such as ACTC1 encoding for actin. The E99K mutation is a single base pair substitution in the ACTC1 gene that results in an amino acid change from glutamic acid to lysine linking to HCM. Despite extensive study the mechanisms mediating many of the associated clinical manifestations, such as interstitial fibrosis, remain unknown and human models are required. To address this, fibroblasts were isolated from E99K patients/ heathy relatives, and hiPSC lines were generated using Sendai virus reprogramming. Corresponding isogenic controls were created for each line by correcting or introducing the mutation using CRISPR/Cas9 gene editing technology. Isogenic pairs were differentiated to cardiomyocytes and subjected to electrical stimulation to capture the cells at a stress-inducing 3Hz contraction frequency. Following 24 hours pacing, conditioned medium was collected and EVs isolated and characterised. The pattern of release (size and quantity) of extracellular vesicles (EVs) produced by diseased cardiomyocytes was significantly altered compared to isogenic control lines. Stressed conditioned medium, containing EVs, was shown to activate fibroblasts into a more secretory active myofibroblast state. We hypothesise that E99K mutant cardiomyocytes modify the release and content of their extracellular vesicles, and are investigating whether how this causes activation of neighbouring cells to result in unexplained disease phenotypes such as fibrosis.

F-1038

NON-INTEGRATING RNA-BASED MODIFICATIONS OF HIPSC-DERIVED CARDIOMYOCYTES - APPLICATIONS FOR OPTOGENETICS, SIRNA KNOCKDOWN AND DOMINANT NEGATIVE EXPRESSION OF MUTATED TRANSCRIPTS

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSCM) are a well accepted model to study drug induced cardiac side effects and efficacy. Xpress.4U is a novel transfection technology that allows for highly efficient and gentle transfer of mRNAs. Due to its optimized composition, it is specifically designed for use of hiPSCM as well as other hiPSC-derived cell types. The mechanism is based on the incorporation of complexed mRNAs into the lumen of fusogenic liposomes. Immediately upon contact these loaded liposomes integrate spontaneously into the membrane of the target cells, releasing their cargo directly into the cytosol. Unlike classic lipofection, this physicochemical driven transfer mechanism bypasses the endocytotic uptake pathway and prevents the nucleic acids from lysosomal degradation. Instead, the nucleic acids are instantly bio-available for their expression. This opens up new opportunities for transient genetic modification of iPSC-derived cells for drug development and disease modeling. Based on high biocompatibility, Xpress.4U also allows for accurate adjustment of expression rate, duration of expression and transfer efficiency. In combination with commercially available techniques to modify/stabilize mRNAs, Xpress.4U driven mRNA transfer demonstrates numerous advantages over DNA plasmid transfection as we have shown in neural and myogenic derived cells. Exogenous expression of Channelrhodopsin 2 in excitable cells can help to overcome the hurdles and artifacts seen with electrical stimulation. As electrophysiological responses are often frequency dependent, this optogenetic functionalization is critical for investigation of frequency-dependent responses of anti-arrhythmic drugs on cardiomyocytes.

Besides optogenetic modification, the Xpress.4U technology applications including siRNA knock down of gene expression, over-expression of genes of interest and dominant negative expression of mutated proteins (i.e. disease modeling) without altering the host cell genotype. For these applications we provide proof of concept data within this presentation.

F-1042

LONG NON-CODING RNA REGULATES HUMAN SPECIFIC CARDIAC DEVELOPMENT

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Cardiogenesis processes in human and animals have differential dynamics, suggesting the existence of species-specific regulators during heart development. However, it remains a challenge to discover human-specific cardiac regulatory genes, given that most coding-genes are conserved. Here, we report the identification of a human-specific long noncoding RNA, Heart Brake LncRNA 1 (HBL1), which regulates cardiomyocyte development from human induced pluripotent stem cells (hiPSCs). Overexpression of HBL1 repressed, whereas knockdown and knockout of HBL1 increased cardiomyocyte differentiation from hiPSCs. HBL1 physically interacted with MIR1 in an AGO2 complex. Disruption of MIR1 binding sites in HBL1 showed similar effect as HBL1 knockout. SOX2 bound to HBL1 promoter and activated its transcription. Knockdown of SOX2 in hiPSCs lead to decreased HBL1 expression, and increased cardiomyocyte differentiation efficiency. Thus,, HBL1 functions as a human-specific regulator to fine-tune human-specific cardiomyocyte development by forming a regulatory network with SOX2 and MIR1.

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ENDOTHELIAL CELLS AND HEMANGIOBLASTS

F-1046

CELL METABOLISM LIMITS MATURATION OF HUMAN IPSC-DERIVED ENDOTHELIUM

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Human induced pluripotent stem cells (iPSCs) are widely used for studying development, scaffold recellularization, disease modelling and drug development. iPSCs have been differentiated into many cell types, including endothelium, however maturation and stabilization of iPSC-derived endothelial cells (iPSC-ECs) remains challenging. We differentiated iPSCs to endothelial cells by inducing mesoderm formation and selection for CD31. iPSC-EC were exposed to prolonged physiological shear stress, which induces EC quiescence, and co-culture with pericytes to provide an instructive environment for maturation. When compared to control microvascular endothelial cells, iPSC-ECs did not align to shear, had increased focal adherens junctions and vWF / angiopoietin2 containing vesicles did not have the appearance of typical Weibel-Palade bodies. Electron microscopy of the iPSC-EC cells confirmed the immature phenotype of the iPSC-EC, with immature mitochondria and Weibel-Palade bodies. Furthermore, these cells failed to express heparan sulfate and hyaluronan at the cell surface, which are critical for vasculature homeostasis and signalling. Since vascular stability and glycocalyx production in endothelial cells is dependent upon glucobiosynthesis, we investigated glucose metabolism in undifferentiated iPSCs, iPSC-ECs and microvascular endothelial cells. iPSC-ECs have reduced mitochondrial function and higher production of reactive oxygen species (ROS), while normal glycolysis and ATP levels are maintained. The observed inadequate iPSC-EC metabolism could be the limiting factor in maturation and stabilization of iPSC-ECs. Maturation of mitochondrial structure and function and thereby lowering ROS may restore the glycocalyx and consequently the endothelial signalling and function.

F-1048

VEGFA PROMOTES ENDOTHELIAL CELLS DIFFERENTIATION FROM HUMAN EMBRYONIC STEM CELLS MAINLY THROUGH PKC EPSILON/ ETA PATHWAY

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Human pluripotent stem cells (hPSCs) have unlimited proliferation capacity and can differentiate into most somatic cell types. We previously described that overexpression of FLI1, as well as activation of PKC simultaneously (FLI1-PKC), could rapidly and efficiently differentiate hPSCs into endothelial cells (ECs). However, the relationship between VEGFa and PKC in hPSCs-ECs differentiation is debating, and the roles of different PKC isoforms remains unknown. In this study, we found that pan-PKC inhibitor has much more impaired function (ECs induction efficiency decreased from 71.1% to 5.4%) of ECs direct differentiation than VEGFa siRNA (reduced to 37.9%), which suggested that PKC play a more important role than VEGFa in hPSCs-ECs process. Meanwhile, overexpression of FLI1 as well as adding VEGFa in hPSCs (FLI1-VEGFa) can acquire 29.1% ECs within 2 days, indicating that VEGFa can partially replace PKC in hPSCs-ECs process. Adding pan-PKC inhibitor in FLI1-VEGFa system could completely suppress ECs induction efficiency to 5.9%. We also use cytokines alone to induce ECs according to Patch's report. Similarly, the addition of pan-PKC inhibitor in cytokines way would also reduce ECs efficiencies (decreased from 76.8% to 3.1%). The above results suggest that PKC play a role downstream of VEGFa in ECs induction process, which is different from previous reports. To further investigate which PKC isoform is mainly involved in converting hPSCs into ECs, siRNAs were used to knockdown 9 PKC isoforms respectively for 2 days in the FLI1-PKC system. Only PKCε or PKCη knockdown could decrease the ECs induction efficiencies from 71.1% to 51.1% or 50.5%. Moreover, RNA-seq and qPCR analysis also showed that only the expression of PKCε and PKCη were robustly upregulated during the FLI1-PKC induction process. Similar results appeared in cytokines (decreased from76.8% to 32.5%) and FLI1-VEGFa (decreased from 29.1% to 16.1%) way to acquire ECs when adding PKCε/η siRNA. In summary, our results suggest that VEGFa promote ECs differentiate from hPSCs, which mainly depends on PKC, especially PKCε and PKCη pathway.

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HEMATOPOIESIS/IMMUNOLOGY

F-1050

MLLT3 GOVERNS SELF-RENEWAL AND ENGRAFTMENT ABILITY OF HUMAN HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSC) regenerate the blood and immune systems upon transplantation, offering a life-saving treatment for many blood diseases. However, HSC transplantation is available for only a fraction of patients due to limitations in HSC quantity and histocompatibility. To achieve robust expansion of HSC for transplantation, a better understanding of the mechanisms governing human HSC self-renewal, and why this program fails in culture, is required. Through transcriptional profiling of human HSC and their non-self-renewing differentiated or cultured progeny, we identified MLLT3/AF9 as a novel HSC regulator. MLLT3 was highly enriched in human HSC from all sources, but downregulated during culture. Knockdown of MLLT3 disrupted the expansion and engraftment of fetal liver and cord blood hematopoietic stem/progenitor cells (HSPC), implying critical function in HSC. Conversely, stabilizing MLLT3 expression in culture using lentiviral vector led to expansion of HSPC that possessed multilineage differentiation ability and superior engraftment capacity. MLLT3 expressing cultured HSPC yielded 14-30 fold higher engraftment than controls, and consistently repopulated the HSPC compartment and all differentiated lineages in bone marrow and peripheral hematopoietic organs. Moreover, culturing MLLT3 overexpressing HSPC for 2 weeks before transplantation resulted in higher engraftment than without culture, implying expansion of functional HSC. Similar to endogenous MLLT3, overexpressed MLLT3 co-localized with active histone marks to promoters of HSC genes, thereby preserving authentic HSC regulatory program in cultured HSPC without reprogramming the cells or causing oncogenic transformation. MLLT3 maintained the expression of key HSC regulators such as HLF, MECOM and MSI2, through direct binding, and indirectly suppressed abnormal activation of immune response and apoptosis genes in cultured HSPC. MLLT3 binding in HSC genes correlated with higher levels of the transcription elongation mark H3K79me2, which is deposited by MLLT3 interaction partner DOT1L. MLLT3 thus represents a novel HSC maintenance factor that links histone reader and modifying activities to regulate HSC genes, and introduces a promising approach to culture HSC for therapeutic applications.

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F-1052

MIR-450B-5P PROMOTES OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE -DERIVED MESENCHYMAL STEM CELLS IN VITRO AND ENHANCES BONE FORMATION IN VIVO BY TARGETING BMP3

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Osteoporosis is a prevailing skeletal disease which is characterized as deterioration of the bone microarchitecture and low bone mass, resulting in reduced bone strength and increased risk of fragility fractures. With the widely use of glucocorticoids and aging population increasing, osteoporosis as a serious public health concern is becoming worldwide. One the prime reasons of osteoporosis is the decrease of osteogenic differentiation of mesenchymal stem cells. Identifying factors which can enhance osteoblastic differentiation of mesenchymal stem cells is very urgent. In this study, we found the expression level of miR-450b was upregulated during osteogenic differentiation of hADSCs. In male C57BL/6J mice with growth and aging, the expression level of miR-450b was dynamic and correlated with the bone formation genes in the femur. We performed a series of gain- and loss-of-function analysis in the process of osteogenic differentiation, and demonstrated that miR-450b not only promoted the process of hADSCs differentiate to osteoblasts *in vitro*, but also enhanced the ectopic bone formation *in vivo*. Bone morphogenic protein 3 (BMP3), as a suppressor member of BMP family in regulation of osteogenesis and bone formation, was identified as a direct target of miR-450b. Downregulation the endogenous expression of BMP3 could mimic the effect of miR-450b upregulation on the osteogenic differentiation of hADSCs. Our findings suggested that using miR-450b to inhibit the expression of endogenous BMP3, thereby promoting bone healing, may be a more effective approach, perhaps at a lower cost, for the treatment of osteoporosis, bone defection and other bone metabolism-related diseases.

F-1054

HYPOXIA PROMOTES HEMATOPOIESIS OF HUMAN PLURIPOTENT STEM CELLS THROUGH SEQUENTIAL HIF1ALPHA ACTIVATION AT STAGES OF MESODERM AND ENDOTHELIAL-TO-HEMATOPOIETIC TRANSITION

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Human early embryonic hematopoiesis occurs in a hypoxic microenvironments before the establishment of a circulatory system. In vitro hematopoietic differentiation from human pluripotent stem cells (hPSCs) provides us a unique approach to explore the mechanism of hypoxia on early hematopoiesis. Although hypoxia is often used in hPSCs differentiation protocol, its role in hematopoiesis is largely unknown. Currently, the leading methods of hematopoietic differentiation from hPSCs rely on stromal cell coculture or embryoid body (EB) formation, which contain the poorly defined components or have the solid sphere-like structure that makes against the infiltration of hypoxia. To better understand the effect of hypoxia on early hematopoiesis, we developed a chemically defined monolayer differentiation strategy. In this stepwise system, we monitored the sequential development of mesodermal progenitors (Brachyury+KDR-), hematopoietic-endothelial progenitors (KDR+CD34+) and CD43+CD41a+ hematopoietic cells. We found that hematopoiesis was significantly enhanced by hypoxia. The gene expression of hypoxia-inducible factor 1α (HIF-1α) was upregulated in two waves, suggesting two distinct effects during hPSCs differentiation. Around the first HIF-1α peaked period, KDR+CD34+ hematopoietic-endothelial progenitors were increased, but not Brachyury+KDR- mesodermal progenitors, suggesting that hypoxia enhanced hematopoietic-endothelial lineage commitment from mesodermal progenitors. Around the second HIF-1α peaked period, hypoxia promoted endothelial-to-hematopoietic transition (EHT) in a HIF-1α-dependent manner while the hemogenic endothelial cells (CD34+CD144+CD73-CD184-) was not enhanced by hypoxia. Interestingly, arterial endothelial cells (CD34+CD144+CD73-CD184+) and arterial-specific gene DLL4, a Notch ligand, were both promoted by hypoxia. Further study indicated HIF-1α suppression impaired DLL4 expression as well as the CD43+ hematopoietic cells generation, which was suppressed by Notch inhibitor. Collectively, we demonstrated for the first time that sequential HIF-1α activation effectively

promoted hematopoiesis by enhancing hematopoietic-endothelial lineage commitment and EHT, which was properly through Notch signals from arterial endothelial niche.

F-1056

MODELING HUMAN B CELL DEVELOPMENT WITH HUMAN PLURIPOTENT STEM CELLS

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The hematopoietic system in the early embryo consists of distinct programs-primitive, erythro-myeloid progenitor (EMP) and definitive- that can be distinguished by site and timing of development and the spectrum of lineages generated. We have successfully modeled human primitive and definitive hematopoiesis in human pluripotent stem cell (hPSC) differentiation cultures and showed that these programs rapidly diverge and are derived from different KDR+ mesoderm populations that can be distinguished based on the expression of CD235a. Additionally, we found that the specification of these mesoderm subsets is controlled by Wnt and activin A/nodal signaling and that through appropriate manipulation of these pathways it is possible to generate populations highly enriched in primitive or definitive hematopoiesis. With access to these populations, we have been able to investigate the onset of human definitive hematopoiesis and show this program transitions though a hemogenic endothelial stage, identified by the emergence of CD34+CD73-CD184- progenitors that display the potential to generate T lymphoid, erythroid and myeloid progeny. In this study, we have taken advantage of these advances to investigate the onset of human B cell development. We found that the CD34+CD73-CD184- progenitors efficiently generate CD19+CD10+ B lineage cells following co-culture on MS5 stromal cells. In contrast, the KDR+CD235a+-derived primitive hematopoietic progenitors showed no capacity to generate B cells, indicating that this potential is restricted to definitive hematopoiesis. When transplanted into the neonatal liver of NSG recipients, these B lymphoid cells colonize both the marrow and spleen where they mature and give rise to CD19+IgM+IgD+ cells. Our current studies are aimed at mapping the origin of the hPSC-derived B cell lineage to determine if these cells develop from a multipotent progenitor.

F-1058

CHARACTERISATION OF THE IMMUNOGENICITY OF REGENERATIVE MEDICINE CELLULAR THERAPIES

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HiPSCs and primary organoids are two promising regenerative medicine cellular therapies. As the routine use of autologous cells is not yet feasible due to practical constraints such as derivation time and cost, bio-banking of allogeneic cell lines is a promising alternative. A robust understanding of the immunogenicity of these cell types is essential prior to clinical use and differences in immune response could help identify the optimal platform. To address this question we compared the immunogenicity of hiPSC-derived cholangiocytes and biliary organoids as proof-of-principle. iPSCs were differentiated into cholangiocytes (iChols) and primary cholangiocyte organoids (COs) were derived from the biliary tree using our established methods. As human leukocyte antigen (HLA) I expression is a major determinant for immunogenicity, HLA I expression was compared in undifferentiated iPSCs, iChols, COs and primary cells using qPCR, immunofluorescence, transcriptomic analysis and flow cytometry. Our results show that HLA I expression increased after differentiation of hiPSCs into iChols. However both iChols and COs exhibited significantly reduced HLA I expression when compared to primary cholangiocytes, suggesting reduced immunogenicity in vitro. COs and iChols were treated with IFN-γ to model the post-transplantation inflammatory milieu and HLA I expression was assessed as before. Both COs and iChols exhibited upregulation of HLA I, comparable to primary tissue, suggesting that the immunogenicity of these cells could be increased following transplantation. To explore this further, autologous and allogeneic COs and iChols were transplanted into humanised mice reconstituted with lymphocytes or haematopoietic stem cells. Lymphocyte engraftment and activation was assessed by flow cytometry. Immunofluorescence analysis was used to evaluate engraftment of the transplanted cells, graft infiltration by lymphocytes and morphological changes to the graft. Preliminary results indicate a comparable immune response between COs and iChols. In conclusion, iChols and COs exhibit reduced immunogenicity in vitro, which is restored after transplantation in response to the inflammatory milieu. These results provide essential information for the use of immunosuppression in the context of cellular therapies.

F-1060

VARIANTS OF DNMT3A CAUSE TRANSCRIPT-SPECIFIC DNA METHYLATION PATTERNS AND AFFECT HEMATOPOIETIC DIFFERENTIATION

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The de novo DNA methyltransferase 3A (DNMT3A) plays a pivotal role in hematopoietic differentiation. It can be spliced into various different transcripts whose exact functions are not fully understood. In this study, we followed the hypothesis that alternative splicing of DNMT3A has characteristic epigenetic and functional sequels. Specific DNMT3A transcripts were either knocked down with short-hairpin RNAs or overexpressed in cord blood-derived human hematopoietic stem and progenitor cells (HSPCs). Knockdown of transcripts 1 and 3 (coding for the same protein) and transcript 2 resulted in 352 and 8,905 CpGs with significant and transcript-specific DNA methylation (DNAm) changes ($n = 3$; adjusted $P < 0.05$). Knockdown of transcript 4, which does not have a methyltransferase domain, did not lead to any changes in DNAm. Upon overexpression of transcripts 1+3 and 2, the vast majority of DNAm changes were regulated in the opposite direction. Furthermore, modulation of DNMT3A transcripts also evoked corresponding and transcript-specific gene expression changes. Functional analysis indicated that particularly transcript 2 (coding for DNMT3A2) activates proliferation of HSPC ($n = 6$; $P < 0.05$). The total number of colony forming units was increased upon knockdown and reduced upon overexpression of DNMT3A2 ($n = 3$). Transcript 4 interfered with formation of erythroid colonies. Notably, in acute myeloid leukemia (AML) expression of the unique exon of transcript 2 correlates with the same transcript-specific DNA methylation and gene expression signatures as observed in ourin vitro experiments. Lower expression of DNMT3A transcript 2 is associated with poor prognosis in AML (Kaplan-Meier $P = 0.019$; univariate Cox $P = 0.016$). Our results demonstrate that specific splice variants of DNMT3A have distinct epigenetic and functional impacts. Particularly DNMT3A2 triggers hematopoietic differentiation and the corresponding signatures are also relevant in AML.

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F-1062

AN INTEGRATED GLOBAL REGULATORY NETWORK OF HEMATOPOIETIC PRECURSOR CELL SELF-RENEWAL AND DIFFERENTIATION

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Systematic study of the regulatory mechanisms of Hematopoietic Stem Cell and Progenitor Cell (HSPC) self-renewal is fundamentally important for understanding hematopoiesis and for manipulating HSPCs for therapeutic purposes. Previously, we have characterized gene expression and identified important transcription factors (TFs) regulating the switch between self-renewal and differentiation in a multipotent Hematopoietic Progenitor Cell (HPC) model system, EML (Erythroid, Myeloid, and Lymphoid) cells. Herein, we report binding maps for additional TFs by using chromatin immunoprecipitation (ChIP)-Sequencing, to address the underlying mechanisms regulating self-renewal properties of lineage-CD34+ subpopulation (Lin-CD34+EML cells). Furthermore, we applied the Assay for Transposase Accessible Chromatin (ATAC)-Sequencing to globally identify the open chromatin regions associated with TF binding in the self-renewing Lin-CD34+EML cells. Mass spectrometry (MS) was also used to quantify protein relative expression levels. Finally, by integrating the protein-protein interaction database, we built an expanded transcriptional regulatory and interaction network. We found that MAPK (Mitogen-activated protein kinase) pathway and TGF-β/SMAD signaling pathway components were highly enriched among the binding targets of these TFs in Lin-CD34+EML cells. The present study integrates regulatory information at multiple levels to paint a comprehensive picture of the HSPC self-renewal mechanisms.

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PANCREAS, LIVER, KIDNEY

F-1066

TRANSPLANTABILITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSC) DERIVED KIDNEY ORGANOIDS FOR USE IN KIDNEY REGENERATION

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The current treatments for patients with kidney failure are suboptimal. Therefore there is an interest in iPSC-based therapies for kidney regeneration. Recently protocols for the in vitro generation of kidney organoids have been developed. In this study, we investigated the transplantability of iPSC-derived kidney organoids. To do so, we generated iPSC-derived kidney organoids and analysed their differentiation status. Two human iPSC lines were grown on mouse embryonic fibroblasts and treated with CHIR99201 (a Wnt agonist) and fibroblast growth factor 9 (FGF9). The resulting organoids showed a 10- to 40-fold increase in mRNA for kidney-specific markers after 18 days of differentiation. Immunostaining confirmed that organoids contained essential renal structures. Moreover, there was a 10-fold increase in the expression of the anion transporters OAT1 and OAT3, suggesting tubular functionality. While the iPSC marker NANOG decreased 15-fold, cMYC, REX-1 and Klf4 remained around baseline levels. The iPSC marker cMYC was highly expressed in a low number of cells spread out in the organoids, which raised questions about the risk of teratoma formation by residual iPSC in the organoids upon transplantation. Therefore, we evaluated the tumorigenic risk of iPSC and iPSC-derived kidney organoids in an immune deficient mouse model. Either iPSC (~0,5x10e6) or cells of iPSC-derived kidney organoids were subcutaneously injected into 4 locations. After 5 and 10 weeks the mice were sacrificed for histological analysis. After 5 weeks, mice that received iPSC showed teratoma formation in 7 out of 8 locations with weights varying from 30 milligrams to 1,08 grams. However, none of the injected organoid aggregates resulted in tumor growth. At 10 weeks the kidney organoid aggregates formed a teratoma in 1 out of 8 locations. Other locations contained intact tubuli suggesting that the organoids remain viable upon transplantation. Interestingly, chondrogenic differentiation had occasionally occurred. Even though there is the indication of residual iPSC in the kidney organoids, the tumorigenic risk carried by iPSC-derived kidney organoids is far lower compared to iPSC. Our results indicate that future application in a transplantation model should be preceded by analysis of tumorigenic risk of iPSC-derived kidney organoids.

F-1068

EXPLORING THE ABILITY OF IMMUNOMODULATED HESC-DERIVED PANCREATIC BETA CELLS TO RESIST IMMUNE REJECTION

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In Type 1 diabetes (T1D) the beta cells of the pancreas, which produce insulin in response to glucose, are destroyed through an autoimmune attack. Pathogenesis arises when complex genetic and environmental factors trigger erroneous recognition of self proteins produced by beta cells as autoantigens. The human major histocompatibility complex molecules (HLA) present the autoantigens to cytotoxic CD8+ T cells, which destroy the foreign-perceived beta cell. An ideal T1D therapy should incorporate both a sufficient supply of beta cells that can correct hyperglycemia, and a means to sustain beta cell survival by suppressing immune responses that cause allograft rejection or autoimmunity. As pancreatic donor tissue is scarce, novel therapeutics have explored methods to replace beta cell loss from pluripotent stem cell sources. While improvements have been made in the generation of functional beta cells from differentiated human embryonic stem cells (hESCs), we still lack technology to counteract the rejection of transplanted allogeneic donor cells, without applying chronic immunosuppression or encapsulation. This stalls the broader utility of stem cells for clinical translation. Here, we describe an approach to resist graft rejection by attenuating the expression of immune regulatory molecules on hESCs through a multilayered genome engineering strategy. We have silenced B2M and CIITA by CRISPR/Cas9 editing in hESCs. These genes control cell surface expression of HLA I and HLA II, which instigate T cell allogeneic responses. Concurrently through a TALEN-based system, we have generated cell lines that overexpress two immune inhibitory molecules, CTLA4 and PDL1, to block pathways of T cell

co-stimulation, either with or without a HLA I- and II-deficient background. We are optimizing differentiation of our immune-modified hESCs into mature beta cells that can correct hyperglycemia to mitigate diabetes, and determining the best strategy of immunomodulation that will confer long-term graft protection in humanized diabetic mouse models that have been reconstituted to contain functional alloreactive human T cells. These studies will facilitate a better understanding into mechanisms that dictate host allogeneic responses and will serve as a basis to generate a universal donor source for diabetes treatment.

F-1070

PROPAGATION OF HUMAN IPSC-DERIVED NEPHRON PROGENITORS IN VITRO

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During development, nephron progenitor forming one million nephrons, a functional unit in the kidney. Nephron consists of glomeruli and renal tubules play a critical role to maintain homeostasis. However, nephron progenitor ceases after birth in mice, and before birth in human when they terminally differentiated to the nephron. Thus, no new nephron formed in adult kidney, which may explain the irreversible nature of diseased kidney. Our lab established the method for induction of nephron progenitors from mouse Embryonic Stem (ES) cells and/or human induced Pluripotent Stem (iPS) cells. For application of using induced nephron progenitors to regenerative medicine, a large number of progenitor cells are required for medical applications such as disease modeling and drug screening. We recently reported that successful propagation of the mouse nephron progenitors, which express transcription factor Six2, by combing Wnt, Fgf, Bmp and LIF at low concentrations . Cells were propagated beyond physiological limits both for cell number and lifespan, and reconstituted the nephron. However, this culture condition enabled partial propagation of induced nephron progenitors from human iPS cells. In order to propagate the human nephron progenitors, we generated iPS cell lines that express GFP in the SIX2 locus by TALEN-mediated homologous recombination and then purified SIX2-positive population from iPS-derived tissues by FACS. Isolated SIX2-positive cells were propagated for 14 days in our currently defined culture condition. Propagated SIX2-positive nephron progenitors formed renal tubules and vascularized glomeruli with the host endothelial cells upon transplantation. Thus, we have developed in vitro conditions for propagation of hiPS-derived nephron progenitors, which will be useful for regenerative medicine.

F-1072

INITIATION OF DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO A HEPATOCYTE LINEAGE BY THE INTRODUCTION OF LIVER-SPECIFIC TRANSFECTION FACTORS

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Transcription factors were investigated to determine the conditions that initiate the differentiation of human induced pluripotent stem (iPS) cells most efficiently. The expression of genes in the adult human liver was compared to that in 201B7 cells (iPS cells) by using cDNA microarray analysis. After referring to literatures, transcription factors were chosen on the basis of whether they are expressed in hepatocytes; 16 transcription factors were chosen. cDNAs of the transcription factors were subcloned into episomal plasmids. Each transcription factor was transfected into 201B7 cells and cultured in ReproFF medium for seven days. Their RNA was isolated, and cDNA was then synthesized for real-time quantitative PCR (qPCR). Ratios of the expression levels of AFP to those of the Nanog gene were calculated. The top four ratios were those of CCAAT/enhancer binding protein alpha (CEBPA), CCAAT/enhancer binding protein beta (CEBPB), forkhead box A1 (FOXA1), and forkhead box A3 (FOXA3). These four genes were transfected into 201B7 cells, which were then cultured in William's E (WE) medium for 14 days. After RNA isolation, cDNA was synthesized and subjected to qPCR for the analysis of AFP, albumin, CYP3A4, and aldehyde dehydrogenase (ALDH) 2 levels. Non-transfected 201B7 cells were used as a negative control. The expression levels of AFP, albumin, CYP3A4, and ALDH2 in the transfected cells were found to be 3.133.8, 8.131.7, 7.832.2, and 3.133.1, respectively, when compared with their expression levels in the negative control. The expression of liver-specific genes was up-regulated in 201B7 cells. In conclusion, transfection of CEBPA, CEBPB, FOXA1, and FOXA3 was suitable for the initiation of differentiation of 201B7 cells to a hepatocyte lineage.

F-1074

CHARTING HUMAN IN VITRO BETA CELL DIFFERENTIATION BY SINGLE-CELL RNA SEQUENCING

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Directed differentiation promises to cure diabetes by replacing the insulin-secreting beta cells that patients lose to autoimmune attack or dysfunction. Protocols for directed differentiation use signaling modulators to guide human pluripotent stem cells toward a beta cell fate through a sequence of progenitors. Thus, several cell types are produced by differentiation such as beta cells, their progenitors and other cell types derived from those progenitors. We comprehensively characterize the transcriptomes of these populations for the first time by performing single-cell RNA sequencing of more than 130,000 cells. We identify novel and expected cell types using both conventional clustering techniques and bootstrapped topic modeling, a new machine learning approach. Our data shows that beta cells produced in vitro express the genes responsible for glucose-stimulated insulin secretion and that they gradually increase their expression of genes associated with in vivo beta cell maturation. We explore the identities of cell types produced prior to and alongside beta cells in several time course experiments. Leveraging previous studies of human cadaveric islets, we compare these in vitro cell types to their in vivo counterparts. We further demonstrate that the same progenitors and differentiated cell types arise in different pluripotent stem cell lines and under different protocols. Using a six-week long time course in the absence of exogenous growth factors, we show that beta cells and other cell types are transcriptionally stable once established. Finally, we conclude by generating a high-resolution time course of endocrine induction to characterize all the transient progenitor states which arise during this process, and to propose a complete lineage map of in vitro beta cell differentiation. Our study creates a comprehensive resource that will inform future efforts to artificially produce beta cells for research and regenerative medicine.

F-1076

SINGLE-CELL RNA SEQUENCING OF PRIMARY HUMAN LIVER FOR ANALYSIS OF CELLULAR DIVERSITY AND IMPROVED HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTE DIFFERENTIATION

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The liver is an organ with a diversity of essential functions including xenobiotic removal, bile acid production, storage of iron and vitamins, and metabolism of glucose and fatty acids. Hepatocytes, which represent the most abundant cell type in the liver, fulfill the majority of these functions. As a result, disorders affecting these cells are life threatening, and end-stage treatment relies upon a limited supply of liver donations for transplant. Thus, the production of hepatocyte-like cells (HLCs) from human pluripotent stem cells (hPSCs) for clinical applications including cell-based therapies and toxicology screens has become a strong research focus. However, hPSC-derived hepatocytes lack the functional repertoire of their in vivo counterpart and are currently unable to satisfy this clinical need. Here, we propose to address this limitation by using single-cell RNA sequencing (scSeq) to compare HLCs with fetal and adult primary hepatocytes isolated from patient tissue. ScSeq offers the opportunity to explore cellular diversity by providing precise transcriptomic information at the single cell level and subsequent insight into molecular signatures that are lacking in current differentiation systems. Accordingly, we have developed a protocol to dissociate primary adult human liver tissue into single-cell suspensions. We applied this method to isolate fresh primary hepatocytes and non-parenchymal cells for scSeq using SmartSeq technology, with the aim to uncover cell-to-cell interactions and diversity within cell populations. ScSeq data of hPSC-derived hepatocytes at sequential differentiation stages was compared to fetal and adult liver scSeq profiles to uncover mechanisms of cellular diversity which may be important in understanding the immature nature of HLCs. We have identified putative hepatocyte subpopulations, including a progenitor population known to reside in adult tissue, with unique proliferation and zonation properties. Overall, our novel approach will identify important regulators involved in late-stage maturation of hPSC-derived hepatocytes and allow us to generate hepatocyte subpopulations in vitro for disease modelling and translational applications.

F-1078

STEM CELL DERIVED HEPATOCYTE TRANSPLANTATION FOR THE CORRECTION OF LIVER DISEASE

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Urea cycle disorders are inborn errors of ammonia detoxification. These result from mutations in the genes for any of the several proteins that comprise the urea cycle. The most common urea cycle defect is ornithine transcarbamylase deficiency (OTCD). Cell-based therapy can provide a partial correction of the phenotype. However, the shortage of available liver organ- and cell-donors increases the need to investigate new potential sources of hepatocytes. A potential source of autologous cells for transplantation could be provided from induced pluripotent stem cells (iPSC) after CRISPR/Cas9 technology-mediated gene editing. The aim of this study was to correct the genetic defect in cells from OTCD patients through gene editing, and to analyze the phenotype of the genetically corrected cells. Fibroblasts from two OTCD patients were reprogrammed using the Yamanaka factors. Disease-causing mutations were identified and corrected through CRISPR/Cas9 technology. Gene correction and the absence of off-targets was confirmed by whole genome sequencing. Hepatic differentiation of corrected and uncorrected iPS cells was performed. mRNA levels were examined and compared with data from 52 adult and fetal cases. Seventeen iPSC lines were generated from two OTCD deficient patients and characterized based on pluripotency markers. Pathogenic mutations affecting the natural splice sites were identified in each patient. CRISPR/Cas9 technology was applied to one iPS line to correct the disease-causing mutation with 3.5% estimated efficiency. Urea cycle function, measured as 15N-Ammonium chloride enrichment of urea, was increased in the genetically corrected iPS-derived hepatocytes as compared to the uncorrected cells. In conclusion, gene edited correction of OTCD cells could provide autologous cells for transplantation if the safety of the procedure can be established.

EPITHELIAL TISSUES

F-1082

FOXO3A PLAYS ROLES IN THE WOUND HEALING THROUGH AUTOPHAGY

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The skin epidermis is located on the outermost layer of the body and plays a role in protecting the human body from harmful stress environment such as ultraviolet rays. Therefore, the skin is constantly exposed to potential injury, and thus wound healing is a vital process for the survival of all higher organisms. Previously, by integrating both loss- and gain-of-function studies of Notch receptors and their downstream target Hes1, we show multiple roles of Notch signaling in the regulation of transit amplifying cells in suprabasal layers. Notch signaling induces differentiation of suprabasal cells via Hes1 independent manner, whereas Hes1 is required for maintenance of the immature status of suprabasal cells by preventing premature differentiation. In addition, we found that Hes1 directly suppressed the expression of Bnip3, whose expression is sufficient to induce terminal differentiation of keratinocytes by induction of autophagy. Furthermore, Bnip3 also has a protective effect in keratinocytes upon ultraviolet B (UVB) irradiation. In this study, we are focusing on FoxO3a functions in the epidermis in this study as FoxO3a is one of the candidates of BNIP3 regulators. Interestingly, Chromatin immunoprecipitation assay revealed that HES1 could directly bind to FOXO3A promoter to suppress the expression. We also found that FoxO3a is expressed in the granular layer of the epidermis but not involved in the regulation of BNIP3 expression. We also found that FoxO3a induces autophagy and differentiation in human primary epidermal keratinocytes. In addition, UVB irradiation was sufficient to trigger the activation of FoxO3a in primary epidermal keratinocytes and dermal fibroblasts. In turn, activated FoxO3a induces autophagy and apoptotic cell death. Interestingly, knockout of FoxO3a accelerates wound healing, migration of dermal fibroblasts, and lamellipodia formation of dermal fibroblasts. Furthermore, lamellipodia formation in wild type fibroblasts was significantly stimulated by lamellipodia A1, an inhibitor of autophagy, indicating that lamellipodia formation was inhibited by autophagy. These data clearly indicated that FoxO3a-induced autophagy has a crucial role in wound healing by regulating keratinocyte differentiation and fibroblast migration.

F-1084

ENGRAFTMENT AND PROLIFERATION POTENTIAL OF EMBRYONIC LUNG TISSUE CELLS IN IRRADIATED MICE WITH EMPHYSEMA

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There is an increasing interest in stem cell transplantation therapy against chronic lung diseases, by using lung epithelial cells or alveolospheres derived from endogeneous lung progenitor cells or induced pluripotent stem (iPS) cells. However, in vivo effects and the optimal transplantation strategy of endogeneous lung progenitor cells or iPS-derived cells have not been well addressed yet. In the case of fetal cardiomyocytes, the developmental stages of transplanted cells were an important factor for efficient transplantation to the heart. To gain insight regarding optimization of lung stem cell transplantation therapy, we investigated whether cell engraftment potential differs between the different developmental stages of the lungs: the pseudoglandular stage (E12.5-E15.5), canalicular stage (E15.5-E16.5), saccular stage (E16.5-P0), and alveolarization stage (P0-P21). After preconditioning with irradiation and elastase to induce lung damage, we infused E15.5 CAG-EGFP whole lung cells. We confirmed engraftent of epithelial cells, endothelial cells, and mesenchymal cells by flowcytometry and immunohistochemistry. The number of GFP-positive epithelial cells increased from day 7 to day 28 after infusion, suggesting cell proliferation after engraftment. Using the same engraftment protocol, we found that E15.5 epithelial cells had the most efficient engraftment potential among E13.5, E15.5, E18.5, P7, P14, and P56 mice. In vitro, E15.5 epithelial cells formed largest alveolospheres among the group. 3´SAGE-seq analyses of sorted epithelial cells in E13.5, E15.5, E18.5, P14, and P56 mice revealed cell cycle genes and cell-cell adhesion genes that were highly enriched in the E15.5 epithelial cells. By using publicly available single-cell transcriptome data, we confirmed that these genes were indeed down-regulated in E18.5 epithelial cells (GSE52583), compared to E14.5 and E16.5 epithelial cells. Our research suggests that in vivo/vitro engraftment and proliferation potential differs between the different developmental stages of the lungs, and cell therapy for lung diseases might be most effective when using epithelial cells with transcriptional traits similar to E15.5 epithelial cells.

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F-1086

HORMONES INDUDE THE FORMATION OF LUMINAL-DERIVED BASAL CELLS IN THE MAMMARY GLAND

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In the mammary gland, the luminal cells are known to be unipotent after birth, contributing only to luminal compartment in normal development. Here, by lineage tracing, we uncovered an unexpected potential of luminal cells that can give rise to basal cells during early pregnancy. These luminal-derived basal cells (LdBCs) persisted through mammary regression and generated more progeny in successive rounds of pregnancies. LdBCs express basal markers as well as estrogen receptor α (ERα). In ovariectomized (OVX) mice, stimulation with estrogen and progesterone promoted the formation of LdBCs. In serial transplantation assays, LdBCs were able to reconstitute new mammary glands in a hormone-dependent manner. Transcriptome analysis and genetic experiments suggested that Wnt/β-catenin signaling is essential for the formation and maintenance of LdBCs. Our data uncover an unexpected bi-potency of luminal cells in a physiological context. The discovery of ERα+ basal cells, which can response to hormone and are endowed with stem cell-like regenerative capacity in parous mammary gland, provides new insights into the association of hormone and breast cancer.

F-1088

CELL ATLAS OF HUMAN UTERUS

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The human uterus is a highly dynamic tissue that undergoes repeated damage repair and regeneration along the menstrual cycle, which make it ideal model to study tissue regeneration and pathological process. Stem/progenitors were shown to be involved in the regeneration of endometrial epithelial and pathogenesis of endometriosis. But the identity and regulatory mechanisms of the uterus epithelial stem/progenitors in vivo remain unclear. Here, we dissected the cell heterogeneities of the human uterus epithelial cells, stroma cells, endothelial cells, myofibroblasts, immune cells and smooth muscle cells from 2735 single cell from the full-thickness human uterus by single cell RNA-seq. Further analysis showed subgroups of ciliated and secretory epithelial cells in the uterus, among which we

found a unique cell cluster that exhibit characteristics of epithelial-mesenchymal transition (EMT) that mainly localized in the upper functionalis of the endometrium. Ordering the cell subpopulations along the pseudo-space revealed cell clusters possess cellular states of stress, inflammation and apoptosis in the upper functionalis cellular ecosystem of the endometrium. Connectivity map among the human uterus subpopulations revealed potential inflammatory and developmental-like signals from the upper functionalis cellular ecosystem of the endometrium, especially from other epithelial clusters, regulating cell plasticity of the EMT-epithelial clusters. This study reconstructed the heterogeneities, space-specific distribution and connectivity map the atlas of human uterus, which might provide insight in the repeated regeneration of endometrial and pathogenesis of endometriosis.

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F-1090

DIFFERENTIAL EFFECTS OF EXOGENOUS IGF-1 ADMINISTRATION ON YOUNG ADULT AND GERIATRIC MICE FOLLOWING LEFT LUNG PNEUMONECTOMY

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The murine unilateral pneumonectomy (PNX) model induces growth of new alveolar tissue in the remaining lung, increasing lung mass and volume 14 days after surgery. This compensatory lung growth response declines as mammal's age. We aimed to determine whether infusion of Insulin-like Growth Factor (IGF)-1 could enhance lung growth in young adult mice and very old mice following PNX. Groups of 5 -8, female C57BL6/J mice aged 12 weeks or 23 months, received both a left sided PNX and the subcutaneous insertion of an osmotic pump, delivering either rhIGF-1 or PBS. To determine changes in lung volume, mice were imaged by micro-Computerised Tomography at days 4,7,14 and 21 and then sacrificed, the right lung inflated, embedded and examined by immunohistochemistry. Following PNX in 12-week old mice, there is a rapid increase in lung volume which approaches pre-operative volumes by day four. IGF-1 supplementation following PNX in 12-week old mice significantly increased Ki67+ and Ki67/SpC+ lung cell numbers, at day 21 post-surgery, but did not affect magnitude of lung volume change compared to PBS treated mice. In contrast in old mice, there was a much less pronounced increase in total lung volume of the right lung following surgery. IGF-1 supplementation following PNX surgery significantly increased the volume of air in the lungs compared to PBS treated old mice, but not to the level of preoperative lung volumes and had no effect on tissue content or cell proliferation. We conclude that exogenous IGF-1 treatment does not accelerate or increase lung growth in young mice or induce lung growth in old mice, following PNX. However, IGF-1 treatment did significantly increase the lung volume and reduce the lung density in old mice.

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EYE AND RETINA

F-1094

GENERATION OF RETINAL ORGANOIDS FROM IPSC ENABLES MODELING OF EARLY RETINAL DEVELOPMENT

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The development of highly organized retinal organoids from induced pluripotent stem cells (iPSCs) is challenging but provides new possibilities for studying the early retinal development, pathophysiology and cellular therapies. However, the architecture of retinal organoids derived in-vitro doesn't completely reflect the in-vivo state of the retina. The differentiation of retinal organoids from iPSCs have been established, which either recapitulate the retinal morphogenesis or maximize the photoreceptor generation. Here, we synchronize the protocol for stepwise generation of retinal organoids which are highly autonomous and efficient. In the study, the iPSCs were efficiently generated from the normal human epidermal keratinocytes (NHEK) using CytoTune-iPS Sendai Reprogramming Kit. The iPSC colonies were observed in 12 days which expressed the pluripotency markers such as Oct4, Sox2, and Nanog. The iPSCs were self-induced to form aggregates like embryoid bodies (EB) that expressed the markers for germ layers like mesoderm (CD34), endoderm (AFP)

and ectoderm (GFAP). Further, the EB was differentiated in a synchronized manner starting from anterior neuroepithelium to eye field primordial, neural retina and subsequently retinal organoids. The in-vitro timeline for all major developmental stage of the retina was established and characterized using specific markers. The three-dimensional retinal organoids were analyzed at different weeks for major types of retinal neurons performing thin section and immunohistochemistry. The retinal organoids starting from 7th week showed the expression of the markers for amacrine cells, retinal ganglion cells, horizontal cells, and photoreceptors, etc. Our study reveals the efficient strategy for developing retinal organoids providing the hope for future medicine.

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F-1096

NOVEL RETINA SPECIFIC LAMININ ISOFORMS RECAPITULATE RETINAL INTERPHOTORECEPTOR MATRIX TO GENERATE HUMAN EMBRYONIC STEM CELL-DERIVED PHOTORECEPTORS

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Photoreceptors offers promising role in retinal cell therapy to restore vision. The use of retinal organoid has been hailed to be the established in vitro retinal differentiation method to generate photoreceptors from the pluripotent stem cells. However, this approach is evidently limited by its reproducibility and efficiency level during retinal organoidogenesis. Furthermore, the existing retinal organoid based protocols are not chemically defined and xenogen-free, which include the use of non-human animal serum and Matrigel®, a murine Engelbreth-Holm-Swarm sarcoma extract that are not considered clinically safe. Therefore, it is critical to generate functional and clinically safe photoreceptors with the prospect of not compromising patient's vision conditions. Currently, we have developed an alternative method that does not require retinal organoids. Our novel alternative approach employs the use of the human recombinant retina-specific laminin isoforms to recapitulate the retinal interphotoreceptor matrix environment. With the support of an analogous retina matrix like surface, the human embryonic stem cells (hESCs) are being efficiently differentiated towards photoreceptor progenitor-like cells. Unlike retinal organoids, this laminin based differentiation method consistently generates photoreceptor progenitors in every single batch of differentiation. These hESCs-derived photoreceptors are shown to be positive for PAX6, VSX2, CRX and RCVRN as early as Differentiation Day 30, based on transcriptome and immunocytochemical analyses (n=9). In contrast, the pluripotency and teratoma transcript markers are shown to be drastically downregulated, suggesting reduced associated risk of teratoma formation. Our novel retina laminin based differentiation method does not involve the formation of retinal organoid. This xenogen-free and chemically defined protocol is compatible with GMP (Good Manufacturing Practice) condition and it reproducibly promotes hESCs to photoreceptor progenitor lineage. Hence, these results suggest that our method may constitute an important step towards the future use of hESC-derived photoreceptors to treat vision loss.

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F-1098

EFFECTS OF MHC CONFORMITY ON CORNEAL EPITHELIAL CELL SHEET TRANSPLANTATION IN NON-HUMAN PRIMATES

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Transplantation of HLA-homozygous corneal epithelial cell sheet (CEC sheet) derived from induced pluripotent stem cells (iPSC) is a potential new treatment of limbal stem cell deficiency. However, the effects of HLA concordance on the CEC sheet transplantation are not addressed yet. Here, we investigated the difference in immune response to the MHC-homozygous CEC sheet and the effect of the donor cell engraftment in MHC-matched and unmatched non-human primates. A CEC sheet donated from a cynomolgus monkey with homozygous MHC haplotypes was transplanted to the limbal stem cell deficiency model of cynomolgus monkeys having heterozygous MHC haplotypes (MHC-matched model) or non-identical MHC alleles (MHC-unmatched model). At 3 months after transplantation, the CEC sheet was engrafted into each model, and there was no obvious difference in immune response in both models. Interestingly, at 6 months after transplantation, exacerbating inflammation and increasing corneal thickness were observed in MHC-unmatched model, but

not in MHC-matched model. In histological examination, CD8 positive cells were observed in the cornea in both models. In contrast to this observation, stratified epithelium in MHC-matched model significantly expressed K12 (specific marker of corneal epithelial cells) than in MHC-unmatched model. Our study confirmed that transplantation of CEC sheet on MHC-matched model was properly remained whereas the sheet on MHC-unmatched model was partially lost. As a result, rejection reaction in MHC-matched model was less sensitive than that in MHC-unmatched model.

STEM CELL NICHES

F-1100

PERICYTES PROMOTE SYMMETRIC CELL DIVISIONS IN THE EPITHELIAL STEM/PROGENITOR COMPARTMENT OF HUMAN SKIN AND ESTABLISH A NORMAL EXTRACELLULAR MATRIX MICROENVIRONMENT

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Our lab has been interested in understanding the cellular and molecular interactions between epithelial cells and their mesenchymal microenvironment during homeostasis, wound repair and cancer. Our previous work has shown that human skin regeneration is attributed to both the intrinsic properties of epidermal stem and progenitor cells, and the cellular composition of their extrinsic mesenchymal microenvironment, leading us to investigate stromal heterogeneity in the epithelial microenvironment . We identified of a rather potent sub-population of mesenchymal stem-cell like cells i.e. $CD45^-$/$FAPa^-$/$VLA1^{bright}$/$CD146^+$/$PDGFRb^+$/$NG2^+$pericytes - previously known to have a role in regulating microvessel structural stability and permeability in both normal and neoplastic conditions. We demonstrated that pericytes had a novel angiogenesis-independent function in normal skin regeneration in 3D organotypic cultures, mediated at least in part by increased deposition of the basement membrane extracellular matrix protein LN511 pericytes. New data from our lab show that pericytes promote symmetric cell divisions within the proliferative compartment of the epidermis resulting in the maintenance of a more normal epithelium displaying greater polarity complete with hemi-desmosomes and basement membrane assembly *in vitro,* driven by proteins secreted by pericytes. We have begun to focus on 3D whole mount imaging of human skin tissue and established that the epidermis is patterned in arrays of rete ridges with a predictable array of microvessels coated with pericytes. Analysis of mitotic epidermal cells with respect to the spatial location of nearby pericytes was undertaken revealing that mitotic cells are predominantly found about 20-50μm from pericytes and that they are distributed throughout the basal layer at the tips and troughs of the rete ridges. We are currently investigating how local variations in the microenvironment particularly the net stromal content of pericytes versus fibroblasts dictate symmetric versus asymmetric cell divisions in the overlying epidermal stem and progenitors during epithelial renewal by imaging whole mounts of skin. These studies provide an insight into how the physical organisation of epidermal cells and their stroma contribute to form and function.

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F-1102

SECRETOME BENEFITS OF HUMAN MESENCHYMAL STEM CELLS FOR WOUND HEALING IN THE EARDRUM

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Surgical treatment of chronic perforations of the eardrum resulting from infections and trauma are expensive and require specialised facilities and expertise. Our studies have previously shown that human adipose-derived mesenchymal stem cells (ADSC) produce an activity that stimulates wound healing in eardrum keratinocytes (hTMk). Bioinformatics analysis was used to explore the molecular mechanism behind the paracrine activity of ADSCs on wound healing to determine the ADSC transcriptome for growth factor gene expression and matched to receptor entities on hTMk. Conditioned media (CM) were collected from normal and hypoxic (<0.1% O2) ADSCs to assess paracrine activity on hTMk proliferation and migration, as well as quantifying specific protein secretions using ELISA. Transcriptomic analysis of ADSC and hTMk were assessed using RNAseq and a bioinformatics pipeline. Differentially expressed genes ($P<0.05$) between hypoxic and normoxic ADSC were filtered through databases for secretome to only account for ligands known to be secreted. Transcriptome data of hTMk were filtered

through databases for known keratinocytes receptors. Both subsets were then matched to a ligand-receptor pair database curated with the FANTOM5 consortium for a final set of ligand-receptor repertoires between ADSC and hTMk. The results showed a total of 492 ligand-receptor pairs with 166 ADSC ligands and 166 hTMk receptors. Of the 492 pairs, 90 contained ligands upregulated >2-fold, 219 down-regulated, 42 turned on, 45 turned off, and 96 had less than 2-fold change in hypoxia. Paracrine activity produced by ADSC under hypoxic conditions showed enhanced wound healing properties of the human eardrum in vitro. Bioinformatics analyses were able to identify and predict the potential paracrine effectors for this wound healing activity. Further experiments are necessary to test and verify potential molecular mechanisms behind wound healing effects of ADSC on eardrum keratinocytes.

F-1104

TRANSCRIPTOME ANALYSIS OF REPLICATIVE SENESCENCE HUMAN CARDIAC PROGENITOR CELLS ATTENUATED BY RAPAMYCIN

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Cardiac progenitor cells senescence has been implicated reduced cardiac regeneration, and better understood senescence-associated changes in human cardiac progenitor cells. Chronic treat with rapamycin indicate suppression of replicative senescence, we investigated changes in their transcriptome using RNA sequencing. RNA sequencing revealed that 437 significantly differential expressed transcripts, which represents 265 transcripts up regulated in senescence and 172 transcripts down regulated by rapamycin. In KEGG pathway mapping, specifically the number of up and down regulated genes involved in cell cycle and PI3K-Akt signaling pathway, Foxo signaling by rapamycin. Also, we confirmed that rapamycin down regulate senescence-associated secretory phenotype (SASP) related transcript. These findings will help to understand rapamycin attenuate human cardiac progenitor cells senescence and provide rapamycin could be suggest development of target of anti-senescence.

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F-1106

MIR-302/MIR-22 SWITCH IS ASSOCIATED WITH IN VITRO AGEING OF HUMAN MESENCHYMAL STEM CELLS

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Significant progress has been witnessed on the use of human Mesenchymal Stem Cells (hMSCs) as an alternative resource for cell replacement therapy and that this requires in vitro expansion to meet the clinical need. However, MSCs undergo in vitro aging, thus limiting their proliferation and differentiation potential in long term culture. The complex interplay of molecular pathways especially microRNA dysregulation and microniche underlying in vitro stem cell aging remain to be explored. In this study, we aimed to determine microRNA signature of primary hMSCs isolated from deciduous pulp (SHED) and Wharton's Jelly (WJ) associated with in vitro aging in culture. SHED and WJSC (Ethic no: DF RD1503/0013-L) were isolated and then cultured in KO-DMEM supplemented with 2 ng/ml bFGF and 10% FBS for 6 passages consecutively. Briefly, total RNAs were isolated from SHED and WJSC at passage 3 and passage 6 and subjected to complete array of mRNA and miRNA footprints, which the data was analysed for mapping the molecular pathways using Ingenuity Pathway Analysis. The results showed that both phenotypic and molecular signatures of primary hMSCs isolated from SHED and WJSC were altered during in vitro passaging, and that it was significantly altered in p6 compared to p3. Both cell types showed higher population time and lower stemness and cell cycle markers in p6 compared to p3. Meanwhile senescence and inflammatory cytokines markers were upregulated. MiRNA profiling showed a set of diverse miRNAs that are deregulated in hMSCs isolated from SHED and WJ that are shown to govern many cellular processes including epigenetics, cell cycle, senescence, proliferative pathways and metabolism. Notably, highly deregulated miR-302 and miR-22 are those implicated in regulation of stemness and stress regulation meanwhile mRNA profiling revealed the dysregulation in G2/M cell cycle regulation and *Tumor Necrosis Factor Receptor 2 pathway.* Our results suggest hMSCs lost their therapeutic potential during in vitro passaging is regulated by a complex miRNA-mRNA network. In summary, discovery of miRNA regulatory network implicated in stem cell aging pave a path for the development of strategies to delay and reverse the aging of MSCs during their expansion, thus produces higher quality cellular resources for clinical applications.

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F-1108

MURINE HEMATOPOIETIC STEM CELLS IN PERISINUSOIDAL NICHES ARE PROTECTED FROM AGING

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Bone marrow (BM) hematopoietic stem cell (HSC) niches are constituted by multiple hematopoietic and non-hematopoietic cells interacting in a complex 3-dimensional architecture. With aging, intrinsic HSC activity decreases, resulting in impaired tissue homeostasis, reduced engraftment following transplantation and increased susceptibility to diseases. However, whether aging affects also the HSC niches impairing their capacity to support HSC function is still largely unknown. Here, by using in-vivo long-term label retention assays we demonstrate that aged labeling retaining HSCs are in the old mice the most quiescent HSC subpopulation with the highest regenerative capacity and cellular polarity, which reside predominantly in perisinusoidal niches. We show that upon aging HSCs are located more distant to multiple types of bone marrow niches, including the endosteal, the megakaryocytic, the arteriolar as well as the periarteriolar Nestin-GFPhigh cell niche, but not from the sinusoidal and perisinusoidal Nes-GFPlow cell niche. Furthermore, we demonstrate that sinusoidal niches and perisinusoidal Nes-GFPlow cells are uniquely preserved in shape, morphology and number upon aging. Using an in-silico model we conclude that the increased distance of aged HSCs from arterioles and Nes-GFPhigh cells depends on the alteration of the niche architecture. Finally, we show that myeloablative chemotherapy can selectively disrupt aged sinusoidal niches, and such disruption is critically linked to hematopoietic failure and decreased survival of aged mice. Overall, our data characterize for the first time the alterations of aged HSC niches and unveil that perisinusoidal niches are uniquely preserved and HSCs localized in these niches are protected from aging. These findings underline that physiological alterations of the BM niche upon aging impact on hematopoiesis and survival particularly in the context of specific (chemo-) therapeutic interventions.

F-1110

THE EFFECTS OF FICOLIN-1 AND FIBRINOGEN-LIKE DOMAIN CONTAINING PROTEINS ON EX VIVO HEMATOPOIETIC STEM CELL EXPANSION

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The insufficient number of Hematopoietic Stem Cells (HSCs) extracted from umbilical cord-blood contributes to poor clinical outcomes for HSC transplantation procedures. Ex vivo expansion techniques have been developed to increase the number of cord-blood HSCs via the promotion of progenitor expansion without differentiation with the use of niche growth factors. Successful expansion of HSCs has been shown with stem cell niche factors such as members of the Angiopoietin-like protein family (ANGPTL-2, 3 and 5). However, these large Fibrinogen-like domain (FLD) containing proteins are readily degraded and form aggregates, making them difficult to purify for ex vivo expansion systems. Comparative protein sequence analysis of ANGPTL-2, 3 and 5 was used to identify other FLD containing proteins which may possess HSC expansion potential. Ficolin1 (FCN1), Intelectin, Fibroleukin and Tenascin XB were identified as candidate proteins containing a FLD with high protein structure similarity to ANGPTL-2, 3 and 5. Flow cytometry results showed that FCN1 had the ability to expand CD34+ HSC ex vivo, with a significant increase in the proportion of CD34+ CD45+ and CD34+ CD133+ HSCs after 16 days compared to expansion in the absence of FCN1. Similar expansion effects were also seen with Intelectin, Fibroleukin and a purified peptide of the FCN1 FLD domain alone (201aa-300aa), suggesting that the FLD domain is likely to be responsible for the HSC expansion potential of this class of proteins. However HSC expansion was not observed with Tenascin XB, suggesting that subtle differences in the FLD sequence or surrounding sequences may lead to differences in HSC expansion potential. The mechanism of action of FLD containing proteins was investigated by RNA sequencing and subsequent pathway analysis using FCN1 treated HSCs across different time points. This study highlights the significant of utilising proteins that mimic the stem cell niche to increase HSC ex vivo expansion.

Funding Source: This work was supported by the funds from Cytomatrix Australia.

F-1112

DETERMINATION OF OPTIMAL CONDITIONS FOR COLONY-FORMING ABILITY OF STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

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Stem cells from human exfoliated deciduous teeth (SHED) are originally referred to as fibroblastoid-colony-forming-cells because one of their characteristics is related to proliferation and differentiation potential. In this study, we developed an optimal protocol for colony-forming-unit (CFU) assay for SHED. The culture conditions including culturing time, seeding cell density and modifications of media supplements were optimized. The CFUs were determined on the colony morphology, sizes and the numbers of colony. Isolated SHED expressed CD44, CD73, CD105 and showed multipotent ability. Higher yield was obtained when SHED was cultured at the density of 500 cells per well of a 6-well plate for 14 days in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS). The number and size of CFU was increased with the increase of FBS concentration in which the transit-amplifying cell (TAC) colonies were the most abundant, followed by the stem cell (SC) and differentiated cell (DC) colonies, respectively. In DMEM with 20% FBS and 100 mM L-ascorbic acid-2-phosphate, the CFU rate was highest with increasing the proportion of SC colonies. The improvement of the culture conditions described herein could be adopted to easily acquire more functional SHED for applying in cell therapy.

Funding Source: This study was funded by Mahidol University.

F-1114

METABOLISM AS AN EARLY PREDICTOR OF HUMAN DENTAL PULP STEM CELL AGING

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Pluripotent stem cells can switch their unique metabolic requirements to facilitate cellular changes but it is not clear if adult stem cells utilize metabolism in a similar manner. Here we studied the metabolism of a human adult stem cell type, dental pulp stem cells (DPSC).

We cultured stem cells from dental pulp isolated from a diverse patient group from surgically extracted third molar with a high percentage of mesenchymal population demonstrated by expression of surface markers, CD29, CD44, CD146 and Stro1. We employed an assay to quantitatively measure the differentiation abilities of these cells into osteogenic and adipogenic lineages. In this study we have identified homeobox protein, Barx1 as a specific marker for DPSC. Furthermore, using high throughput proteomic analysis we identified markers for DPSC populations with accelerated replicative senescence. In particular, we show that the transforming growth factor-beta (TGF-b) pathway and the proteins for muscle contraction are upregulated in fast aging DPSC, indicating a loss of stem cell character and initiation of terminal differentiation. Importantly, using metabolic flux analysis, we identified a metabolic signature for fast aging DPSC. This metabolic signature is predictive since it can be observed prior to replicative senescence phenotypes. Hence, the present study identifies Barx1 as a DPSC marker and dissects the first predictive metabolic signature for DPSC aging.

NEURAL DEVELOPMENT AND REGENERATION

F-2002

SWI/SNF COMPLEX MUTATIONS IN INTELLECTUAL DISABILITIES

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SWI/SNF or BAF complexes are large, multi-subunit protein complexes composed of a core ATPase, either BRG1 or BRM, and up to 15 associated subunits. Many of the subunits are members of gene families, which are alternatively used to impart cell type- and developmental stage-specific activities. For example, during neural differentiation from embryonic stem cells (ESCs), the BRG1 ATPase is downregulated, while its homolog BRM is upregulated. BAF complexes are thought to use energy generated through ATP hydrolysis to alter the open/closed state of chromatin by moving or displacing histone octamers. However, the mechanism by which the BRM ATPase regulates neural differentiation therein is unknown. This is particularly relevant because heterozygous mutations in the BRM ATPase domain cause Nicolaides-Baraitser syndrome (NCBRS), an intellectual disability syndrome. To address this, we engineered human ESCs to carry either a BRM K755R or R1159Q mutation commonly found in NCBRS and performed in vitro neural differentation. As expected, BRMK755R/+ and BRMR1159Q/+ ESCs proliferate normally and maintain their cell identity. However, we found that mutant neural progenitor cells (NPCs) have morphological defects and proliferated significantly slower than WT NPCs. In addition, mutant NPCs expressed reduced levels of NPC specific markers such as PAX6 and SOX2. To assess the impact on chromatin remodeling, we performed ATAC-seq on WT and BRM mutant NPCs and found thousands of changed accessible sites with strong overlap between the two mutants. Surprisingly, decreased accessible sites largely overlap with H3K4me1 and H3K27Ac, enriched at enhancers, indicating that BRM-containing BAF complexes are critical for maintaining enhancer accessibility. SOX and RFX family binding motifs were highly enriched in sites that lost accessibility, suggesting that binding of neural-specific transcription factors is likely impacted. Indeed, RNA-seq analysis revealed decreased expression of genes involved in neural development and increased expression of genes involved with extracellular matrix organization. These data suggest that BRM-containing BAF complexes are required for proper neural differentiation by facilitating access of neural transcription factors to transcriptional enhancers specifying neural cell fate.

F-2004

DETERMINING FETAL- AND HESC-DERIVED DOPAMINERGIC GRAFT COMPOSITION THROUGH SINGLE-CELL RNA SEQUENCING OF TRANSPLANTED CELLS AFTER 6 MONTHS OF IN VIVO MATURATION

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The prospect of using stem cell based treatment for Parkinson's disease (PD) is edging closer. Following transplantation of stem cell derived dopaminergic (DA) progenitors in animal models of PD the cells mature, re-innervate the brain, restore synaptic connections with host neurons and release dopamine. Motor recovery of the transplanted animals is dependent on a sufficient number of cells with an authentic ventral midbrain (VM) DA identity being present within the graft. However, although highly homogenous VM progenitor populations are used for transplantation, not all cells acquire a mature VM DA identity after transplantation. The DA component of the graft is typically around 20-40% and the identities of the remaining cells within the graft remains largely unknown. In this study we set out to investigate the cell composition of both fetal VM - and hESC-derived grafts after 6 months maturation in vivo in the adult rat brain. The graft core was re-isolated by microdissection, dissociated into a single cell suspension followed by FACS based on GFP and/or

size and granularity. The cells were analyzed by single-cell RNA sequencing (scRNAseq). The transcriptomic analysis of fetal VM transplants revealed 3 major clusters of cells; neurons, astrocytes, oligodendrocytes, while the hESC-derived grafts contained astrocytes and neurons but no oligodendrocytes. Additionally, the hESC-derived grafts contained a fourth group of cells that expressed markers of perivascular fibroblast-like cells, and the identities of these groups could be further validated by immunohistochemistry. A more detailed transcriptional analysis is underway and once completed, this study will provide us with a unique unbiased assessment of graft composition that has not been possible to conduct until now. It will also provide an in-depth comparison between mature fetal- and hESC-derived DA grafts and will result in an increased understanding of graft diversity and composition.

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F-2006

A NOVEL APPROACH TO TRANSCRIPTIONALLY SEQUENCE HUMAN-PLURIPOTENT STEM CELL-DERIVED TRANSPLANTS IN ANIMAL MODELS

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Our increasing ability to fate restrict human pluripotent stem cells (PSC) into defined lineages makes them a valuable resource for transplantation, targeted at tissue repair. One of the most advanced applications in this regard, is the generation of hPSC-derived dopamine progenitors for transplantation in Parkinson's Disease. Despite rapid progress in both the generation and transplantation of hPSC-derived dopamine progenitors, means to assess these, and other human xenografts, have been limited. Efforts to transcriptionally profile transplants have largely relied upon laser capture to isolate the grafted tissue, however such approaches either include high proportions of host-derived cells (the consequence of trying to obtain cells within the graft core and periphery) or isolate only the central region of the graft (in an effort to reduce host contamination). To circumvent this challenge, we have developed a novel technique that allows us to specifically sequence the transplanted human cells within a xenograft, by recognising differences in gene homology between species. To demonstrate this technique, we implanted undifferentiated hPSC, and hPSC-derived dopamine progenitors, assessing grafts after 1 and 5 months. RNA Sequencing of the grafts, and validation by quantitative PCR and immunohistochemistry confirmed efficiency of the technique with, for example, teratoma-forming grafts displaying high expression of proliferative and trilinage potential genes. In contrast, dopaminergic progenitor grafts showed characteristic developmentally immature gene expression at 1month (e.g. EN1, FOXA2, OTX2) whilst graft maturation resulted in expression of VMAT, DAT, synaptophysin etc. More importantly, the RNAseq revealed a number of novel genes that may be critical in understanding graft composition, maturation and functional integration. In conclusion, we developed a new technique for transcriptome profiling in xenografts of human pluripotent stem cells in animal models.

Funding Source: NHMRC - National Health and Medical Research Council - Australian Government

F-2008

MITOGENIC CONTROL OF HUMAN NEURAL STEM CELL FATE

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Modelling human neurological development requires reliable cellular systems. Human neural stem cells are traditionally grown in the presence of EGF and FGF. Both EGF and FGF are known to influence positional identity and proliferation of neural progenitor populations. To study the requirements of human neural stem cells we use neuro epithelial stem cells (NESc) derived from human induced pluripotent stem cells (hiPSCs). However, these NES cells adopt a hindbrain identity over passages. Hence we will investigate to what extent EGF/FGF composition can be utilized to control and influence the identity of NES cell populations. Preliminary data, where either EGF or FGF2 was removed from NES cultures showed continued proliferation also in these conditions, indicating that endogenous signalling maintains continued self-renewal or only one of the factors are essential. Cells expanded in either EGF or FGF2 also have the capacity to differentiate upon complete growth factor removal. Interestingly, response to differentiation is altered depending on previous culture conditions, which indicate enhanced cell fate control by optimal culture conditions during in vitro expansion of NES cells. In 2D differentiation experiments, NES cells grown in only FGF2 exit cell cycle faster upon differentiation but generate quite similar number of neurons compared to NES cells growth in normal EGF and FGF2 conditions. NES cells expanded in EGF only also differentiate faster upon growth factor removal however, the differentiation seem to be biased to glia fates on the expense of neuronal fate. Currently, experiments are ongoing whereby NES cells grown in different conditions are labelled with a lentiviral GFP construct in order to track them after transplantation into a human brain organoid.

So far these GFP positive cells survive the transplantation and currently we are investigating if they follow similar differentiation fate as seen in 2D differentiation. Understanding the role that common mitogens EGF and FGF play in human stem cell culture will aid in the non-invasive manipulation of neural stem cells and therefore, provide an optimal starting point in guiding the required cell type for transplantation purposes.

F-2010

SUBGROUPS OF MOUSE NEURAL STEM CELLS HAVE DIFFERENTIAL BASAL MTORC1 ACTIVITY AND DISTINCT RESPONSES TO TSC2 LOSS

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Subependymal giant cell astrocytomas (SEGAs) present in a subset of patients with Tuberous Sclerosis Complex (TSC), a neurodevelopmental disorder caused by mutations in TSC1/2. Interestingly, SEGAs are consistently located along the ventral portion of the ventricular-subventricular zone (V-SVZ) stem cell niche, suggesting a tight connection between anatomic location and disease pathogenesis. Neural stem/progenitor cells (NSPCs) in the V-SVZ are the proposed cell(s) of origin for these tumors. These NSPCs possess a positional identity that arises early in development, is defined by the expression of location-specific transcription factors (TFs), and predicts the type of progeny they produce. Since TSC is a disease of increased mTORC1 signaling, this suggested that signaling activity may be closely linked to positional identity. Therefore, we hypothesized that cells from the dorsal and ventral V-SVZ are differentially susceptible to mutations driving TSC pathogenesis, and that they harbor distinct mTORC1 signaling profiles. Upon removal of Tsc2 in the dorsal V-SVZ, mice died perinatally from seizures whereas in mice with Tsc2 removed in the ventral V-SVZ, nodular protrusions resembling patient tumors were observed. Dorsal and ventral lesions from TSC patients also displayed differential expression of location-specific TFs, consistent with findings in mouse. Higher basal mTORC1 signaling was observed in ventral V-SVZ cells compared to matched dorsal counterparts. This pattern was apparent in acutely isolated NSPCs, retained in cultured NSPCs, and visible in immunofluorescent staining of mouse and human tissue. This work reveals that positional identity also includes stereotypic basal signaling activity and that differing levels of growth pathway signaling in subregions of the V-SVZ are connected to distinct predispositions to disease phenotypes.

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F-2012

THE EFFECT OF QUERCETIN ON PROLIFERATION, PROTEASOME ACTIVITY AND NRF2 PROTEIN LEVELS IN EMBRYONIC NEURAL STEM/PROGENITOR CELLS OF RAT

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Despite the relatively unknown mechanisms of neurodegenerative diseases, progressive neural cell loss or cell death is common in all these diseases. Due to lack of effective treatments for neurodegenerative conditions, new and efficient therapeutic approaches are required. Neural stem cells (NSCs) can potentially be valuable in developing effective drugs to improve neurogenesis and endogenous cellular repair in above-mentioned conditions. Since one suggested method for cell replacement is pharmacologic stimulation of endogenous NSCs, flavonoid compounds such as quercetin have been a candidate for this purpose. These compounds can potentially effect neurogenesis and they also show connections with some important neurogenesis mechanisms. Based on this, we hypothesized that different concentration of quercetin can affect viability and proliferation of neural stem/progenitor cells in 14-16 days old rat fetuses. To test our hypothesis, we evaluated the correlation between quercetin and Nrf2 protein levels and proteasomal activity as two much-debated mechanisms in neurogenesis. We isolated and cultured the ganglionic eminence of 14-16 days old rat embryos. After confirmation of the neural stem/progenitor cells existence, cells were treated in three doses of quercetin (1,5,15 µM) and afterwards, viability, proliferation, proteasomal activity, and Nrf2 protein level were evaluated respectively by MTT assay, Brdu assay, proteasomal activity kit and western blotting. Our preliminary results indicate that quercetin increases the viability and proliferation of embryonic NSCs. We are currently evaluating the Nrf2 protein level, proteasomal activity and their relationship with increase in proliferation after treating with quercetin. This study can shed light on quercetin effect on neural stem/progenitor cells and it raises the possibility of using quercetin as a potential drug for treatment of neurodegenerative diseases and other disorders accompanied by neural cell loss. In addition, this study can demonstrate the proteasomal system and Nrf2 pathway as potentially new therapeutic drug targets for neurodegenerative conditions.

F-2014

WATER-SOLUBLE ARGINYL-DIOSGENIN ANALOG RESCUES ADULT MICE HIPPOCAMPAL NEUROGENESIS IMPAIRED BY MICROGLIA-MEDIATED INFLAMMATION VIA INHIBITING JNK MAPK SIGNALING

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Microglial-mediated inflammatory responses are well-known to inhibit neurogenesis in the DG of the adult hippocampus and therapeutic intervention to suppress microglial activation could be an effective strategy to restore the hippocampal neurogenesis. We have reported that hydrophilic modification of diosgenin showed increased anti-inflammatory activity in LPS-stimulated microglia. Here, we investigated the effect of another water-soluble arginyl- diosgenin analog (Arg-DG) on the adult hippocampal neurogenesis using a central LPS-induced inflammatory mice model, along with the fundamental mechanisms in vivo and in vitro using LPS-stimulated microglial BV2 cells. Arg-DG (0.6 mg/Kg) attenuated LPS-impaired neurogenesis by promoting the proliferation and differentiation of neuron stem cells (NSCs) and suppressing apoptosis of NSCs via suppressing the production of LPS-induced pro-inflammatory cytokines in the dentate gyrus of hippocampus. In the in vitro study, Arg-DG also inhibited the production of nitric oxide (NO), nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression through inhibiting the expression of the pro-inflammatory cytokines such as interleukin (IL)-6, IL-1beta and tumor necrosis factor alpha (TNF-alpha). Anti-inflammatory effect of Arg-DG was regulated by NF-kB and MAPK JNK signaling in LPS-stimulated microglial BV2 cells. Besides, Arg-DG ameliorated LPS-impaired neurogenesis and inhibits the expression of pro-inflammatory cytokines via inhibiting JNK MAPK signaling in vivo. Taken together, these results suggested that Arg-DG might have potential agent to treat various neurodegenerative disorders resulting from microglia mediated-neuroinflammation.

F-2016

L-PROLINE-MEDIATED NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS: AN IN VITRO MODEL OF EMBRYONIC NERVOUS SYSTEM DEVELOPMENT

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During the early stages of mammalian development, cells of the embryo transition through a series of cell populations including the inner cell mass, primitive ectoderm, definitive ectoderm and neurectoderm before giving rise to cells that make up the nervous system. Due in part to the complexity of differentiation, the small size of the embryo and its relative inaccessibility in utero, the molecular mechanisms underlying the formation of these cell populations, and thus development of the neural lineage, are relatively poorly understood. We therefore developed a protocol using mESCs to recapitulate mammalian nervous system development in vitro using the amino acid L-proline. D3 and 46C-Sox1-GFP mESC cell lines were cultured as embryoid bodies (EBs) with time-dependent additions of 400 μM L-proline and 10 μM SB431542 over 9 days. On Day 9, EBs were seeded down under serum-free conditions and allowed to differentiate for a further 6 days, after which they were assessed for the presence of neural cell types. Using qPCR (data expressed as peak log2 fold change, n≥3), mESCs differentiated to Dnmt3b+ (3 3 0.5) and Fgf5+ (5 3 0.3) primitive ectoderm between Days 3-5, followed by Penk1+ (1 3 0.6) and Pard6b+ (2 3 0.1) definitive ectoderm between Days 5-7. From Days 5-9, a Sox1+ population of neurectoderm (2.5 3 0.3) was produced, at the expense of Mixl1+ mesendoderm (-2.1 3 1.2). By Day 9, flow cytometry analysis showed that 68 3 2% cells cultured in L-proline were Sox1-GFP+, and immunofluorescence imaging indicated that this expression was maintained in some cells at the core of EBs cultured until Day 15. At Day 15, 61 3 14% and 68 3 9% (n≥3) of D3 and 46C EBs, respectively, were found to be neural cells types including Nestin+ and BLBP+ neural progenitors and NeuN+ neurons. Taken together, in the presence of L-proline, neurectoderm production from mESCs was achieved prior to the addition of SB431542, thus providing evidence for an instructive model of neurectoderm induction. This is the first study, to our knowledge, that shows that a simple amino acid acts like a typical growth factor to induce differentiation at multiple stages of mammalian development.

F-2018

OPTIMISING CULTURE CONDITIONS FOR EXPANSION AND LONG TERM PROPAGATION OF MOUSE ENTERIC NEURAL PROGENITOR CELLS

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The enteric nervous system (ENS) plays an essential role in gut motility. Diseases of the ENS result in bowel motility disorders that are some of the most challenging clinical conditions to manage. Cell therapy offers the potential to treat gastrointestinal motility disorders caused by enteric neuropathies. We have previously shown that following transplantation into the colon of recipient mice, enteric neural progenitors isolated from fetal or postnatal mouse gut proliferate, migrate and differentiate into a variety of neurochemical and functional types of neurons that innervate the gut musculature. However, translating these rodent studies to human infants will require massive in vitro expansion of these endogenous enteric neural progenitors. Here we examined the ability of enteric neural progenitors to be grown in adherent culture rather than typical neurosphere cultures, and the influence of various extracellular matrix and growth factor combinations on their proliferation and differentiation in vitro. Cells grown on a combination of poly-L-ornithine (PLO) and laminin showed a significant increase in total cell number when compared to fibronectin, Matrigel or laminin alone. PLO+laminin also supported neuronal differentiation, with a significant increase in HuC/D-positive cells (neurons) compared to control conditions. Addition of endothelin-3, Wnt3a-conditioned medium, or Wnt3a and R-Spondin-conditioned media resulted in increased proliferation (EdU-positive cells) and total cell numbers compared to control conditions. Treatment of enteric neural progenitors with BMP7 resulted in increased neuronal differentiation, as well as increased cell clustering. Our data demonstrate isolated endogenous enteric neural progenitor cells can be grown as an adherent monolayer, and that the extracellular environment influences their proliferation and differentiation.

F-2020

SCHWANN CELL LAMELLIPODIA REGULATE CELL-CELL INTERACTIONS AND PHAGOCYTOSIS

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While it is well known that lamellipodia in Schwann cells (SCs) are crucial for myelination, their other biological functions remain largely uncharacterised. Two types of lamellipodia exist in SCs: axial lamellipodia at the outermost edge of the cell processes, and radial lamellipodia appearing peripherally along the entire cell. We have previously shown that radial lamellipodia on olfactory glia (olfactory ensheathing cells (OECs)) promote cell-cell adhesion, contact-mediated migration and phagocytosis. Here we investigate whether lamellipodia in SCs have similar roles. Using live-cell imaging, we show that the radial lamellipodia in SCs are highly motile, appear at multiple cellular sites and rapidly move in a wave-like manner. We found that axial and radial lamellipodia have strikingly different roles and are regulated by different intracellular pathways. Axial lamellipodia initiate interactions with other SCs and with neurons by contacting radial lamellipodia on SCs, and budding neurites/axons. Most SC-SC interactions resulted in repulsion, and lamellipodial activity did not affect cell migration rate. Thus, SC lamellipodia, unlike those in OECs, do not promote contact-mediated migration. We show that lamellipodia are crucial for SC-mediated phagocytosis of both axonal debris and bacteria, and demonstrate that inhibition of lamellipodial activity by blocking the Rho/Rac pathways also inhibits phagocytosis. We also show that heregulin, which induces SC differentiation and maturation, alters lamellipodial behaviour but does not affect phagocytic activity. Overall the results show that SC lamellipodia are important and essential for both cell interactions and phagocytosis.

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F-2022

PROTOCADHERIN 15 REGULATES OLIGODENDROCYTES PROGENITOR CELL PROLIFERATION, PROCESS EXTENSION AND SELF-REPULSION

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Oligodendrocyte progenitor cells (OPCs) proliferate and generate oligodendrocytes (OLs) while maintaining a relatively even distribution throughout the central nervous system (CNS). However, the signals that regulate OPC self-repulsion are poorly understood. Protocadherin 15 (PCDH15) is a member of the cadherin superfamily of transmembrane proteins that mediate calcium-dependent cell adhesion, and mutations in PCDH15 have been implicated in Usher syndrome and decreased PCDH15 expression is also associated with more aggressive glioma. Microarray and RNA sequencing data show that Pcdh15 mRNA is more highly expressed by OPCs than any other cell type in the CNS, and using immunohistochemistry we have determined thatOPCs express PCDH15 in vivo. However, the role of PCDH15 within the normal CNS, and more specifically in OPCs, is unknown. Using an shRNA-mediated gene knockdown approach, we determined PCDH15 suppresses OPC proliferation in vitro. In cultures with reduced PCDH15 expression, OPCs more readily incorporated EdU and extracellular signal-related kinase (ERK) activation was increased. Moreover, reducing PCDH15 expression significantly altered the physical interactions that occurred between adjacent OPCs. Specifically, it increased actin polymerization within OPC processes, increased filopodial contact time and reduced the frequency of lamellipodial extrusion and retraction (increased veiling time), while also markedly reducing the number of lamellipodia-like processes produced by each OPC. This phenotype was not affected by ERK activation, indicating that PCDH15 suppresses proliferation and promotes self-repulsion through distinct downstream signaling pathways. We have utilized CRISPR/Cas9 technology to generate a Pcdh15 $^{fl/fl}$transgenic mouse line, to examine the importance of Pcdh15 for OPC function in vivo.

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F-2024

TRANSPLANTING STEM CELL DERIVED V2A INTERNEURONS TO TREAT THE INJURED SPINAL CORD

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Traumatic spinal cord injury (SCI) results in extensive loss of neurons and glia at the site of injury. Among the many cells lost post-injury are spinal interneurons (SpINs), which are known to mediate anatomical and functional plasticity. With a focus on traumatic cervical SCI and the resulting respiratory deficits, the present work tests whether transplanting SpINs can promote repair and recovery. Building upon our recent discovery that V2a SpINs contribute to anatomical plasticity after injury, these experiments test the hypothesis that transplanting neural progenitor cells (NPCs; neuronal and glial lineage-restricted cells) enriched with stem cell derived V2a INs, can contribute to the formation of novel neural networks that enhance respiratory plasticity post-injury. Adult rats received a lateralized, mid-cervical contusion injury (Infinite Horizon Impactor, 200 kilodynes), which disrupts the phrenic motor circuit controlling the diaphragm, the primary respiratory muscle. Cultured NPCs derived from the embryonic (E13-14) rat spinal cord were enriched with stem cell-derived, Chx10-driven, V2a INs and allowed to aggregate for 2 days in vitro. These cellular aggregates (~120 micron diameter) were then transplanted into the lesion epicenter 1 week post-injury. Anatomical and functional analyses were performed one month following transplantation. Both NPC and V2a IN donor cells survived, differentiated and integrated with the injured host phrenic circuitry, as assessed by immunohistochemistry. NPC-derived glia migrated both rostral and caudal to transplant epicenter, and donor V2a INs extended neurites both rostral (up to 8.6mm) and caudal (up to 7.5mm) as measured from the edge of the transplant. A proportion (12.2%) of V2a donor cells expressed neuronal marker NeuN, while NPCs yielded both NeuN positive neurons and GFAP positive glia. Functional diaphragm electromyography demonstrated increased muscle (diaphragm) output one month following transplant, compared to injured, vehicle-treated animals. These ongoing studies not only test the efficacy of a promising therapeutic strategy, but also offer insight into the neuronal phenotypes that can be effective for neural transplantation to repair injured neural circuits.

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NEURAL DISEASE AND DEGENERATION

F-2026

NLGN4 REGULATES EXCITATORY SYNAPTIC TRANSMISSION IN HUMAN NEURONS

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Mutations in the Neuroligin 4 gene (NLGN4) are associated to Autism Spectrum Disorder (ASD) and have almost complete penetrance. The effect of these mutations has been problematic to study because of the poor conservation and very low expression levels of the NLGN4 gene in rodents. Here we report the generation of engineered isogenic human neurons derived from embryonic stem cells (ESC) carrying mutations in the NLGN4 gene. Mutations were introduced by adeno associated virus AAV - mediated homologous recombination with efficiency up to 40%. Synaptically competent excitatory and inhibitory neurons were generated by using specific transcription factor cocktails developed in our laboratory. We found that the NLGN4 mature protein is expressed by inhibitory and only a subset of excitatory neurons. We found that the both NLGN4 knock-out and NLGN4-R704C neurons have exaggerated excitatory post-synaptic currents (EPSC) but normal inhibitory (IPSC). Interestingly, this is in sharp contrast with previous studies in mouse neurons. The mutant neurons have normal cell morphology but abnormal synapses number suggesting a role of NLGN4 also during synaptogenesis. Moreover, Co-Immunoprecipitation (Co-IP) shows the interaction of NLGN4 with a subunit of the AMPA receptor. This is the first report of the study of NLGN4 in the context of human neurons. Our data suggest that NLGN4 mutations predispose to autism, at least partially, by inducing an abnormal synaptic activity by modulating the AMPA receptor that may be amenable to pharmacological intervention.

F-2028

MACROPHAGE-DERIVED EXTRACELLULAR SUCCINATE LICENSES NEURAL STEM CELLS TO SUPPRESS CHRONIC NEUROINFLAMMATION

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Neural stem cell (NSC) transplantation can influence immune responses and suppress inflammation in the central nervous system (CNS). Metabolites such as succinate modulate the phenotype and function of immune cells, but whether and how NSCs are also activated by such immunometabolites to control immunoreactivity and inflammatory responses is unclear. Here we show that transplanted somatic and reprogrammed NSCs ameliorate chronic CNS inflammation by reducing succinate levels in the cerebrospinal fluid, thereby decreasing mononuclear phagocyte (MP) infiltration and secondary damage. Inflammatory MPs release succinate which activates succinate receptor 1(SUCNR1)/GPR91 on NSCs, leading them to secrete prostaglandin E2 and scavenge extracellular succinate with consequential anti-inflammatory effects. Thus, this study uncovers a succinate-SUCNR1 axis that clarifies how NSCs respond to inflammatory metabolic signals to inhibit activation of type-1 MPs in the chronically-inflamed brain.

Funding Source: Wellcome Trust, Italian Multiple Sclerosis Association (AISM), United States Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP), European Research Council (ERC) and Bascule Charitable Trust.

F-2030

ESTABLISHMENT OF MICROFLUIDICS PLATFORM FOR COMPARTMENTALIZED NEURONAL CULTURE WITH AXONAL PROPAGATION VELOCITY MEASUREMENT

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Studying axonal physiology, pathology and electrophysiology of the nervous system in vitro can be complex due to the random orientation of axonal network and presence of cell bodies within the axonal network. Microfabrication and microfluidics technology enable generation of multi-chamber in vitro cell culture platforms that allow compartmentalized cell culture and guidance of cell growth. These characters give several advantages for microfluidic platforms over traditional cell culture methods. The aim of this study was to establish a microfluidic platform for neurons with isolated axonal culture that can be used for measuring the propagation velocity of action potential. Microfluidic platform was manufactured from polydimethylsiloxane (PDMS). The platform contains compartments for neuronal somas and axons and compartmentalization is achieved by restriction tunnels whose variable dimensions (height and length) was tested. Human pluripotent stem cell (hPSC) -derived cortical neurons were cultured in the platform. The growth of axons through restriction tunnels was followed by phase-contrast imaging, and nuclear staining of living cells using DAPI was performed to evaluate the somal penetration through restriction tunnels. Results showed that the growth of axons through restriction tunnels was height-dependent. Even though axons passed through the lower tunnels their amount and extension capacity were poor compared to axons that traversed the higher restriction tunnels. Neuronal somas were unable to penetrate through lower restriction tunnels but in higher restriction tunnels the somal penetration was length-dependent. LIVE/DEAD assay showed that neuron culture in the platform was viable and immunocytochemical staining confirmed the neuronal nature of the cells. Using microelectrode array (MEA) system, the propagation velocity of action potential of hPSC-derived neurons in the platform could be measured within the biological range. In conclusion, the developed microfluidic platform is suitable for the culture of hPSC-derived neurons enabling axonal isolation and action potential measurement. The platform has potential use in the field of axonal biology and axonal electrophysiology.

F-2032

PROTEOMIC PROFILING OF THE EFFECT OF MCT8 MUTATION HAS ON BRAIN MICROVASCULAR ENDOTHELIAL CELLS PRODUCED FROM PATIENT IPSCS

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It is now widely known the variable mutations that can occur within the solute carrier family 16 member 2 (SLC16A2) gene results in structural and functional changes in the cell-membrane transporter protein Monocarboxylic acid Transporter 8 (MCT8) resulting in detrimental phenotypic effects in patients. The transporter's mutation is predominantly responsible for affecting the transport of thyroid hormone T3 across the membrane of several cell types, limiting the maturation of neuronal cells and manifesting as severe neurodevelopmental deficiency. It has been hypothesised that the brain microvascular endothelial cells, which make up the blood-brain barrier, is one of the crucial sites affected by the MCT8 transporter mutation as it limits the overall amount of T3 thyroid hormone available in the brain tissue. This study aimed to explore the effect of MCT8 mutations on the proteome of 5 variations of brain microvascular endothelial cells (BMECs) which were generated using induced pluripotent stem cells. Protein extracts were tryptically digested and the resultant generated peptides were analysed by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). Proteomic differences were seen in the post hoc systems biology based analysis which reveals the large proteomic profile shifts which can be attributed to the MCT8 mutations. The interaction network analysis allowed for an in depth pathway analysis identifying changes in key interactions across cell lines.

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F-2034

EXCITATION-INHIBITION BALANCE DEPENDENT SYNCHRONOUS ACTIVITY IN MOUSE IPSC-DERIVED NEURONS

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The excitation/inhibition (E/I) balance in cerebral cortex plays an important role in brain functions. When the E/I balance is disturbed by external stimulation, homeostasis is maintained by plasticity in case of that is sufficiently small. However, if the disturbance exceeds the range that can be compensated for by plasticity, it will lead to the onset of diseases such as epilepsy and schizophrenia. Thus, it is important to know the range where E / I balance is maintained by homeostasis in elucidation of disease pathogenesis and drug discovery. In this study, we aim to elucidate the relationship between the E/I balance in the cell population and the synchronous activity pattern of the neurons network composed of excitatory and inhibitory neurons differentiated from mouse induced pluripotent stem cells (iPSCs). First, neural cells in dorsal and ventral cortex were induced from mouse iPSCs, and the proportion of glutamatergic and GABAergic neurons was evaluated by immunocytochemistry. Next, these cell populations were seeded on microelectrode arrays (MEAs), which is a measuring device with multiple electrodes integrated in a cell culture dish, and

the spontaneous activity was recorded. As a result of evaluating the change in the activity pattern over time, synchronized activity across the network was detected from the excitatory group from the second week. On the other hand, remarkable synchronous activity was not detected from the inhibitory population. As a next step, we will evaluate synchronous activity patterns in cell populations with various E/I balance, which is constructed by mixing ventral and dorsal neurons with various ratios.

F-2038

INCREASED OXIDATIVE STRESS BUT NO AMYLOID OR TAU PATHOLOGY IN IPSC-DERIVED NEURONS FROM SPORADIC ALZHEIMER'S DISEASE PATIENTS

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Mitochondrial dysfunction is a prominent feature of Alzheimer's disease (AD) and increased production of reactive oxygen species (ROS) has been described in postmortem brain samples and animal models. Moreover, altered mitochondrial complex composition and imbalances in mitochondrial dynamics, namely fission and fusion, have been reported. However, these observations were made at a late stage of disease and the inability to examine an early, presymptomatic phase in human neuronal cells impeded our understanding of cause and consequence of mitochondrial dysfunction in AD. We used human induced pluripotent stem cell-derived neuronal cells (iN cells) from sporadic AD (SAD) patients and healthy control subjects (HCS) and found higher ROS production in patient cells. This was accompanied by increased levels of oxidative phosphorylation chain complexes, whereas mitochondrial fission and fusion proteins were unaffected. Surprisingly, these effects neither correlated with Aβ nor phosphorylated or total tau levels. Synaptic protein levels were also unaffected in SAD and HCS iN cells. The results of this study give new insights into neuronal changes in a presymptomatic phase of AD and suggest an integral role for mitochondrial dysfunction in AD even before the appearance of amyloid and tau pathology.

F-2040

NEURONAL DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS FROM A PATIENT WITH HYPOMYELINATION AND EPILEPSY REVEALS MUTATIONS IN THE GCN5L1 GENE CAUSING MITOCHONDRIAL DEFECTS

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GCN5L1 [GCN5 (general control of amino acid synthesis 5)-like 1; also known as Bloc1s1], is an acetyltransferase linked to the regulation of mitochondrial respiratory chain protein acetylation, but is also a constituent of the BLOC complex involved in the assembly of organelles such as lysosomes. Whole genome sequencing of a patient who presented with hypomyelination and epilepsy identified compound heterozygotic mutations in the GCN5L1 gene that were predicted to be pathogenic. Fibroblasts from the proband were reprogrammed into foot-print free iPSC using Sendai virus and multiple clones exhibited the expected normal karyotypes, expression of pluripotency markers and tri-germ layer differentiation potential in teratoma and embryoid body assays. Cortical neuronal cultures derived from GCN5L1 mutant iPSC exhibited normal cortical layer differentiation potential. However, analysis of key parameters of mitochondrial function (Seahorse) revealed increased mitochondrial oxygen consumption as well as increased mitochondrial proton leak. Altered mitochondrial ultrastructure and evidence of increased mitochondrial fragmentation indicates that mutation of GCN5L1 affects both mitochondrial protein makeup and mitochondrial turnover. In conclusion, we have established an iPSC model of a novel, poorly understood neurological disease and present the first evidence to show that a mutation in GCN5L1 is linked to mitochondrial bioenergetic defects. This model will now be used to further investigate disease mechanisms, and for screening drugs that may ameliorate mitochondrial defects.

F-2042

MODELLING CDKL5 DISORDER USING PATIENT CELLS: HUMAN BRAIN CELLS IN A DISH

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The CDKL5 disorder is a rare X-linked neurodevelopmental disorder characterised by severe intellectual disability and infantile onset epileptic encephalopathy. There is no treatment for this devastating disorder and little is known about mechanisms underlying its pathogenesis. To bridge the large knowledge gap relating to the biology of CDKL5 we are generating an in vitro model for CDKL5 disorder. We have derived patient-specific neurons and isogenic controls using CRISPR-Cas9 technology and iPSC generation. These iPSC's express well-established markers of pluripotency and are karyotype normal. We have successfully differentiated both patient cells and isogenic controls into neurons, allowing us to examine how loss of CDKL5 affects neurite outgrowth, microtubule dynamics, gene expression and to search for novel kinase targets in neurons as a consequence of CDKL5 deficiency. Our research using a "disease in a dish" approach will contribute to the fundamental knowledge into the biology of the CDKL5 disorder using these techniques. Uncovering the biological pathogenesis of CDKL5 deficiency is vital to aid in the development of new therapeutics for this debilitating disorder. Relating to this, a major aim of this project is to use iPSCs to determine whether microtubule instability is a feature of CDKL5 deficiency, as has been shown in the related disorder, Rett syndrome, which is caused by mutations in Methyl CpG-binding Protein 2 (MECP2). Current research from the Christodoulou laboratory on MeCP2 is focusing on reversing the Rett neurological phenotype by restoring microtubule dynamics using pharmaceutical inhibition of a microtubule modifying protein, HDAC6. We propose that MeCP2 and CDKL5 may share similar cellular functions, and therefore therapeutic approaches used for restoring microtubule dynamics in MeCP2 deficiency may also be of benefit in the CDKL5 disorder. The outcomes of this project will potentially be of translational importance for other severe neurodevelopmental disorders where microtubular trafficking is of critical importance.

F-2046

SHORT TANDEM REPEATS SUSCEPTIBLE TO PATHOGENIC INSTABILITY LINKED TO CHROMATIN DOMAIN DISRUPTION

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More than 25 inherited neurological disorders are caused by the unstable expansion of repetitive DNA sequences termed short tandem repeats (STRs). A fundamental unresolved question is why some STRs are susceptible to pathologic expansion, whereas thousands of repeat tracts across the human genome are relatively stable. Here, we unexpectedly discover that nearly all disease-associated STRs (daSTRs) are located at boundaries demarcating 3D chromatin domains. We identify a subset of boundaries with markedly higher CpG island density compared to the rest of the genome. daSTRs specifically localize to ultra-high-density CpG island boundaries, suggesting they might be hotspots for epigenetic instability or topological disruption upon STR expansion. Fragile X Syndrome patients exhibit severe boundary disruption and loss of CTCF occupancy in a manner that correlates with the degree of Fmr1 silencing and daSTR length. Our data uncover higher-order chromatin architecture as a new dimension in understanding repeat expansion disorders.

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CANCERS

F-2048

BMP PATHWAY AS THE MASTER REGULATOR OF THE SLOW CYCLING, CHEMO-RESISITANT CANCER STEM CELLL COMPARTMENT IN HUMAN EPITHELIAL CARCINOMA

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Resistance to chemotherapy is a major prognostic challenge in majority of cancers. The existence of functionally distinct subpopulations in tumours, know as intra-tumour heterogeneity with differential response capacities to drugs, plays a central role in chemotherapy resistance/evasion. Cancer stem cells have been described as a major functional compartment in the heterogeneous context of tumours with major roles in tumour initiation, drug resistance and metastasis. In this study, we have used human epithelial ovarian carcinoma as a model, to analyze the molecular and cellular dynamics of tumour cells during chemotherapy response, focusing on the BMP pathway as a key player. Using in vitro and in vivo gain and loss of function assays and single-cell tracking and transcriptomic approaches, we highlighted a slow cycling subpopulation with cancer stem cell properties within tumour cells, with the capacity to resist/evade chemotherapy. We further demonstrate a key role for an autocrine bone morphogenic protein (BMP) signaling pathway as part of the maintenance network for this compartment that regulates the slow cycling/quiescent properties, chemo-resistance and epithelial-mesenchymal transition signatures. Further single cell tracking and transcriptome analyses confirm that the de novo sensitization to chemotherapy observed through inhibition of BMP pathway in the tumour cells, majorly results from release of the slow cycling cancer stem cell compartment, by adoption of active cell cycle progression patterns and chemo naïve gene signatures. Our findings strongly support a novel role for this signaling pathway at the core of chemo-resistant cancer stem cell regulation that could further refine therapy regimens through targeted therapeutic approaches.

F-2050

A SUBSET OF CANCER CELLS IN RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA TUMORS EXPRESS PLURIPOTENCY AND CANCER STEM CELL MARKERS

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Recessive dystrophic epidermolysis bullosa (RDEB) is a skin blistering disease caused by mutations in the COL7A1 gene. RDEB patients present with severe blistering and fragility of the skin and mucous membranes, leading to pseudosyndactyly, susceptibility to infections, esophageal strictures, and aggressive cutaneous squamous cell carcinoma (SCC). SCC accounts for more than two thirds of RDEB patient deaths due to the development of multiple primary tumors and the accelerated progression of these tumors to a metastatic state. Identifying the genes and pathways involved in RDEBSCC development and progression will increase the ability of physicians to identify pre-cancerous lesions, design more effective, targeted therapies to slow or stop tumor growth, and potentially lead to the development of preventative measures. This study aimed to characterize the transcriptomes of RDEBSCC samples and non-cancerous keratinocytes using RNA-seq to identify conserved genes and pathways in RDEBSCC development. RDEBSCC cells showed significantly increased expression of four pluripotency genes, SOX2 (q=9.34E-06, log2FC= +7.668), TERT (q=3.08E-06, log2FC=+8.206), FOXD3 (q=0.001, log2FC=+5.103), and SALL4 (q=0.001, log2FC=+2.288), numerous members of the HOX and FOX transcription factor families, and two genes previously identified as markers of cancer stem cells (CSCs), AIM2 (q=8.32E-06, log2FC=+4.456) and ABCB5 (q=0.001, log2FC=+4.661), relative to non-cancerous, primary RDEB keratinocytes. AIM2 expression was also significantly increased (q=0.0486, log2FC=+3.088) in primary RDEB keratinocytes compared to keratinocytes from wild-type, bone-marrow-matched siblings, suggesting that AIM2 could be involved in cancer development and progression in RDEB keratinocytes. Flow cytometry of RDEBSCC cells showed that only a small subset of RDEBSCC cells (~0.5%) expressed high levels of both AIM2 and ABCB5, suggesting that there may be a CSC population in these tumors. These results, in combination with previous

studies on CSC populations in tumors, suggest that targeting the cells expressing these CSC markers may be effective in slowing or even stopping the growth and spread of these tumors in RDEB patients.

F-2052

ENRICHMENT AND CHARACTERIZATION OF CLINICALLY RELEVANT STEM-LIKE TUMOR-INITIATING CELLS (TICS) BY GENERATING PATIENT DERIVED XENOGRAFT (PDX) MODELS OF HUMAN METASTATIC CANCERS

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Tissue heterogeneity and increased genomic instability associated genetic/epigenetic variability were shown to confer metastatic advantage to certain clones in oncogenesis and this is also influenced by tumor microenvironments. We hypothesize that targeted therapy failures and emergence of therapy-resistant clones will be dependent on cancer types and are also specific to metastatic sites in patient-specific fashion. Thus, traditional treatments of metastatic cancers based on primary tumor biology may not be long-lasting. It is difficult to characterize metastasis *in vivo* as culture cell line derived animal models recapitulate neither the original tumor heterogeneity nor its specific metastasis patterns. Therefore, we have developed patient derived xenograft (PDX) models of metastatic cancers such that human tumor stroma can be maintained and tumor metastases can be followed in real time of clinical trials. We have focused our investigations on bone metastases of many common carcinomas of prostate, breast, lung, and kidney etc, as well as rare cancers of particularly mesenchymal cell origin such as osteosarcoma, Ewing sarcoma, chordoma, and uveal melanoma. We exploited the PDX approach to amplify primary tumor tissues with limited (up to 3) successive passaging of PDXs of metastatic tumors to conveniently enrich the otherwise rare populations of stem-like tumor initiating cells (TICs) in the institutional review board approved pre-consented clinical patients' specimens. Routinely, TICs are characterized by pluripotent stem cell characteristics such as efficiency of oncosphere formation *in vitro* and tumor initiation ability in vivo. We have also investigated the expression of novel markers, such as Sox2, Oct4, TRA 1-160, etc., in epithelial- and CD44, CD99, etc in mesenchymal-tumor derived spheroids. In addition, we compared the enhanced metastatic ability of PDXs generated in humanized mice with immuno-compromised controls and respective exosome protein profiles, determined by mass spectrometry. We also present some preliminary immuno-profiling data of fresh tumor specimens and their respective PDXs for macrophages, T-cells, dendritic cells and stromal fibroblasts of human origin. Associated potential pitfalls and trouble shootings are discussed in broader audience interests.

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F-2054

IDENTIFICATION OF PROTEIN INTERACTORS FOR A NOVEL LINCRNA AND ELUCIDATING THEIR ROLE IN REGULATION OF MITOSIS AND STEMNESS

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A novel long non-coding RNA was identified by us from mouse melanoma cell line CloneM3. This RNA exists as a pair of overlapping sense (Ginir) and antisense (Giniras) transcripts of 612 bases and we find that the levels of the individual RNA transcripts - Ginir and Giniras are critical for maintaining genomic stability in mouse cells. This transcript pair is spatial-temporally regulated during mouse embryonic development. Ectopic expression of the sense long non-coding RNA transcript in mouse embryonic fibroblast cells causes generation of stemness phenotype. RNAseq data indicated upregulation of genes enriched for the GO terms related to cell cycle progression and pluripotency in Ginir over expressed cells as compared to control cells. Using RNA Affinity Pulldown experiments and RNA immunoprecipitation, we have identified putative interacting protein partners of Kif family for Ginir and we here demonstrate that they play important roles in cytokinesis. Our studies show that cells ectopically expressing Ginir exhibit defects in the localization of these proteins at midbody during late telophase and cytokinesis in high frequency as compared to normal fibroblasts. Interaction of these proteins with the noncoding RNA caused perturbed proper assembly and function of midbody during cytokinesis. Any deregulation in this process caused defective cell division. In conclusion, we demonstrate role of Ginir/Giniras pair in regulating mitosis and stemness through its interactions with proteins of the Kinesin family.

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F-2056

TARGETING THE T LAK CELL ORIGINATED PROTEIN KINASE BY OTS964 SHRINKS THE SIZE OF POWER-LAW CODED HETEROGENEOUS GLIOMA STEM CELL POPULATIONS

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Glioblastoma resists chemoradiotherapy, then, recurs to be a fatal space-occupying lesion. The recurrence is caused by re-growing cell populations such as glioma stem cells (GSCs), suggesting that GSC populations should be targeted. This study addressed whether a novel anti-cancer drug, OTS964, an inhibitor for T-LAK cell originated protein kinase (TOPK), is effective in reducing the size of the heterogeneous GSC populations, a power-law coded heterogeneous GSC populations consisting of glioma sphere (GS) clones, by detailing quantitative growth properties. We found that OTS964 killed GS clones while suppressing the growth of surviving GS clones, thus identifying clone-eliminating and growth-disturbing efficacies of OTS964. The efficacies led to a significant size reduction in GS populations in a dose-dependent manner. The surviving GS clones reconstructed GS populations in the following generations; the recovery of GS populations fits a recurrence after the chemotherapy. The recovering GS clones resisted the clone-eliminating effect of OTS964 in sequential exposure during the growth recovery. However, surprisingly, the resistant properties of the recovered-GS clones had been plastically canceled during self-renewal, and then the GS clones had become re-sensitive to OTS964. Thus, OTS964 targets GSCs to eliminate them or suppress their growth, resulting in shrinkage of the power-law coded GSC populations. We propose a therapy focusing on long-term control in recurrence of glioblastoma via reducing the size of the GSC populations by OTS964.

F-2058

TARGETING SOLID TUMORS AND CANCER STEM CELLS WITH PROSTHETIC ANTIGEN RECEPTOR MODIFIED T-CELLS

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Over the past decade, it has become increasingly clear that harnessing a cancer patient's T-cells to destroy tumor cells will be an important weapon in an oncologist's therapeutic arsenal. Our group has envisioned the potential of chemical biology and protein engineering to non-genetically modify T-cell membranes. We have shown that two dihydrofolate reductase molecules ($DHFR^2$) fused to a single chain antibody (scFv) or peptide can spontaneously self-assemble upon the addition of the chemical dimerizer, bis-methotrexate (BisMTX), into highly stable multivalent targeted chemically self-assembled nanorings (CSANs). A unique feature of our approach is the ability to remove the CSANs from the T-cells by incubation with the FDA-approved non-toxic antibiotic trimethoprim, thus allowing us to deactivate the modified cells pharmacologically. In addition, we have shown that the CSANs have negligible immunogenicity in mice and do not induce naïve human T-cell anergy (i.e, unresponsiveness). Recently, we have prepared anti-EpCAM/anti-CD3 CSANs and anti-CD133/anti-CD3 CSANs. EpCAM has been shown to be a ubiquitous marker of a number of epithelial cancers, including breast, prostate, lung, pancreatic and colon cancer, while CD133 has been shown to be a marker for a number of types of cancer stem cells. The bispecific CSANs rapidly (min) and stably (days) bind to CD3 on T-cell membranes, thus forming *Prosthetic Antigen Receptors (PARs) T-cells*. Upon incubation of the PAR T-cells with EpCAM+ and/or CD133+ cancer cells, T-cell activation and selective cell killing was observed. *Furthermore, we demonstrated with murine xenograft model of triple negative breast cancer that the anti-EpCAM and anti-CD133 PAR T-cells are able to permanently eradicate tumors in animals.* Further analysis revealed that CD133+ TNBC cells function as CSCs both in vitro and in vivo. In addition, we observed that the loss of the CD133+ cells, both in vitro and in vivo, results in primary cells de-differentiating into populations of CD133+ CSCs. Therefore, tumor eradication could only be observed when T-cells were directed to kill both primary tumor and CSCs. Results of ongoing studies of the mechanism of primary TNBC tumor de-differentiation into CSCs, as well as the behavior of PAR-T-cells in vivo will be presented.

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F-2060

PEROXIREDOXIN II PROMOTES RAS-INDUCED LIVER TUMORIGENESIS THROUGH RECIPROCAL REGULATION OF FOXM1

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Peroxiredoxin II (Prx II), an antioxidant enzyme, is elevated in several human cancers. Although Prx II has been suggested to be related with hepatic tumor development and progression, the mechanisms by which Prx II participates in are not known. Here, we investigated whether Prx II plays an important role in oncogenic RasG12V-induced hepatic tumorigenesis. We examined the expression of Prx II in hepatocellular carcinoma (HCC) cells and tissues from H-rasG12V transgenic mice and HCC patients using immunoblot, quantitative real-time polymerase chain reaction, and immunohistochemistry. The effect of Prx II in tumorigenesis was investigated using soft agar assay and H-rasG12V/Prx II-/- mice. Chromatin immunoprecipitation and reporter assay were used to find the relationship between Prx II and forkhead Box M1 (FoxM1) induced by H-rasG12V. Expression of Prx II was elevated in H-rasG12V transformed HCC cells and tumor tissues from H-rasG12V transgenic mice. H-rasG12V/Prx II-/- mice exhibited a significantly reduced number and size of tumors. Overexpression of Prx II promoted growth of colony formation in soft agar. The expression of Prx II was correlated with that of FoxM1 in HCC patients and H-rasG12V mice. We identified that FoxM1 is a direct transcription factor of Prx II. Interestingly, Prx II modulated FoxM1 expression through ERK phosphorylation. This mutual regulation between Prx II and FoxM1 contributes to sustain Raf/MEK/ERK pathway and cyclin D1 activation. Prx II is an important downstream target of FoxM1 in H-rasG12V transformed HCC cells and this novel Prx II/ERK/FoxM1 signaling axis promotes hepatocarcinogenesis.

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F-2062

SINGLE-CELL TRANSCRIPTOMES REVEAL REGULATORY FACTORS OF STEM CELL INTRACOLONY HETEROGENEITY

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A human embryonic stem cell (hESC) colony is tacitly considered as a "clonal" expansion of a stem cell, and a homogeneous cell population. However, several recent studies, including ours showed morphological and molecular heterogeneity among cells within a hESC colony. This intracolony heterogeneity is not yet fully appreciated by the stem cell community. Intracolony heterogeneity not only impacts the expansion and clinical applications of stem cell, but more importantly suggests that there is a molecular mechanism to regulate the division of individual cells within a colony. With microfluidic devices, we obtained high quality single-cell cDNA from H9 cells within a colony and performed deep RNA-seq for 20 million pair-end reads. With this deep RNA-seq, we are able to perform valiant calls similar to analysis with traditional bulk lysate to identify single-nucleotide polymorphism (SNP) in individual cells. With these single-cell SNP, we clustered single H9 cells by SNP similarity to identify active dividing cells and senescent cells. By comparing the transcriptomes between the clusters of dividing vs senescent cells, we identify potential stem cell secretory factors that regulate cell division within a H9 colony. Such intracolony factors can regulate stem cells within a colony and force them into senescence. Manipulating these factors could provide an effective and safe method to control cancer stem cell proliferation and senescence within a tumor. Those factors are ideal intervention targets for control tumor growth in a systematic approach by targeting the stem cells within a tumor.

CHROMATIN AND EPIGENETICS

F-2064

VERIFICATION OF LONG-TERM GENETIC STABILITY DURING SUBCULTURE AFTER CELLULAR UPTAKE OF NANOPARTICLE IN STEM CELLS

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Many researchers have long sought to overcome major challenges in the treatment of nanoparticles, including intracellular delivery, biocompatibility and activation. In particular, the genetic stability of nanoparticles in stem cell therapy is becoming increasingly important. Herein, we fabricated functional nanoparticles by coating heparin gels on Quantum dot nanoparticles, and based on this, bind the sox9 gene and transfer it into stem cells, and reported intracellular genetic stability. The genetic stability of nanoparticle internalized by hMSCs were confirmed by chromosomal analysis, CNV analysis and mRNA profiling. The stem cells with internalized nanoparticles in the early stage were found to retain genetic stability even after passage.

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F-2066

CELL CYCLE-SPECIFIC ACCUMULATION OF 5-HYDROXYMETHYLURACIL ON ALPHA SATELLITES INDICATES ITS POTENTIAL ROLE IN ESTABLISHMENT OF CENTROMERIC CHROMATIN IN HUMAN PLURIPOTENT STEM CELLS

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5-Hydroxymethyluracil (5hmU) is an enigmatic DNA modification that has previously been regarded as a by-product of normal cellular metabolism in mammals. A recent study demonstrated that 5hmU is produced via oxidation of thymine by Ten-eleven Translocation (TET) enzymes in the DNA of mouse embryonic stem cells (ESCs). Although this finding implied that 5hmU may act as a bona fide epigenetic mark in ESCs, potential biological roles of this modification are currently unclear. Here, employing sensitive mass spectrometry we show that 5hmU is accumulated in the DNA of human pluripotent stem cells (hPSCs) during mitosis. In line with this, our immunostaining experiments and genome-scale profiling of 5hmU reveal predominant association of this modification with centromeric alpha satellites in hPSCs. Moreover, we demonstrate that 5hmU is enriched not only at tandem repeats-containing centromeres but also at the centromeres comprised of non-tandem-repetitive sequences in chicken DT40 cell line. Collectively, our data indicate cell-cycle-specific and likely alpha satellite-independent association of 5hmU with centromeric chromatin and suggest that this modification may potentially be involved in epigenetic determination of centromeres in vertebrates.

F-2068

DISTINCT EPIGENETIC PROGRAMS REGULATE CELL STATES IN PLURIPOTENCY AND DIFFERENTIATION TRAJECTORIES

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Human pluripotent stem cells (hPSCs) provide an opportunity to interrogate key aspects of embryonic development. Recent studies using pre-implantation human embryo revealed that the cells undergo dramatic epigenetic remodeling in the earliest steps of development. We hypothesized that epigenetic changes in pluripotency and in the first steps of differentiation may be defined and then modeled in hPSCs at high temporal frequency revealing mechanisms controlling cell commitment. Using RNA seq data, we identified three transcriptionally distinct and sequential steps as hPSCs self-renew. hPSCs progressed from state 1 with a mesendodermal bias through an intermediate state 2 to state 3 with neurectodermal bias. When passaged, cells in state3 re-set to state1 within 24 hours to repeat this temporal progression. Coinciding with the resetting of pluripotency in state 1, there is a burst of expression of genes regulating autophagic cytoplasmic degradation and increased autophagic flux. Pharmacological perturbation shows that proper regulation of autophagy is required for the transition from state 1 to 2. To establish the chromatin status of these transcriptionally distinct states and developmentally biased states, we investigated an activating (H3K4me3), repressive (H3K27me), and heterochromatin associated (H3K9me3) histone modification. All three of the histone modifications showed global level changes across the three states but only minor changes in peak locations in ChIP-seq. The peak locations of H3K4me3 or H3K27me3 correlated with changes in gene expression as the cells exit the pluripotency to differentiation. These data suggest that global levels of histone modification

and the peak location of the histone modifications are two separate mechanisms that shape the molecular and transcriptional structure of pluripotency. Future studies will test if our paradigm can be generalized to understand further steps of differentiation.

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F-2070

CANONICAL WNT SINGALING MODULATES PLURIPOTENCY EPIGENETIC STATE THROUGH CNBP1

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Canonical Wnt signaling is pivotal for early embryo development, stem cells self-renewal and differentiation. b-Catenin nuclear translocation is the key event following canonical Wnt signaling activation, how it regulates gene expression in different cellular context is not well understood. We used proteomics to explore b-Catenin binding partners in the nucleus of naïve state mouse embryonic stem cells (mESCs) and identified several previously unreported b-Catenin nuclear binding proteins (CNBPs), including epigenetic modifiers, RNA binding proteins and protein kinases. β-Catenin nuclear binding protein1 (CNBP1) is a component of the mammalian COMPASS/Trithorax (trxG) complex regulating histone H3 Lysine4 methylation, it is highly expressed in naive mESCs and downregulated upon differentiation. ShRNA knocking-down CNBP1 in ESCs resulted in reduced expression of pluripotency genes and loss of self-renewal ability. CNBP1 deletion by CRISPR in mouse zygotes led to embryo death before gastrulation. CNBP1 knock-out blastocysts contained inner cell mass (ICM) but could not give rise to ESCs. Transcriptome profiling of ICM and trophectoderm (TE) cells in CNBP1 knock-out embryos revealed significant change in key pluripotency, early lineage regulator, chromatin remodeler, metabolic gene expression. Using newly developed ChIP-seq technologies suitable for small number of cells, we found that histone H3K4me3 marks was initially reduced in selective genome regions in ICM cells, then diminished genome wide as embryo implant and initiate differentiation. Our results demonstrated that CNBP1 is required for early mammalian development. Specifically, it mediates H3K4me3 modification in a spatiotemporally regulated manner, thus form a direct link between canonical Wnt/b-Catenin pathway and the epigenome to regulate stem cell states and fate decisions.

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F-2072

SIMPLE SEQUENCE REPEATS (SSRs) - GUARDIANS OF THE CHROMATIN GALAXY

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Establishment, maintenance and dynamic rearrangement of euchromatin and heterochromatin domains in the genome guide the processes of development and differentiation in organisms. Simple Sequence Repeats (SSRs) or Microsatellites are short tandem repeats of 1-6 nucleotide motifs, with the repetitive unit occurring between 10-20 times. SSRs occur in most organisms, in coding and non-coding DNA. In the human genome, they are twice as abundant as the protein-coding DNA, but their functions are unknown. They are used widely in genetic linkage mapping analysis and the triplet repeat disorders caused by aberrant expansions in SSRs are well known. Studies suggest that SSRs may have essential roles in gene regulation and genome organisation. Chromatin domains in the genome are defined by DNA elements called Insulators/boundary elements. They function as enhancer-blockers, which prevent communication between a promoter and enhancer when placed between them and/or barriers, which protect a flanked transgene from position-dependent silencing. The globin insulator, the Drosophila Gypsy insulator and the boundaries of Drosophila Hox loci are well-known insulators. Though many insulator proteins in Drosophila are known, the CCCTC-repeat binding factor, CTCF is the only insulator protein identified in vertebrates. It functions as a transcription factor and chromatin organising protein and is shown to be critical for stem cell proliferation. We tested whether SSRs are capable of enhancer-blocker and barrier activities. We selected 23 human SSRs based on their 'length preference' (Ramamoorthy S. et al. 2014) and cloned the oligos into vectors for cell-based functional assays to study their cis-regulatory potential. The 23 SSRs which are mostly intergenic or intronic modulated promoter activity in transient luciferase assays in six different cell lines, in a cell-type-dependent manner. 15 of 23 SSRs showed insulator activity in K562 cell line. Electrophoretic Mobility Shift Assay (EMSA) using nuclear extracts from Drosophila embryos and human cell lines revealed that these SSRs bind specific proteins. Using biotin-affinity purification and proteomics approach, we aim to identify novel SSR-binding proteins, which may have important roles in organisation and regulation of the genome.

ORGANOIDS

F-2074

DEVELOPMENT OF 3-DIMENSIONAL HUMAN CORTICAL SPHEROID PLATFORMS WITH HIGH HOMOGENEITY AND FUNCTIONALITY FOR HIGH THROUGHPUT AND HIGH CONTENT SCREENING

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The human cerebral cortex is organized in a complex 3-dimensional (3D) structure comprising different neural cell types. The coordinated work of these different cell types is key for brain function and homeostasis. Recently, much work has been focused on obtaining 3D brain organoids in an attempt to better recapitulate the brain development/function in vitro. However, current protocols may lead to variable organoid size and function, making the use of these powerful tools impractical in a drug screening scenario. Here we describe the development of a highly homogenous human induced Pluripotent Stem ell (hiPSC)-derived cortical spheroid screening platform in 384 well format, composed of cortical neurons and astrocytes. Immunofluorescence analysis indicated that these derived neurons and astrocytes display key markers of cellular identity as well as maturity, such as synaptic proteins and glutamate transporters. Viability assays carried out with compounds with known mechanism of action indicated scalability and feasibility of the assays, with results comparable to a standard 2D model employing the same culture composition. Kinetic, high trhoughput calcium flux analysis performed in a in a Fluorometric Imaging Plate Reader (FLIPR) highlighted that the spheroids present quantifiable, robust and uniform spontaneous calcium oscillations, measured. The calcium signal was modified with excitatory and inhibitory modulators coherently and in a highly reproducible fashion, confirming the presence of a functionally integrated glutamatergic/GABAergic circuit. High speed confocal imaging confirmed homogenous calcium oscillations at the cellular level, whereas multielectrode array (MEA) analysis demonstrated robust synchronous neurophysiological activity at the network level. Additionally, these cortical organoids are amenable to immunostaining in suspension, enabling scalable high content image-based assays focused on key protein markers. Altogether, the developed 3D cortical spheroid platform can be easily implemented as a reliable high throughput screening platform to investigate complex cortical phenotypes *in vitro*, as a reliable high-throughput screening platform for toxicology studies, disease modeling and drug testing.

F-2076

TESTES IN A DISH: DIRECTING PLURIPOTENT STEM CELLS TO HUMAN TESTIS CELL LINEAGES

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At six weeks of human embryonic development, the gonads develop in a bipotential form that subsequently differentiates into either testes or ovaries, depending on the embryo's sex chromosome constitution. Disruption of normal gonad development and differentiation often results in Disorders of Sex Development (DSD) in humans. Genomic analysis of these patients has identified variants in both known and novel DSD genes. However functional analysis of these variants is severely hampered by the lack of a human embryonic gonad cell line. Recent studies have differentiated human pluripotent stem cells (hPSCs) into renal lineages. Aggregates of these cells, termed kidney organoids, grown in vitro recapitulate features of functional embryonic kidneys (Takasato et al., 2015). Given the shared developmental origin of gonad and kidney, we have developed a protocol for the differentiation of hPSCs into male gonad cells, to create "testes in a dish". Starting with male hPSCs, we differentiated these cells in monolayer culture in two stages (WNT signalling activation followed by FGF/BMP signalling activation), inducing intermediate/lateral plate mesoderm-like cells. Prolonged culture of these differentiated cells showed that by day 12 they exhibit a strong bipotential gonad expression profile (LHX9+/WT1+/ GATA4+), shown by qRT-PCR and immunofluorescence staining. Furthermore, at day 18 of culture these cells showed upregulation of markers for two testis lineages, Sertoli (SOX9+) and steroidogenic (STAR+) cells. This was accompanied by a decrease

in bipotential gonad-specific marker LHX9, indicating commitment to a testis fate. These data provide evidence for the induction of male gonad lineages from hPSCs; additional characterisation will be performed to test their function in sex steroid production and germ cell maintenance. We are also exploring organoid culture methods to stimulate differentiation of additional testis lineages and testis cord-like structures. These exciting results will be presented and their implications for DSD patients discussed.

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F-2078

INSIDE YOUR BRAIN: NEW FRONTIERS IN THE STUDY OF NEURODEVELOPMENTAL DISORDERS

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The brain is the most complex organ in the vertebrate body and it is evident that its development is finely regulated, both temporally and spatially. The cerebral cortex, in particular, is characterized by unique anatomical, cellular, molecular and genetic features, and the complex interplay of these contributing factors underpin its assembly during fetal development. However, genetic mutations and environmental insult can disrupt fetal brain development and cause brain disorders. To date, animal models have been extremely informative for the characterization of genetic factors which influence human brain development and disease. However, one clear limitation to this approach is to model the effects of disease-associated genetic mutations in the development of the human fetal cerebral cortex, which is clearly distinct to other mammals such as mice. Here, we report our preliminary results from experiments designed to generate three-dimensional (3D) cerebral organoids from human embryonic stem cell lines so as to model human fetal brain development. We find that our cerebral organoids exhibit features of self-organization, tissue differentiation, and neurogenesis. In addition, we will discuss our strategy to engineer disease-associated mutations to candidate genes which underlie human disorders of brain growth, such as microcephaly.

F-2080

RESCUE OF MOUSE ACUTE LIVER FAILURE BY HUMAN LIVER ORGANOIDS GENERATED WITH SINGLE DONOR DERIVED MULTIPLE CELLS

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Acute liver failure (ALF) is a life-threatening disease with a high mortality rate. Treatments or devices that can stimulate hepatic regeneration and minimize the risk of complications associated with ALF are limited. Here, we demonstrate a new treatment for ALF by transplantation of functional liver organoids (LOs) generated with single donor derived human induced pluripotent cells (hiPSC) endoderm, endothelial cells (ECs) and mesenchymal cells (MCs). Umbilical cord (UC) is a promising source of ECs and MCs, and UC derived ECs (UC-ECs) and MCs (UC-MCs) could provide a multicellular microenvironment that promoted LO differentiation. Using a non-viral method, we reprogrammed UC-ECs into hiPSCs in a feeder-free culture system, and the resultant EC-hiPSCs efficiently differentiated into definitive endoderm and hepatic lineages. Simultaneously plating EC-hiPSCs endoderm, UC-ECs and UC-MCs in a three-dimensional micro-patterned culture system yielded single donor cells derived LOs (SDC-LOs) that could be induced to differentiate into functional LOs with enhanced metabolic capacity as compared to EC-hiPSC-derived hepatic-like cells. When these SDC-LOs were transplanted into the kidney capsule of ALF mice, they rapidly assumed hepatic functions and improved the survival rate of the mice. These results demonstrate that these functional LOs generated from single donor cells can alleviate liver damage in ALF and has a promising application in liver regenerative therapy.

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F-2082

GENERATION OF AN INFLAMMATORY BOWEL DISEASE MODEL SYSTEM USING HUMAN IPS CELL-DERIVED INTESTINAL ORGANOIDS

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Inflammatory bowel diseases (IBDs) are intestinal diseases represented by Crohn's disease and ulcerative colitis. The number of IBD patients has been increasing in recent years. IBDs are known to be caused by abnormal production of cytokines that regulate the intestinal immune system. Especially, inflammatory cytokines, such as TNF-α, produced by abnormally accelerated macrophages in Crohn's disease cause damages of intestinal epithelial cells. However, currently, there is no in vitro evaluation system that mimics intestinal mucosal damages due to IBDs. Therefore, in this study, we conducted whether human iPS cell-derived intestinal organoids can mimic the pathogenesis of IBDs by treatment of TNF-α for 96 hours. We confirmed that the intestinal organoids contained various intestinal cells such as enterocytes, intestinal stem cells, goblet cells, enteroendocrine cells, Paneth cells and mesenchymal cells. By treatment of TNF-α, mRNA expressions of enterocyte marker villin, goblet cell marker MUC2, and water channel AQP3 decreased. On the other hand, fluctuations of their expressions were suppressed by treatment of infliximab (IFX), which is an antibody of TNF-α used in clinical practice. There was no change in expressions of other intestinal cell marker genes. mRNA expression of inflammatory markers TNF-α and IL-1β, and intestinal stem cell marker OLFM4 was increased by treatment of TNF-α, and their fluctuations were suppressed by treatment of IFX. In immunofluorescent staining, the epithelial cell damage and collapses of tight junction structures were confirmed. In addition, caspase-3 positive cells were increased by treatment of TNF-α, and it was also observed that apoptosis cells were dropped in the lumen of intestinal organoids. Furthermore, the permeation of a nonabsorbable marker FD-4 into the intestinal organoids was confirmed in treatment groups of TNF-α and EGTA, but not in that of IFX. These results suggested that human iPS cell-derived intestinal organoids could reflect the mucosal damage, which is one of the pathogenesis of IBDs, and evaluate the effect of therapeutic agents of IBDs such as IFX.

F-2084

MODELING HUMAN HEART MUSCLE AND NEURONAL CO-DEVELOPMENT IN BIOENGINEERED HEART MUSCLE

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Recent work in our lab showed the ability of human pluripotent stem cells (hPSC) to self-assemble into a force generating bioengineered heart muscle (BHM) with spontaneous co-development of sympathetic neuron-like structures in long-term cultures (day 60). We hypothesized that the neuronal co-development in BHM can be guided by directed differentiation of neurogenesis, resulting in a complete heart-neuron tissue with enhanced contractile performance. BHM was generated from hPSCs (5x105 cells/BHM) under stage-specific directed cardiac differentiation. Mechanical loading was introduced on day 13 followed by defined neuronal induction from culture day 29 onwards. Isometric force measurement was performed on day 60 to assess BHM contractility under defined conditions. Enzymatic dispersion of BHMs was performed to determine total cell count and composition via flow cytometry. Neuronal gene expression was confirmed using qPCR and immunohistochemistry. Previous results revealed that BHMs closely mimic key steps of in utero heart development with the induction of mesoderm, followed by specification towards cardiac lineages. At culture day 60, BHMs subjected to neuronal induction (8 days), neuronal expansion (7 days) followed by neuronal differentiation (14 days), showed improved contractility (maximal force of contraction in mN: 0.4130.05 vs 0.2730.03 for Control; n=26; p=0.06). Flow cytometry results revealed significantly higher survival of cardiomyocytes marked nuero-enhanced BHM group (% α-actinin+ cells: 2233 vs 1332 for Control; n=15; p<0.05). On the molecular level, neuronal markers such as PAX6 and MAP2 were highly upregulated in neuro-enhanced BHM. Detailed morphological studies confirmed MAP2 positive staining, presence of neuronal rosettes and fine neuronal outgrowths in close proximity to networks of striated cardiomyocytes in day 60 neuro-enhanced BHMs. Taken together, our study provides first evidence for the directed organization of human BHM with enhanced neuro-cardiac interface. The engineered co-development of cardiac mesoderm and ectoderm appears to recapitulate the complex process of autonomic innervation of heart muscle during fetal heart development.

F-2086

GENERATION OF SCALABLE MIDBRAIN ORGANOIDS FROM EXPANDABLE STEM CELLS ALLOWS ADVANCED IN VITRO MODELING FOR PARKINSON'S DISEASE

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Models of the human midbrain are critical for understanding human development as well as for identifying and testing novel therapeutics for neurodegenerative diseases such as Parkinson's disease. However, the human midbrain is a complex 3-dimensional structure composed of multiple cell types, including neurons, astrocytes and oligodendrocytes, which has been difficult to model in vitro, particularly in a scalable manner. Here, we describe a novel approach to obtain large numbers of high quality midbrain organoids, derived from our unique midbrain floor plate neural progenitor cells (mfNPCs) that can be differentiated with high efficiency in classical 2D cultures. Using these cells, we report the generation of midbrain-specific organoids containing astrocytes, oligodendrocytes, and midbrain dopaminergic neurons, which can be used for disease modeling. 3D organoid cultures of mfNPCs form functional neuronal networks and secrete dopamine. Importantly, midbrain organoids derived from Parkinson's disease specific stem cells reveal disease relevant phenotypes. mfNPCs, enable research to study human midbrain development, modeling of region-specific neurodegenerative diseases, and high throughput drug screening using organoids.

TISSUE ENGINEERING

F-2088

GUIDED SELF-ASSEMBLY OF SOX2+SOX9+ HUMAN LUNG PROGENITORS INTO SIMPLIFIED DEVELOPMENTALLY INSPIRED TUBULAR ARCHITECTURE

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Self-assembling organoids from human pluripotent stem cells mimic aspects of lung organogenesis. These systems suggest that cellular architecture exerts an effect on cellular differentiation. We used simplified but developmentally relevant architecture to probe this affect on cellular differentiation. The tubular architecture of lung during the pseudoglandular stage of development was simulated using soft lithography to create 100μm diameter tubes with a depth of 127311.2μm in a silicon polymer, PDMS. Lung progenitors (LPs) characterized via expression of NKX2.1, FOXA2, SOX2 and SOX9 formed single-cell lined tubes that were mostly SOX9+ SOX2- (74.632.9%). In contrast, cells cultured for the same period on flat PDMS were predominantly SOX2+SOX9+ (82.632.8%). Cells in larger 400μm tubes were similarly mostly SOX2+SOX9+ (85.733.7%). To ensure that decreased SOX2 expression was not due to architecture-created concentration effects LPs were grown on 100μm PDMS posts and were still primarily SOX9+SOX2-(78.137.2%). Compared to flat, cells in tubes displayed significantly altered cell morphology by reduction in cell elongation (4.530.3μm to 3.330.2μm). Furthermore, in 400μm tubes, cells were found to be like flat with respect to cell elongation (4.630.2μm). We speculate that there was increased cellular tension because there was increased phospho-myosin light chain in 100μm tubes. Pharmacologic manipulation of tension within cells through inhibition of ROCK1 resulted in an increase of SOX2+SOX9+ lung progenitors in 100μm tubes (85.5310.8) as did myosin II activity (blebbistatin) and actin polymerization (cytochalasin D). These data suggest a role in cellular tension driven by ROCK1 and/or actomyosin in driving the loss of SOX2+SOX9+ in tubular cultures. Cells exposed first to tubular architecture and then to proximal or distal airway inducing conditions in transwells gained expression of distal epithelial markers (SP-C, SP-B) but not proximal markers (SOX2, p63, MUC5AC). In contrast, cells from flat culture can express both proximal and distal markers. Simplified, developmentally relevant architecture impacts fate choice during directed differentiation. The role that shape plays requires further evaluation in more complex architecture to further assess its role in differentiation.

Funding Source: CIHR Training Programme in Regenerative Medicine; McLaughlin Centre; The Henry White Kinnear Foundation; Medicine by Design

F-2090

TUNING SUBSTRATE STIFFNESS TO UNLOCK THE PARACRINE POTENTIAL OF MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) have significant therapeutic potential due to their differentiation potential, as well as the immunomodulatory and pro-regenerative effects of their secretome. Cues from the extracellular environment, including physical stimuli such as substrate stiffness, are strong drivers of MSC proliferation and differentiation, but their effects upon

MSC paracrine activity are largely unknown. MSC secretome could form the basis of future MSC therapies and in this study, we proposed to test the effect of substrate stiffness on MSC paracrine signalling. We used a model system of human bone marrow MSCs seeded onto soft (0.6 kPa) and stiff (70 kPa) polyacrylamide gels. RNASeq of MSCs cultured in these soft/stiff substrates showed significant changes in genes involved in actin cytoskeleton, mechanotransduction, cell morphology, and cell movement. Conditioned medium comprised of paracrine factors released by MSCs cultured in soft and stiff substrates were collected. Secretome profiling of these showed significantly increased levels of IL-6 and IL-8 on soft substrates and increased levels of Osteoprotegerin, MCP-1, and TIMP-2 on stiff substrates. The effects of these paracrine factors were tested on MSC proliferation, differentiation and immunomodulatory activities. Conditioned media from MSCs on soft substrates was found to significantly increase adipogenic and osteogenic differentiation as well as macrophage phagocytosis activity compared to stiff substrates. Our results show that differences in physical stimuli alter the content of the MSC secretome and its effects on both differentiation and tissue-regeneration, highlighting that the culture environment should be carefully considered when developing MSC-based therapeutics. Our insights provide a new opportunity to engineer the cellular microenvironment to increase therapeutic potential of MSC paracrine factors and open up the potential for tailor-made secretome therapeutics.

F-2092

AUTOLOGOUS BONE MARROW-DERIVED MESENCHYMAL STEM CELLS ASSOCIATED WITH PLATELET-RICH PLASMA FOR DENTAL IMPLANT OSSEOINTEGRATION: AN EXPERIMENTAL STUDY IN MINIPIGS MANDIBLES

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Tissue engineering has been applied to overcome the obstacles encountered with bone regeneration for the placement of dental implants. Using a minipig model, this study evaluated the effect of autologous bone marrow-derived mesenchymal stem cells (BM-MSCs) associated with platelet-rich plasma (PRP) on bone regeneration for osseointegration of dental implants immediately placed in post-extraction sites in minipigs mandibles. BM-MSCs from 04 male and adult Brazilian minipigs were isolated from the iliac crest and expanded in culture. At surgery, undifferentiated MSCs were trypsinized and centrifuged and the pellet of MSCs was used along the autologous PRP to fill the socket at the experimental side prior to implant placement (10 x 106 cells/ socket). Each animal received 04 implants in the control side and 04 in the experimental side, totalizing 32 implants. The specimens were analyzed radiographically and histomorphologically, determining the implant lost rate (ILR), the bone-implant contact (BIC) and bone density inside the threads (BDIT). The ILR, the BIC and the BDIT in control and experimental sides were respectively 25.0% and 18.7% (p=0.686); 39.0 and 27.7 (p=0.110); 46.8 and 36.5 (p=0.247). The use of BM-MSCs+PRP before the implant placement showed a lower implant loss rate, although there was no significant positive effect on bone regeneration and osseointegration of dental implants in minipigs mandibles.

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F-2094

SEEDING DENSITY AND GLUCOSE CONCENTRATION AFFECT HUMAN DENTAL PULP STEM CELL GROWTH IN POLYCAPROLACTONE ELECTROSPUN SCAFFOLDS

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An optimal relationship between cell density and glucose availability is required in cell cultivation to avoid cell starvation and toxic osmolality levels. However, monolayer cell cultivation protocols have been applied to scaffolds without considering that different metabolic uptake of nutrients may occur in these two conditions. The aim of this work has been to study the effect of seeding density and glucose concentration in human dental pulp stem cell growth in polycaprolactone (PCL)

electrospun scaffolds. Scaffolds of PCL with 15 mm of diameter were produced by electrospinning. 5 x 10E4 (low density, LD) and 1 x 10E5 (high density, HD) cells were seeded onto each scaffold. Meanwhile, 1.25 x 10E4 (LD) and 2.5 x 10E4 cells/well (HD) were seeded on 24-well culture plates. The cultivation was realized with Low-glucose (LG) Dulbecco's modified Eagle's culture medium (DMEM) supplemented with 10 % fetal bovine serum and antibiotics or High-glucose (HG) medium. The cell metabolic activity on various times of cultivation was measured by WST-8 colorimetric test and the cell number was estimated by comparison to a standard curve. Both scaffolds and wells seeded with HD exhibited major cell density on first day of cultivation. With LG medium, the LD samples showed a higher initial growth than the HD ones, with a normalized final cell number for the scaffolds (μs) and wells (μw) of 3.3 and 16.2 (>μs = 0.78 and μw = 8.1, obtained with HD). In the HG medium, higher cell numbers were observed, with μs = 11.7 and μw = 26.3. In addition, the HD groups showed higher cell growth when the HG medium was used (μs = 4.7 and μw =17.7). It can be concluded that all the groups exhibited higher cell numbers on the last day of analysis in HG conditions and that the wells showed a higher proliferation rate than the scaffolds. Even though all the groups exhibited a state of homeostasis during cultivation, the stagnation of proliferation occurred later in the HG than in the LG conditions. These results demonstrate that glucose concentration and cell seeding density are important factors that should be evaluated in order to obtain better in vitro culture conditions.

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F-2096

ESTABLISHING SKELETAL MUSCLE CELL LINES AND DISEASE MODELS USING IMMORTALIZED GENES

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Human primary skeletal muscle myoblasts have a limited proliferative capacity in cell culture and cease to proliferate after several passages. This characteristic of the primary skeletal muscle remains a hurdle in the study of muscle myoblasts and muscle diseases. We examined the effects of several oncogenes on the immortalization and differentiation of primary cultures of human skeletal muscle myoblasts. Retroviruses containing a human telomerase reverse transcriptase (hTERT), SV40 large T antigen(LT) and human papilloma virus type 16 (HPV-16) early function (E6 and E7) genes very efficiently immortalize myogenic cells. Cell lines immortalized by retrovirus maintained a sustained proliferation and a very high differentiation capacity. Cell lines immortalized by each gene were screened for cell lines suitable for muscle research. We have established a muscle disease model using immortalized cell lines. The cell line that we obtained may be a useful tool for increasing our knowledge of the genetic and biochemical events involved in the processes of satellite cells and myoblasts growth and differentiation. Moreover, it appears to be a suitable model for pharmacological and toxicological studies related to muscle diseases relevant to humans.

F-2098

APPLYING HUMAN MESENCHYMAL STROMAL CELLS IN SPRAYS - AN IN VITRO FEASIBILITY STUDY FOR CELL THERAPY

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Cell-based therapies of lung diseases like acute respiratory distress syndrome and chronic obstructive pulmonary disease utilizing mesenchymal stromal cells (MSCs) is increasingly under experimental investigation. Normally, the MSCs are administered intravenously or by intratracheal instillation. A novel approach is to administer the cells intratracheally through aerosol administration. In previous studies, we developed a flexible endoscopic aerosolization device, a combination of a bronchoscope with a catheter and pressurized air, which can be used to administer cells within the lungs. Previously, we sprayed respiratory epithelial cells. No changes in viability and in vitro ciliary development were observed. In this study, we evaluated the feasibility of aerosolizing MSCs using this endoscopic spraying device. Cell viability was evaluated with fluorescent live/dead staining with calcein AM and propidium iodide. Subsequent cell proliferation and cytotoxicity (apoptosis and necrosis) were assessed for up to 6 days. To assess long-term influence of spraying on MSC differentiation, aerosolized cells were cultured for up to 14 days. With flow cytometry, we evaluated if aerosolization leads to changes in expression of mesenchymal-typical markers. Cell viability immediately after spraying was decreased to 70 % compared to non-sprayed cells. Interestingly, laminar tube flow of cells through the catheter decreased cell survival already at the same time point (not significant). Aerosolization changed proliferation and cytotoxicity only within the first days. By means of morphology, spraying did not show a long-term effect on MSCs after aerosolization. A minimal value for aerosolization defined in the literature is a survival rate of more than 50 %, which is easily met with our device. Thus, there is great potential for

this aerosolization approach for intrapulmonary MSC-based cell therapies. This spraying approach can further increase the efficiency of stem cell therapies with high local cell concentrations.

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F-2100

NO MODULATES POSTNATAL MSC MIGRATION

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Nitric oxide (NO) is a diffusible free radical, which serves as a pluripotent intracellular messenger in numerous cell systems. NO has been demonstrated to regulate actin dependent cellular functions, and functions as a putative inductive agent in directing stem cells differentiation. In this study, we investigated the effect of exogenous NO on the kinetics of movement and morphological changes in postnatal bone marrow-derived mesenchymal stem cells (MSCs) in a wound-healing model of cellular migration. Cellular migration kinetics and morphological changes of the migrating MSCs were measured in the presence of an NO donor (S-Nitroso-N-Acetyl-D, L-Penicillamine, SNAP). Two experimental conditions were assessed, in which SNAP (200 μM) was applied to the MSCs. In the first experimental group (SN-1), SNAP was applied immediately following wound formation, and migration kinetics was determined for 24 hours. In the second experimental group (SN-2), MSCs were pretreated for 7 days with SNAP prior to wound formation and the determination of migration kinetics. The displacement curves were further analyzed by non-linear regression analysis. The migration displacement of the controls and NO treated MSCs are best described by a two parameter exponential functions expressing difference constant coefficients. Additionally, changes in the fractal dimension (D) of migrating MSCs were correlated with their displacement kinetics for the three groups. These data suggest that NO may function as a stop migration signal without inducing cell death or apoptosis by disordering the cytoskeletal elements required for cell movement and proliferation.

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F-2102

HLA KNOCKOUT IN HUMAN EMBRYONIC STEM CELLS

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More and more tissues are created in laboratories by differentiating stem cells into various cell types with defined, specific characteristics paving the way for an expansion of cell-based therapies. Unfortunately, allogenic transplantations require donor-host HLA-matching as well as immunosuppressive drugs to reduce the risk of rejection. This is mainly caused by mismatched HLA molecules that are recognized as foreign by the host's T lymphocytes, leading to T cell activation and clearing of the graft. The HLA genes are highly polymorphic transmembrane proteins. HLA class I proteins require -microglobulin for presentation on the cell surface, making it an ideal target for gene editing. Furthermore, upon inflammation in the presence of cytokine interferon gamma (IFN-), cells may also upregulate HLA class II molecules, which are recognized by the host immune system causing rejection of the transplanted tissue. We hypothesized that knocking out a key regulator of HLA class II genes would generate a cell type unable to express HLA class II molecule even in the presence of IFN-. We exploited the CRISPR/Cas9 technology to edit the well-conserved -microglobulin gene and the main regulator Class II Major Histocompatibility Complex Transactivator (CIITA) thereby generating human embryonic stem cell (hESC) lines lacking HLA class I and class II, respectively. To test whether these hESCs were incapable of presenting HLA molecules on the cell surface we sought to characterize different single cell derived-hESC clones to determine if the induced mutation had led to a protein knockout through western blot, immunohistochemistry, qPCR and Fluorescence-Activated Cell Sorting (FACS). The resulting genome editing demonstrated that HLA class I surface presentation had been abrogated by the introduced mutation. Next, we are characterizing the potential HLA class II knockout hESC clones and seeking to generate a double-knockout line as well as investigate their potential to differentiate into different cell types as this could have an impact on future biomedical applications.

F-2104

APPLICATION OF THE PLATELET-RICH FIBRIN SCAFFOLDS FOR ENGINEERING THE CHONDROCYTES DERIVED FROM CARTILAGINOUS GRAFT FOR CARTILAGE REGENERATION

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Cartilage injury is a common problem of clinical practice because cartilage is important for many anatomical structures within human body. Cartilage is an avascular tissue with limited capacity of self-repair, these chondral lesions are prone to continuously progress and result in osteoarthritis. To prevent further degeneration of articular surface, many treatments have been developed to promote cartilage healing. The scaffolds composed of platelet-rich plasma are promising in enhancing the healing process by offering growth factors to nourish cells. It has been applied for recruiting cell populations for cartilage repair process. Platelet-rich fibrin (PRF) scaffold is a second generation of platelet concentrate produced from autologous blood because there is no need of anticoagulant supplement and immediate centrifugation for PRF activation, and accordingly can be used as a fibrin biomaterial containing a high concentration of growth factors. The effects of PRF on cartilage regeneration were evaluated both in vitro and ex vivo by engineering the chondrocytes derived from autologous cartilage graft. It was found that the PRF improved the chemotaxis, proliferation, and viability of the cultured chondrocytes. The gene expression of the chondrogenic markers, including type II collagen and aggrecan, revealed that PRF induced the chondrogenic differentiation of cultured chondrocytes. PRF increased the formation and deposition of the cartilaginous matrix produced by cultured chondrocytes. The efficacy of PRF on cell viability was comparable with that of fetal bovine serum. In animal disease models, morphologic, histological, and objectively quantitative evaluation demonstrated that PRF combined with cartilage granules was feasible in facilitating chondral repair and regeneration. It was concluded that the migration, proliferation, viability, and differentiation of chondrocytes could be enhanced by PRF. The data altogether provide evidence to confirm the feasibility of applying PRF scaffolds to engineer chondrocytes derived from autologous cartilage graft for cartilage repair and regeneration.

F-2106

METABOLIC CUES FOR STEMNESS MAINTENANCE

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Stem cells are characterized by their 'stemness', which is their capacity to reproduce themselves indefinitely (self-renewal) and to differentiate into specialized cell types (pluripotency). Bone marrow is the most easily accessible source of mesenchymal stem cells (MSCs). The ability to develop into terminally differentiated phenotypes on reception of appropriate signalling cues makes bone marrow-derived MSCs (BMSCs) attractive candidates for tissue engineering strategies. However, ex vivo expansion of BMSCs has met with limited levels of success as the cells tend to lose their 'stemness', especially after 20 to 40 population doublings. Our present study utilised a cell culture cocktails with a combination of fibronectin (FN), fibroblast growth factor 2 (FGF2) and bone morphogenetic protein 4 (BMP4) to manipulate the microenvironment which showed a profound effect on the expression patterns of the stemness markers including Sox2, Oct4 and c-Myc. We further investigated cellular metabolism, an important but relatively less explored area related to 'stemness', using gas chromatography mass spectrometry (GCMS) approach. Our results revealed a significant increase of intermediate metabolites and analytes in the metabolome, such as L-proline and L-threonin, indicating the metabolite profiles can sensitively reflect the changes in the levels of genes/proteins that differentially expressed between treated and untreated groups. It suggests that the manipulation of microenvironment can lead to a more primitive cell state and investigations into the underlying molecular mechanisms and metabolic pathways hold great therapeutic promise for tissue regeneration and regulation of ageing, as well as novel strategies to combat degenerative disorders.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

F-2110

COMMUNICATION TRIALS BETWEEN THE PUBLIC AND THE SCIENTIFIC COMMUNITY CONDUCTED BY THE JAPANESE SOCIETY FOR REGENERATIVE MEDICINE

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Successful generation of human induced pluripotent stem cells (iPSCs) in 2007 gained significant public attention in Japan. Subsequently, news regarding research on iPSCs and iPSC-derived products captured the media limelight. Interest in iPSCs has influenced both the public and scientists. Terms such as iPSCs and regenerative medicine (RM) are used commonly, and public support for stem cell research (SCR) and RM has increased. However, the strength of expectations does not necessarily lead to the accuracy of knowledge, but rather the proof of scientific effects is inadequate, and there is even the possibility of becoming a hotbed for actions suspected of securing safety. Since scientifically accurate and adequate information is not always available, for example, regarding self-entitled "safe stem cell therapy clinics," Lau et al. (2008) indicated that patients availing such treatments are at risk. On the other hand, consultations on practitions about private clinics doing regenerative medicine and cell transplantation were distributed to various public institutions such as MHLW, AMED, various universities, etc. and could not be integrated in Japan.Therefore, the Japanese Society for Regenerative Medicine (JSRM) conducted a survey to investigate the attitudes of scientists and citizens in order to know what kind of scientists are focusing on communication with the public for shareing appropriate information on stem cell research and regenerative medicine. In addition, JSRM established a telephone consultation window for receiving various consultations on regenerative medicine this year. Through this survey, we were able to clarify the difference between "want to know" and "want to know" existing between ordinary people and scientists. In a knowledge-based society, communication concerning science and technology is an important issue when it comes to establishing appropriate levels of trust between experts, the public, and other stakeholders. In this presentation, I would like to point out the importance of information sharing that the expert group conducting innovative medical research should fulfill to society, introducing the communication activities on regenerative medicine carried out by JSRM.

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CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

F-2114

POTENTIAL ANTI-INFLAMMATORY MECHANISM OF ACTION OF HUMAN MESENCHYMAL STROMAL CELLS IN KNEE OSTEOARTHRITIS PATIENTS RESULTS IN OVERALL IMPROVEMENT IN PAIN AND SYMPTOMS

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We initiated the first Canadian clinical trial using autologous bone marrow-derived mesenchymal stromal cells (MSCs) to treat 12 patients with Kellgren-Lawrence III/IV knee osteoarthritis (KOA). Patients (mean age of 56, range 46-65; 7/12 male) received 1, 10 or 50 million MSCs in a single intra-articular injection without any

adverse events. Overall, patients had improved disease-specific Knee Injury and Osteoarthritis Outcome Score (KOOS) and Western Ontario and McMaster Universities Osteoarthritis (WOMAC) scores at 12 months, relative to baseline (p=0.023 for pain, p=0.007 for symptoms, p=0.042 for function in daily living, p=0.012 for quality of life, p=0.092 for sports/recreation and p=0.042, 0.013 and 0.003 for WOMAC function, pain, and stiffness, respectively). Individual analyses show that 8/12 patients had a Minimal Clinically Important (MCI) change of 10 points relative to baseline in pain and symptom indices at 12 months. This clinical improvement appears to correlate with interim MRI analysis of resolution of synovial inflammation suggestive of anti-inflammatory mechanism of action of MSCs. Full 12-month MRI analyses are pending. There was a decrease in levels of pro-inflammatory monocytes/macrophages in the synovial fluid at 3-month post-MSC injection relative to baseline, concomitant with increased levels of PGE2 and decreased levels of IL12p40, suggestive of MSC-mediated inflammation resolution. Analyses of 12 autologous MSCs showed elevated gene and protein expression of anti-inflammatory markers that correlated with clinical patient responses suggesting that donor heterogeneity is partially responsible for the observed clinical differences. In conclusion, autologous MSCs result in significant improvements in overall KOOS and WOMAC scores at 12 months. A dose of 50 million MSCs resulted in better outcomes based on individual patient analyses. For the first time, we show that MSC injection resulted in less inflammatory synovial environment, which correlated with patient reported outcomes.

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F-2116

EVALUATION OF INTRA-ARTICULAR ADIPOSE DERIVED MESENCHYMAL STEM CELL THERAPY IN THE TREATMENT OF SYMPTOMATIC KNEE OSTEOARTHRITIS - A RANDOMISED CONTROLLED TRIAL (PRELIMINARY OUTCOME)

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Osteoarthritis is a degenerative condition and a leading cause of pain and disability. Current medical treatments are aimed at symptomatic control and do not achieve disease modification. Surgical interventions including total joint arthroplasty can be unpredictable. Pre-clinical and early clinical trials have shown initial evidence of safety and efficacy of mesenchymal stem cell (MSC) therapies. We present the results of a randomised controlled trial on the use of autologous adipose derived MSCs (AdMSC) in the treatment of symptomatic knee osteoarthritis. We intended to enroll and randomise 40 participants with symptomatic and radiological confirmed knee osteoarthritis unresponsive to conservative treatments into 4 groups of 10 participants. The 3 treatment groups included treatm6nt with autologous AdMSCs by intra-articular injection of a single 100×10^7 MSCs, two injections of 100×10^6 (baseline and 6months) and five injections for 40×10^6 (baseline, 1month, 2 month, 3mnth and 6month) respectively. The control group continued conservative management. Clinical outcome was observed over a period of 12 months and included validated pain and functional outcome scores such as the Numerical Pain Rating Scale and Knee Injury and Osteoarthritis Outcome Score. Structural outcome was assessed using Magnetic Resonance Imaging quantitative assessments including T2mapping, cartilage volume and a modified International Cartilage Repair Society Score. The treatment groups receiving a single and two injection protocol respectively and the control group were equally assigned 10 participants. The treatment group receiving five injections of 40×10^6 was ceased due to observed reproducible lack of treatment tolerability. Participants in the treatment groups receiving a single or two injection protocol showed statistical and clinical significant pain and functional improvement in comparison to control until completion of follow-up at 12months. Autologous AdMSC therapy resulted in significant pain and functional improvements. AdMSC therapy appears to be a safe and potentially effective therapy for symptomatic knee osteoarthritis and may also delay disease progression. Importantly it appears injection protocols may determine tolerability of this treatment.

Funding Source: Magellan Stem Cell provided laboratory services for the isolation and expansion of AdMSCs Melbourne Stem Cell Centre provided clinical support for the running of the trial.

F-2118

ENDOMETRIAL REGENERATIVE THERAPY WITH eMSCS IN REPETITIVE IMPLANTATION FAILURE INCREASES CLINICAL PREGNANCY RATE IN VITRO FERTILIZATION

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Autologous stem cell treatment is a promising cellular therapy in reproductive medicine. Considering that the MSCs have properties of angiogenesis and can promote wound healing, our objective was to test endometrial parameters using endometrial mesenchymal stem cells (eMSCs) therapy in patients with Repetitive Implantation Failure (RIF). We selected 30 patients with RIF resistant to estrogen and evaluated their progress before and after the eMSCs treatment. Endometrial parameters were evaluated by flow cytometry (LB, NK, CD4+ and CD8+), pathological anatomic analysis and endometrial thickness (ET). Data was statistically analyzed using the Paired Dichotomic Data Chi-Square McNemar Test and Wilcoxon Test. From each patient we isolated and cultured *in vitro* MSCs cells obtained by biopsy. At confluent stage, the cells were characterized using flow cytometry (CD34, CD45, CD73, CD90, CD105, CD19, and HLA-DR). When the cell population reached passage 4 and when flow cytometry results showed less than 0.3% for CD34, CD45, CD19 and HLA DR and more than 99.9% for CD73, CD90, and CD105 markers, they were transferred using a transvaginal-transmyometrial technique under ultrasound guidance using Towako transfer set. Results obtained showed that there were significant differences in all endometrial parameters studied before and after eMSCs treatment. Flow Cytometry data for LB, NK, CD4+ and CD8+ cells shows p values of 0.0021, 0.0039, 0.0022 and 0.004 respectively. Regarding endometrial thickness values of 5.64 vs. 7.49 mm, p=0.000156 and 7.25 vs. 10.48 mm, p=0.000003 were obtained both in unstimulated and overstimulated patients in 14-16 day of the cycle. Finally, we observed clinical pregnancy in 66% (20/30) of studied cases and a live birth rate of 46% (14/30). Beyond these promising results, more studies are necessary to evaluate the role and effects of eMSCs-treatment in RIF patients.

Funding Source: Our project was subsidized by Tersoglio Foundation and private contributions.

F-2120

LAMINATED GRAPHENE LAYERS PROMOTE OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS

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This study was conducted to evaluate the effects of laminated graphene layers on osteogenic differentiation of human adipose-derived mesenchymal stromal cells (hADSCs). Using a chemical vapor deposition technique, the laminated graphene layers were synthesized on the cell culture slide glass. Image of scanning electron microscope showed the increment of laminated graphene layer number, which accompanied with forming of 30 to 50 nanometer width wrinkles on the graphene surface. Three primary hADSC lines subpassaged different times were provided for monitoring osteogenic differentiation, which confirmed by an alizarin-red staining and real-time RT-PCR. PLOC-GLM in SAS program was employed for statistical analysis. Osteogenic differentiation of the hADSCs were significantly ($p<0.001$) enhanced on the graphene layers than compared with on no layer (control) on the significantly enhanced when they were cultured on the graphene layers. As the graphene layer number increased, the induction greatly enhanced. Reatime RT-PCR detected upregulated expression of osteogenesis-related genes such as runx2, osteocalcin. Significant increase in runx2 and osteocalcin expression in the cells being cultured on the laminated graphene group compared with the control. ($p<0.0007$ for runx2, $p<0.0001$ for osteocalcin). These results suggest that laminated graphene layers may promote osteogenic differentiation of hADSCs.

Funding Source: This work was supported by the BK21 plus program (Seoul national university, Department of Agricultural Biotechnology) through the National Research Foundation (NRF) funded by the Ministry of Education of Korea

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

F-2122

SINGLE-CELL TRANSCRIPTOMIC ASSESSMENT OF STEM CELLS RE-ISOLATED FROM MURINE EMBRYOS TO DETERMINE THE INFLUENCE OF MICROENVIRONMENTS ON STEM CELL DEVELOPMENTAL TRAJECTORIES

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Stem cells representative of the trophectoderm (TE; TSCs) and inner cell mass have made it possible to investigate processes governing embryogenesis in vitro. We previously used these models to investigate the role of microRNAs in cell fate specification resulting in the derivation of a new, mural TE-like cell type (mTELCs, Nosi et al., 2017). These cells exhibit a reduced differentiation capacity in vitro, yet are able to follow their expected developmental trajectory in vivo. Chimeric embryos were generated by injecting fluorescently labeled TSCs or mTELCs into morulae. A subset of embryos were screened at the blastocyst-stage to assess cell contribution potential. Remaining embryos were transferred into pseudo-pregnant female mice to allow further development in vivo, and dissected at 6.5dpc. Both TSC and mTELCs contribute exclusively to the TE monolayer of blastocysts. In post-implantation embryos, TSCs predominantly contribute to the extra-embryonic ectoderm (ExE) compartment with low-frequency contribution to the Reichert's membrane (RM)-comprised of trophoblast giant cells deriving from the mural TE, whereas mTELCs only localized to the RM. The RM-containing epiblast (Epi) was micro-dissected away from the ExE of 6.5dpc embryos, generating two fractions. Single cell suspensions were made from these fractions which were further analyzed by flow cytometry. In TSC-injected embryos, fluorescent cells were enriched in the ExE fraction and not the Epi, whereas embryos injected with mTELCs revealed a higher proportion of fluorescent cells in the Epi compared to the ExE fraction. We are currently performing single-cell transcriptomic analysis of TSC and mTELCs "re-isolated" from embryos. The profile of these embryo-isolated cells will be compared to those of TSCs and mTELCs cultured in stem cell promoting or differentiating conditions prior to embryo injections. This in-depth interrogation of mTELCs aims to unravel fundamental mechanisms of embryo implantation mediated by the complex interaction between the mural TE and endometrium. Further, we aim to understand how the microenvironment drives differentiation of TSCs and mTELCs down their appropriate developmental trajectories. These results will have wider implications on how closely in vitro conditions accurately mimic in vivo environments.

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F-2124

SOX17 AND BLIMP1 EXPRESSION IN GERM LINE PRECURSORS OF THE RABBIT EMBRYO IN VIVO

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The germ line segregates early from the somatic cell lineage during embryonic development in all animal phyla in the form primordial germ cells. In mammals a combination of signals from extraembryonic and embryonic tissues specifies the area where the differentiation of primordial germ cell (PGC) precursors is initiated. Most molecular details of this process have been uncovered in the mouse and some of the key players were detected in other mammals as well. In the human, the Sry-related transcription factor Sox17 appears to be a key regulator during PGC specification at least in vitro and acts upstream of Blimp1; intriguingly, this seems not to be the case in mice. To see whether Sox17 is an early germ cell marker in other mammals as well we analyzed its expression in the developing rabbit embryo in vivo. A comparison of Blimp1 and Sox17 expression in in-situ-hybridized embryos revealed matching distribution of positive cells in the peripheral epiblast at the posterior margin of the embryonic disc, which - according to our previous growth factor expression analysis for BMP2 and BMP4 - may also be considered to belong to extraembryonic parts of the embryo. We hypothesize that - similar to the situation in man - Sox17 may indeed be expressed in precursors of rabbit primordial germ cells. Whether Sox17 is acting upstream of Blimp1 in the rabbit as well will be subject of future experiments.

F-2126

EFFECT OF GDF8 DURING IN VITRO PRODUCTION OF PORCINE CHIMERIC EMBRYO

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Since the last decade, the studies for generation of chimeric organs have been advanced remarkably with the production of chimeric animals using pluripotent stem cells (PSCs). However, the distribution rate of

injected cells into an embryo is still low, and this is considered as one of the major problems toward the effective production of chimeric animal. The purpose of this study is to investigate the effect of growth differentiation factor 8 (GDF8) on porcine chimeric embryo development. Prior to experiment, we established mCherry-tagged porcine iPSCs (mCh-iPSCs). The mCh-iPSCs were injected into 8 cells or morula stage of in vitro fertilized (IVF) embryos using micromanipulator. The manipulated chimeric embryos were cultured porcine embryonic stem cell medium (DMEM F10/low glucose with 15% FBS) for 18h and then transferred to fresh porcine zygote medium 5 (PZM5 with or without 150 pg/mL of GDF8). To investigate the GDF8 supplementation during in vitro culture (IVC) of chimeric embryos, we were accessed their blastocyst formation rate, total cell number and injected cells distribution ratio and SOX2 gene expression levels. After day 4 of IVC of the chimeric embryo, the GDF8 supplement group was shown significantly higher blastocyst formation rate (19.3%31.9 VS 13.0%31.7) than the control group. Using the blastocyst chimeric embryos, we evaluated distributions of the injected cells by immune staining of SOX2 as porcine inner cell mass (ICM) marker. Although no significantly different SOX2 gene expression was detected between GDF8 and control, the GDF8 supplement group was shown significantly increased SOX2 per mCherry expression cells ratio than control (49.2%312.8 VS 20.7%38.2). In conclusion, the supplementation of GDF8 during IVC of porcine chimeric embryos improved developmental competence and enhanced the distributions of injected cells into ICM in chimeric embryos at the pre-implantation stage.

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F-2128

CSRP2BP PLAYS AN IMPORTANT ROLE IN REGULATING HUMAN EMBRYONIC STEM CELLS DIFFERENTIATION

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The molecular mechanism of human embryonic development is complex and sophisticated. Many efforts have been devoted to characterizing the underlying mechanism of early embryonic development, but it is still remained poorly understood. CSRP2BP is a histone acetyltransferase. Previous studies showed that disruption of CSRP2BP expression in mouse lead to embryonic lethality. However, there is no report about its role in development of human embryo. Our current data revealed that CSRP2BP plays an important role in regulating embryogenesis. CSRP2BP influences cell cycle, germ layer differentiation, and the formation of embryonic bodies of human embryonic stem cells (hESC). Knockdown, overexpression and knockout of CSRP2BP in hESCs were generated by lentivirus and CRISPR technologies to study the function of CSRP2BP in human embryonic development.Embryonic bodies were used to study the effects of knockdown, overexpression and knockout of CSRP2BP on hESCs pluripotency and differentiation. Flow cytometry was used to examine the cell cycle. Western Blot, CHIP were used to find out Histone and binding site of CSRP2BP in human embryonic stem cells. RNA-seq was used to examine the gene expression panel which might be regulated by CSRP2BP. We demonstrated that CSRP2BP was significantly enriched in hESCs. CSRP2BP had an impact on the differentiation and pluripotency of hESCs, especially affecting its differentiation into mesoderm. Meanwhile, CSRP2BP specifically acetylated histone H3 in hESCs. Although we show that CSRP2BP is very important in maintaining normal human embryo differentiation, the understanding of working mechanism of CSRP2BP in hESCs is still very limited. More studies will be performed to explore its underline mechanism.This study was the first time to explore the role of CSRP2BP in hESC. Our work will help to increase understanding of early human embryonic development.

TECHNOLOGIES FOR STEM CELL RESEARCH

F-2130

DEVELOPMENT OF A FEEDER-FREE PSC CULTURE SYSTEM ENABLING TRANSLATIONAL & 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 existing cGMP-manufactured passaging reagents, permitting routine clump and single cell passaging methods. Upon expansion, PSCs are shown to maintain normal PSC properties, including morphology, pluripotency, karyotype, and trilineage differentiation potential. Additional information on applicability of scale-up strategies in adherent culture using high capacity cell factories is also provided. Together this system provides a consistent, feeder-free PSC culture medium for translational and clinical research.

F-2132

GENERATION OF FLOW CYTOMETRY IDENTITY STANDARDS FOR HPSCS AND MSCS

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The National Institute for Biological Standards and Control (NIBSC) is a global leader in the characterisation, standardisation and control of biological medicines. NIBSC aims to assure the high quality of biological medicines worldwide, including Advanced Therapy products. There is an urgent need for standards to assess production and safety issues. This need is especially evident at the level of cell-based products, such as Pluripotent Stem Cells (PSCs) and their derivatives (e.g. Mesenchymal Stem Cells (MSCs)).Flow cytometry is widely used as an analytical technique to examine and identify different cell types based on the expression of cell surface and intracellular molecules. Conventionally, beads are used to calibrate a flow cytometer's laser source, optics, and stream flow but these are not suitable as a cell identity profile standard, nor can be used to check antibody specificity. Supplementary, different cell control samples are normally analysed in parallel to identify nonspecific antibody binding. We have been developing controls to reliably monitor cell-based products variability and quality. To do so, we have used the NIBSC8 human PSC (hPSC) line as a high quality starting material for assessing heterogeneity of other pluripotent cell cultures as well as to derive MSC-like cells. This line was initially cultured in pluripotency conditions and lyophilised at one million cells per vial. The vials were reconstituted and tested for specific stem cell markers at various time points over a year to determine stability. The vials were also sent to various groups for external testing. The data collected revealed long term stability and stable cell population profiles that were similar to fresh PSC samples, thereby confirming the suitability of the use of these lyophilised samples as flow cytometry PSC controls. Subsequently, the same procedure was tested for hPSC-derived MSCs. These samples were also reconstituted in house and distributed to external groups and, in this case, tested for specific mesenchymal stem cell markers. . Preliminary results also show suitability of these samples as flow cytometry standards. In summary, we discuss the development, production and testing of hPSC and MSC standards for flow cytometry. Currently, the standards are to be used an identity test specifically for hPSCs and MSCs.

F-2134

COMPARISON OF THE EFFECT OF EXTRACELLULAR VESICLES (EVS) ISOLATED FROM HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS IN A TRADITIONAL 2D ADHESION CULTURE VERSUS A 3D HUMAN PLATELET LYSATE GEL MODEL

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Mesenchymal stromal cells (MSCs) play a role in wound healing. However, the mechanism of action is unclear and perhaps is mediated in part by MSC-derived extracellular vesicles (EV's) rather than direct cell-cell contact. Recent reports indicate that EV's isolated from MSC's in a 3D spherical culture possess different healing properties than EV's isolated from MSC's grown in 2D culture conditions. Therefore, the effect of MSC EVs isolated form 2D culture was compared to EV's isolated from

MSCs grown in 3D culture on the proliferation of Human MSCs. EVs were isolated by ultracentrifugation from media conditioned for 24 hrs by 3 MSC lines cultured under 2D or 3D conditions. The EV were characterized using Nanosight, the Nanodrop, TEM, and BCA Assay. The effects of 2D vs 3D EVs on MSC proliferation was compared using MTT Assay. EV were similar in size (average population approximately 150 nm) and protein content (ng/ml) across 2D vs 3D samples. MTT assay shows 2D EVs appeared to have a greater inhibition of MSC proliferation compared to 3D EVs. This preliminary data suggests that cell culture conditions may affect the physiological properties of EVs isolated from MSCs and that 2D EVs inhibit MSC proliferation more than EVs from 3D culture.

F-2136

IMPROVED CRYOPRESERVATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS VIA ADHERENT VITRIFICATION: A DUAL-CENTER STUDY

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Human induced pluripotent stem cells (hiPSCs) are an important tool for regenerative medicine, basic scientific research, disease modeling, and compound testing. Efficient cryopreservation is a major challenge in stem cell research. Conventional cryopreservation via slow-rate freezing in suspension results in low recovery rates of hiPSCs. We tested whether vitrification, an efficient cryopreservation by ultra-fast cooling rates by immersion in liquid nitrogen, increases post-thawing viability in a selection of hiPSCs form Parkinson's disease and control. A surface-based vitrification approach of hiPSCs avoids enzymatic cell detachment prior to cryopreservation, prevents dissociation of colonies and hence reduces post-thawing recovery time. Adherent vitrification was previously shown for human embryonic stem cells (hESCs) using the so-called TWIST substrate, that combines culture, vitrification, storage, and post-thawing cultivation in a single device. This work represents a dual-center study and compares adherent vitrification on the TWIST substrate as an improved cryopreservation approach for hiPSCs to conventional slow-rate freezing in suspension. Fluorescence-activated cell sorting (FACS) analysis and immunocytochemistry (ICC) for pluripotency markers were performed. FACS analysis of the surface marker Tra-1-60 showed significant increase of surviving hiPSCs after adherent vitrification. Moreover, vitrified hiPSCs showed higher post-thawing viability and preserved confluency on the TWIST substrate. Experiments were further performed using small molecule neural progenitor cells (smNPCs), to test the applicability of adherent vitrification for hiPSC-derived cells . These data suggest that adherent vitrification is an improved cryopreservation technique for hiPSCs and derivatives, compared to the state-of-the-art slow-rate freezing in suspension.

F-2138

IDENTIFICATION OF SMALL MOLECULES THAT PROMOTE CELL PROLIFERATION UNDER 3D CELL CULTURE CONDITIONS

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Three-dimensional (3D) cell culture methods are expected to provide an environment closer to the in vivo situation and elicit more physiological cellular behavior than two-dimensional (2D) monolayer cell cultures do. The 3D methods are widely applied to culture stem cells or organoids. However, cells cultured in 3D cell culture systems almost always show poorer proliferation compared to 2D monolayer cell culture systems, which causes practical problems in cell based research or assay systems. To solve this issue, we have recently developed FCeM, a novel 3D cell culture medium using natural polymer gellan gum, which is able to disperse cells in medium without increasing viscosity and elasticity. We found that gellan gum built network structure in medium, which led to keep single cells and spheres dispersed while suppressing excessive cell aggregation. In this study, we have performed a phenotypic high throughput screening using FCeM for human ovarian cancer cell line SK-OV-3 to identify small molecules that promote cell proliferation under 3D cell culture conditions. As a result of screening, we obtained several desired small molecules named GA-00X series. They promoted the proliferation of not only tested cancer cells but also human mesenchymal stem cells when added to FCeM. They also showed growth-promoting effect in various 3D culture methods (e.g.

round bottom low attachment microplate and hanging drop method). In conclusion, we identified GA-00X series as a consequence of high throughput screening that employed 3D cell culture medium FCeM. GA-00X series promoted the proliferation of various cells in wide variety of 3D culture systems. 3D cell culture methods using GA-00X series will contribute to development of novel and efficient technologies in the field of stem cell culture.

F-2140

DEVELOPING AN ECTOPIC TISSUE FOR SYSTEMIC DELIVERY OF BIOLOGICS TO TREAT MULTIPLE SCLEROSIS

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Many human diseases will be treatable with cell-based regenerative medicine. However the widespread adoption of this treatment approach requires a cost-effective, off-the-shelf product that is safe and has been proven efficacious in both experimental models and clinical trials. To address some of these issues, we are creating "designer" therapeutic cells that have been engineered with novel functions and can be customized for specific diseases or applications. Here we take advantage of two proprietary technologies developed in the Nagy lab in Toronto: the FailSafe system, which enables the establishment of a long-term and stable ectopic tissue under the skin of mice through selectively eliminating the proliferating component of a teratoma, and induced allograft tolerance, which permits survival of the ectopic tissue in MHC-mismatched recipients (C. Monetti and J. Harding abstracts, respectively). By incorporating expression of drug-inducible transgenes into mouse embryonic stem cells, we show that terminally differentiated cells forming a dormant ectopic tissue can serve as a "factory" for the controlled and sustained release of a single or multiple disease-modifying biologic(s). As proof-of-concept in a disease-specific setting, we are employing functional bioassays and experimental mouse models of multiple sclerosis (MS) to assess biologics that block inflammatory cytokines essential to the development and progression of autoimmune-mediated demyelination. We are further combining these anti-inflammatories with biologics targeted towards enhancing endogenous pathways for central nervous system repair and regeneration. Importantly, our approach not only ensures the engraftment of transplanted cells and sustained in vivo production of the biologics, but also permits an assessment of efficacy across the diverse mouse strains that are used to model different pathogenic mechanisms underlying MS.

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F-2142

A MICROORGANISM EXTRACT REGULATES CHONDROGENIC DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS

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Since dental pulp stem cells (DPSCs) have multipotency, high proliferation rates, and accessibility, these stem cells are one of the attractive sources of adult stem cells for therapeutic purposes. There are various microorganisms in a human gastrointestinal tract. Some microorganisms enhance human health and immunity. There are several studies reporting that microorganism extracts affect adipogenic and osteogenic differentiation. However, little is known the effect of the microorganism extracts on chondrogenic differentiation. In this study, the effect of microorganism extract on chondrogenic differentiation of DPSCs was investigated. Microorganism extract was prepared by froze and dried method. The effect of microorganism extract on cell viability at various concentrations was assessed. Micromass and pellet culture were used to induce chondrogenic differentiation of DPSCs for 14 days. The culture medium was changed every 2-3 days with 50 μg/ml microorganism extract. The differentiation abilities were examined by qRT-PCR, alcian blue staining, and glycosaminoglycan (GAG) assay. The expression level of early (SOX9, COL2A1, and ACAN) and late (COL10A1, RUNX2, and MMP13) stage chondrogenic marker genes in microorganism extract-treated (MET) group were significantly higher than that in control group for 10 and 5 days, respectively. MMP13 is also known as a member of proteases that degrade the various components of the extracellular matrix (ECM). Interestingly, the expression level of MMP13 was decreased in MET group when compared with the control group from Day 7 to 14. Low level of MMP13

gene in MET group represents that chondrogenic ECM is maintained by microorganism extract. In addition, alcian blue staining intensity and GAG contents in MET group were significantly higher than that in all control group. In conclusion, our data demonstrate that the treatment of microorganism extract enhances chondrogenic differentiation of DPSCs.

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F-2144

REAL-TIME MONITORING OF GLUTATHIONE IN LIVING CELLS REVEALS A REQUIREMENT OF ITS HIGH LEVELS FOR MAINTAINING STEM CELL FUNCTION

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Glutathione is the most abundant non-protein thiol in cells, functioning as an antioxidant and a redox regulator. Under oxidative-stress conditions, decrease of glutathione levels elicits the activation of redox signaling pathways that alters cellular functions. However, lack of probe for monitoring of glutathione levels in living cells hindered the mechanistic investigation of its role in the pathogenesis of various diseases. Here, we demonstrated that cyanoacrylamide-based coumarin derivatives (Fluorescent real-time thiol tracer; FreSHtracer) reversibly react with glutathione, and their conjugates exhibit spectral shifts and concentration-dependent increases of fluorescence intensity that enable real-time ratiometric measurement of glutathione levels in living cells. Confocal fluorescence microscopy images revealed heterogeneity in glutathione levels in subcellular compartments as well as in the cell population. Real-time monitoring of glutathione in cells treated with hydrogen peroxide showed dynamic changes of its levels in subcellular organelles. Moreover, a subpopulation of stem cells with higher glutathione levels fractionated by flow cytometry exhibited increased activities of self-renewal and cell migration, and showed improved therapeutic ability in an experimental asthma model. Thus, FreSHtracer is a powerful tool to monitor glutathione dynamics at subcellular levels which could advance our understanding of cellular responses to oxidative stress in living cells.

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F-2146

DONOR AGE DEPENDENCE STUDY WITH A NOVEL STRATEGY TO LABEL MOUSE DERIVED MESENCHYMAL STEM CELLS IN VITRO AND IN VIVO USING DENDRIMER NANOPARTICLES IN HUNTINGTON'S DISEASE

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Mesenchymal stem cells (MSCs) are multipotent stem cells known to have potential therapeutic effects for Huntington's disease (HD), a fatal late-onset neurodegenerative disorder caused by the degeneration of medium spiny neurons in the brain. Our previous study showed that the therapeutic effects of bone-marrow MSCs (BM-MSCs) depends on numbers of passages prior to transplantation. We have shown that higher-passaged (HP) MSCs (P40 to P50) delayed the onset of motor, cognitive, and neuropathological loss in HD mice, due to the release of brain-derived neurotrophic factor (BDNF). However, there are adverse effects associated

with HP-BM-MSCs (i.e, chromosomal abnormalities and an unacceptable safety profile) restricting its clinical utility. Given this, the present study looked at 2 critical aspects - the donor age and the passage number of the BM-MSCs in the context of alleviating motor deficits in R6/2 HD mice. Our findings indicate, once again, that HP-BM-MSCs had the best behavioral outcomes, however, the cells obtained from 5-week- old mice alleviated the motor deficits in HD more effectively than did the BM-MSCs from 10 months old mice. Our previous study also identified the biomarker profile of HP-BM- MSCs sub-population (at P45) that is therapeutic to HD mice. The current study was also directed towards obtaining the same therapeutic biomarker profile of HP-BM-MSCs sub-population in a lower passage (LP) BM-MSCs by selecting and sorting the cells for various markers. Our results showed that our sorted MSCs achieved the HP-BM-MSCs therapeutic biomarker profile at P13 (instead of P45) thereby making them safer to be used for in vivo rodent transplantations and clinical trials. In addition, our current work with the fluorescently tagged G4 PAMAM dendrimer nanoparticles (~4nm) have shown that dendrimers can be a good candidate for safer bio-labeling. Following their uptake by the cells, we showed that the BM-MSCs were still able to proliferate and differentiate, proving that the dendrimers do not compromise the stemness of MSCs. Moreover, we also transplanted the Cy5.5 tagged dendrimer MSCs into C57BL/6J mice and were able to track them in the brain using in vivo imaging system. Therefore, our results proved that dendrimers could be an alternate method to label and track the best suitable MSCs for treating HD.

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F-2148

HIGHLY EFFICIENT TRANSFECTION OF HUMAN PLURIPOTENT STEM CELLS USING PEPFECT14 FOR TARGETED GENE SILENCING

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Human pluripotent stem cells are an invaluable tool to investigate the basic developmental and cellular processes. In addition, their potential in cell therapy, drug discovery and disease modeling is highly appreciated. Though human embryonic stem cells have been used in research for years, their modulation using targeted gene expression is hard to achieve and is often with low efficiency or high toxicity. Here we report a novel approach for low-cytotoxicity highly efficient gene silencing using RNAi and cell-penetrating peptide. By using PepFect14 and fluorescently labeled siRNA we have achieved the transfection efficiency of 90%. In addition, treatment of hES cells with PepFect14 and OCT4-specific siRNA resulted in loss of OCT4 expression in 80% of cells, whereas the control siRNA with PepFect14 had no effect on OCT4 expression, as confirmed by flow cytometry, immunofluorescence and RT-qPCR. In other experiments, the transfection of B2M-specific siRNA with PepFect14 resulted in 50% downregulation of B2M as confirmed by flow cytometry and RT-qPCR. Although with some drawbacks, the use of PepFect14 and siRNA shows great promise in modulating human embryonic stem cell gene expression and may be applicable to other cell types of high developmental potential.

F-2150

CHARACTERISING STEM CELL NICHES IN TISSUE SECTIONS USING FLUORESCENCE MULTIPLEXING AND SPECTRAL UNMIXING

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The importance of stem cell niches are well established, however there has been less success in the characterization of niche(s) responsible for the regulation of stem cell behavior. The limitations in studying stem cell niches in situ are largely due to the complexity in identifying stem cells and niche cells on a single section, requiring multiple markers to identify these cell populations. Whilst multi-labelling is simple with FACS, it does not characterize the niche. In contrast, immunofluorescence can visualize the niche, but the degree of complexity increases exponentially with every added cell marker. Furthermore, immunofluorescence studies usually require the use of primary antibodies that are derived from different species to enable sufficient amplification and detection of each cell marker. We have used immunofluorescence multiplexing to overcome the limitations of these two techniques to identify distinct hematopoietic stem cell and multipotent progenitor cell niches in sections of paraffin-embedded mouse bone marrow. Following primary antibody labelling, a tyramide-conjugated fluorophore binds covalently to the tissue localized at the cell marker of interest. Heat treatment then removes the antibody complex but leaves the covalently bound fluorophore in place. This permits subsequent labelling of a new tissue marker with a different antibody and tyramide-conjugated fluorophore. The process is repeated until all cell markers have been labelled, and primary antibodies derived from the same species can be used together on the same sample. Imaging is performed on a confocal microscope using a spectral detector and spectral unmixing is used to identify the unique spectral fingerprint of each fluorophore within the tissue. The combination of these two techniques allows for the labelling of at least six cell markers plus DAPI. This new technology allows the visualization and quantification of the interactions between stem and progenitor cells with their niches in

2D and potentially 3D. The flexibility of this technique allows it to be readily adapted to identify other cells/markers of interest (including reporter mice). It can be used for frozen or paraffin sections and is a valuable tool for researchers studying a wide range of stem cells in different tissues, including human biopsies.

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F-2152

THE DEVELOPMENT OF SINGLE-CELL PREPARATION DEVICE FROM CELL AGGREGATES AND ORGANOIDS

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Induced pluripotent stem (iPS) cells are promising cell sources for regenerative medicine. The stirred suspension culture using a bioreactor system is an efficient method for the large-scale expansion of human iPS cells. We designed a bioreactor series covering 100 folds the volume of culture from 5mL to 500mL. These our bioreactors are suitable for cell aggregates formation and proliferation of iPS cells, growth and maturation of organoids. Cell aggregates and organoids grown by stirred suspension culture are dispersed to single cells by enzyme treatment. The process of dispersing into a single cell is generally carried out manually by using a pipettor. In such this process, the dispersed cells easily die due to shear stress, and the cell recovery rate remarkably decreases. We developed the device for single-cell preparation from organoids. This device has a simple cylinder shaped rotating body in 60 mL plastic bottle. The rotating body has a gap of several millimeters from the inner wall of bottle, and the liquid between the wall surface and the rotating body generates laminar flow (couette flow) in the low-speed region and complicated spiral flow (Taylor vortex flow) in the high-speed region. In addition, the liquid continues to receive a constant shearing force according to the rotation speed of rotating body. By using this tool, it is possible to disperse single cells from cell aggregates and organoids in the enzyme solution more efficiently and reproducibly than manual work using a pipettor. In this meeting, we show the design of this device and discuss the recovery efficiency of single cells from aggregates of iPS cells.

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F-2154

ACCELERATING GENOME ENGINEERING OF INDUCED PLURIPOTENT STEM CELL LINES FOR DISEASE MODELING BY IMPLEMENTING AUTOMATED WORKFLOWS

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Using state-of-the-art genome editing tools, such as Cas9 protein and synthetic guide RNAs, the genome of human induced pluripotent stem cells (hiPSC) can now be easily edited to introduce genetic defects related to disease. These genome edited hiPSC can then be differentiated into for example cardiomyocytes or neurons, which can be implemented to model disease in vitro for basic research or drug discovery. While in general genome editing of hiPSC has become standard practice, higher throughput, larger scale and consistent generation of genome edited hiPSC lines remains challenging due to the complex nature of hiPSC culture conditions. Furthermore, manual picking of hundreds of colonies to isolate clonal lines carrying the desired genomic edit remains a labor intensive process. We therefore sought to implement automation to standardize and facilitate key steps covering the majority of the genome editing workflow in hiPSCs. Of the required steps to generate a clonal, genome edited hiPSC line, we so far achieved full automation of single cell seeding, expansion and consolidation, simply relying on available hiPSC products, a cell sorter and a simple liquid handler. Using SNP introductions to model disease as a genome editing example in hiPSC, we demonstrate, using these automated workflows, that clonal genome edited hiPSC lines can be derived reproducibly across multiple hiPSC backgrounds, with high first-time-right rates that drive throughput and scale. These methods did not affect the karyotype of the generated hiPSC lines, which furthermore maintained their typical pluripotency characteristics and potential to differentiate into specialized cells, allowing us to study the biology of the genomic changes made. By implementing existing and novel innovations in the hiPSC product space, such as StemFlex hiPSC medium, RevitaCell supplement and rhLaminin-521 matrix, we identified automated ways to drive the throughput, scale and consistency of the genome editing workflow in hiPSC.

F-2156

GENOME-WIDE CRISPR SCREEN IDENTIFIES ZIC2 AS AN ESSENTIAL GENE THAT CONTROLS THE FATE OF MESODERMAL PRECURSORS TO HUMAN HEART PROGENITORS

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Cardiac progenitor formation is one of he earliest committed steps of human cardiogenesis and requires the cooperation of multiple groups of genes governed by developmental signaling cascades. To determine the key regulators for cardiac progenitor formation, we have developed a two-stage genome-wide CRISPR screen. We mimicked the progenitor formation process by differentiating human pluripotent stem cells (hPSCs) into cardiomyocytes, monitored by two distinct stage markers of early cardiac mesodermal formation and commitment to a multipotent heart progenitor cell fare; MESP1 and ISL1 respectively. From the screen output, we compiled a list of 15 candidate genes. After validating 7 of these, we identified ZIC2 as an essential gene for cardiac progenitor formation. ZIC2 is known as a master regulator of neurogenesis. hPSCs with ZIC2 mutated still express pluripotency markers. However, their ability to differentiate into cardiomyocytes has greatly reduced. RNA-Seq profile reveals that they have switched to an alternative mesodermal cell fate. Our results provide a new link between ZIC2 and human cardiogenesis and documents the potential power of genome-wide unbiased CRISPR screens to identify key steps in human heart progenitor fate determination during human cardiogenesis with hPSC model systems.

F-2158

CONTINUOUS HARVEST SYSTEM FOR HUMAN ES AND IPS CELLS EXPANSION CULTURED ON BIOMATERIALS IMMOBILIZED WITH THERMORESPONSIVE NANOSEGMENTS

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Stem cells are an attractive prospect for regenerative medicine and tissue engineering.1 However, typical stem cell cultivation are still based on batch-type culture, which is laborious and expensive.2 Here, we developed a partial detachment stem cell culture system by using thermoresponsive nanosegments, a polymer having low critical solution temperature (LCST), which were coated on the surface of cell culture dishes for continuous stem cell harvest. This method enables cell aggregates or cell sheets to be obtained in culture medium without applying an enzymatic digestion. The themoresponsive nanobrush surfaces are composed by three copolymers having polystyrene, which are (a) thermoresponsive poly(N-isopropyl acrylamide), PNIPAAm and (b) biocompatible polyethylene glycol methacrylate (PEGMA). P[St-NIPAAm] (poly[styrene-co-N-isopropylacrylamide]), and P[St-PEGMA] (poly[styrene-co-polyethylene glycol methacrylate]) were prepared via reversible addition-fragmentation chain transfer (RAFT) polymerization. In this study, we successfully cultured human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) on the thermoresponsive surface and continuously harvested for ten cycles. The hESCs and hiPSCs have high pluripotency and differentiation ability by immunostaning analysis and embryoid body (EB) formation after ten cycles. In addition, over 60% of hESCs and hiPSCs could be detached from the surface after 30 minutes of low temperature incubation (7-8°C), and 95-100% hESCs and hiPSCs could be obtained by 1-2 times pipetting. This continuous culture system prevents hESCs and hiPSCs from enzymatic digestion damages and allows cells maintain their pluripotency on the surface. The continuous harvest of stem cells should downsize the equipment requirements for stem cell culture and simplify the culture process. Moreover, we also can scale up cell numbers by shifting 2D to a novel 3D culture system, which will be a great benefit to its clinical application in regenerative medicine.

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F-2160

PROLIFERATION CONTROL CULTURE OF HUMAN ES/IPS CELLS

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Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are expected as a source of regenerative medicine, including stem cell based drug discovery. We developed novel culture medium containing D-Xylose (Xylose medium) to suppress proliferation of hESCs and hiPSCs. In this study, we investigated characteristics of the cells cultured with Xylose medium by comparing it with the cells in

conventional culture. Human iPSCs and hESCs were commonly maintained on inactivated mouse embryonic fibroblasts (MEFs) with normal human ES/iPS medium. To control proliferation, the cells were passaged and then cultured on MEFs with Xylose medium. Although the hiPSCs and ESCs formed typical tightly compacted colonies in conventional method, the morphology of the cells cultured with Xylose medium became less dense. Interestingly the changed morphology was restored to its original state after returning to normal human ES/iPS medium. TEM analysis revealed that the hiPSCs cultured with conventional method were tightly close to each other without gaps. However, hiPSC colony cultured with Xylose medium formed gaps among the cells. On the other hand, the cells after changing back to normal medium returned to the adhesive state. After proliferation control culture, various derivatives of three primary germ layers were observed in the teratomas formed in SCID beige mice. In addition, human iPSCs cultured with proliferation control method by Xylose medium maintained a normal karyotype. By using Xylose medium, suppressing cell proliferation on non-working days have great benefits for researchers.

PLURIPOTENCY

F-2162

KEY PATHWAYS REGULATING THE GROWTH OF HUMAN PLURIPOTENT STEM CELLS IDENTIFIED THROUGH GENOME-WIDE CRISPR/CAS9 SCREENING IN HAPLOID CELLS

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Growth and survival of human pluripotent stem cells (hPSCs) depend on the expression of a set of essential genes and the activity of key pathways. Deciphering the roles of these essential genes and growth-regulating pathways in hPSCs along with their significance for the pluripotency network stands as crucial to the optimization of growth conditions and the assessment of the quality of hPSCs for regenerative medicine. We have recently identified essential and growth-restricting genes in hPSCs through a genome-wide loss-of-function genetic screen that combined the use of CRISPR-Cas9 technology and haploid hPSCs, targeting 18,166 protein coding genes with nearly 180,000 single guide RNAs. Here, we aimed to investigate the functions of a set of these essential genes that have an enriched expression in hPSCs and hPSC-selective growth-regulating pathways. We demonstrate that the growth and survival of hPSCs were inhibited by the knockouts of the putative DNA-binding protein VRTN, the selenium metabolism enzyme SEPHS1 and the DNA replication factor DSCC1. Transcriptome analysis upon knockdown of these essential genes with enriched expression in hPSCs showed downregulation of expression of multiple hESC-enriched genes and suggested regulatory roles for these genes in different cellular processes: energy metabolism for DSCC1 and SEPHS1 and mitosis for VRTN. Our previous work on growth-restricting genes identified in our genome-wide loss-of-function screen demonstrated two prominent pathways as the leading growth-regulatory pathways: ROCK-pathway and p53-mTOR pathway. We now extend our analyses on these pathways and show that hPSCs are more sensitive to growth regulation by the mTOR pathway as compared with somatic and cancer cells. We demonstrate that mTOR sensitivity of hPSCs can be attributed mostly to mTORC1 complex which regulates MAPK pathway in hPSCs but not in somatic cells. mTORC1 also regulates transcription of TP53 and CDND1 in hPSCs, thereby regulating apoptosis and cell cycle progression, respectively. Overall, we have defined novel regulators of pluripotency that function in different cellular processes and hPSC-specific regulatory mechanisms of pathways that are shared across many cell types.

F-2164

REGULATION OF CYCLIN E1 EXPRESSION IN HUMAN PLURIPOTENT STEM CELLS AND DERIVED NEURAL PROGENY

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Human pluripotent stem cells (hPSCs), like embryonic and induced pluripotent stem cells (hESCs and hiPSCs) show unique cell cycle characteristics, such as a short doubling time due to an abbreviated G1 phase. Whether or not the core cell cycle machinery directly regulates the stemness and/or the differentiation potential of hPSCs remains to be fully uncovered. If so, manipulation of cell cycle effectors may represent an additional tool by which in vitro maintenance or differentiation of hPSCs may be controlled for use in regenerative medicine. To date, several scenarios describing the atypical cell cycle of hPSCs have been suggested, and therefore there is still controversy over how cyclins, master regulators of the cell cycle, are expressed and regulated. Furthermore, the cell cycle profile and the expression pattern of major cyclins in hESCs-derived neuroprogenitors (NP) have not been studied yet. Therefore, herein we characterized the expression pattern of major cyclins in hPSCs and NP. We determined that all studied cyclins mRNAs expression levels fluctuate along cell cycle. Particularly, after a thorough analysis of synchronized cell populations, we observed that cyclin E1 mRNA levels increased sharply in late G1 concomitantly with cyclin E1protein accumulation in hPSCs and NP. Additionally, we demonstrated that cyclin E1 mRNA expression levels involves the activation of MEK/ERK pathway and the transcription factors c-Myc and E2Fs in hPSCs. Lastly, our results reveal that proteasome mediates the marked down-regulation (degradation) of cyclin E1 protein observed in G2/M by a mechanism that requires a functional CDK2 but not GSK3β activity.

F-2166

THE REMOVAL OF FIBROBLAST GROWTH FACTORS PROMOTE HEPATOBLAST DIFFERENTIATION FROM HUMAN iPS CELL-DERIVED DEFINITIE ENDODERM CELLS

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Human induced pluripotent stem cell-derived hepatocyte-like cells are expected to be utilized in pharmaceutical research. In general, human induced pluripotent stem (iPS) cells are differentiated into hepatocyte-like cells through definitive endoderm cells and hepatoblast-like cells using various growth factors that are essential for liver development. Although recombinant bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) are widely used in the hepatoblast differentiation, hepatoblast differentiation process has not been fully modified. In this study, we examined the roles of BMPs and FGFs in the hepatoblast differentiation from human iPS cells. Surprisingly, the gene expression levels of hepatoblast markers (alpha-fetoprotein and cytokeratin 7) were upregulated by the removal of FGFs. In addition, the percentage of hepatoblast marker (cytokeratin 19)-positive cells was increased by the removal of FGFs (from 87% to 94%). On the other hand, the percentage of the definitive endoderm marker (Sry-related HMG-box gene 17)-positive cells was significantly decreased (from 21% to 10%), suggesting that residual definitive endoderm cells were decreased by the removal of FGFs. Furthermore, the hepatocyte differentiation potency was also significantly increased by the removal of FGFs. To examine whether FGF signals are completely unnecessary for the hepatoblast differentiation, the expression levels of endogenous FGF ligands and receptors were examined. The definitive endoderm cells highly expressed the FGF ligand, FGF2, and the FGF receptor, FGFR1. To examine the role of endogenous FGF signals, an FGFR inhibitor was treated during the hepatoblast differentiation. The hepatoblast differentiation was promoted by using FGFR inhibitor, suggesting that endogenous FGF signals are also unnecessary for the hepatoblast differentiation. In conclusion, we found that FGF signals are not essential for hepatoblast differentiation. We believe that our finding will be useful for generating functional hepatocyte-like cells for pharmaceutical applications.

F-2170

EFFECTS OF OCT4 KNOCKOUT ON THE ENHANCER LANDSCAPE IN MOUSE EMBRYONIC STEM CELLS

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The transcription factor Oct4 is vital for maintaining pluripotency in mouse embryonic stem cells (mESC). Alterations in Oct4 expression are known to precede differentiation of mESC and loss of pluripotency. The role of Oct4 in pluripotency has been extensively studied, however, the mechanism of how Oct4 maintains pluripotency remain to be elucidated. Here we use transient transcriptome sequencing (TT-seq) on inducible Oct4 knockout mESC to study the immediate

changes in the transcriptome upon Oct4 knockout. TT-seq allows us to study nascent RNA and assess changes in short-lived non-coding RNAs. Dissecting the kinetics of RNA synthesis during Oct4 knockout is to provide insights into the molecular function of Oct4 in mESC.

F-2172

DECODING PLURIPOTENCY AND TOTIPOTENCY BY NOVEL LINC-RNAS

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The enormous value of stem cell-based therapy has attracted broad attention and prompted its applications in clinical regenerative medicine. Topipotent cell, which is capable of developing into a complete organism, is expected as an important tool in regenerative medicine and disease modeling. 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 cell 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 totipotent cells. 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.

F-2174

DISCOVER THE REGULATORY MODEL OF SETDB1 IN DIFFERENT PLURIPOTENT STATE IN MOUSE EMBRYONIC STEM CELLS

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Setdb1, known as a Histone H3 Lysine 9 (H3K9) methyltransferase, plays an important role in maintaining mouse embryonic stem (mES) cell's identity by repressing trophectoderm differentiation. Previously research shows that Erk1/2 inhibitor pD0325901, GSK3 α/β inhibitor chir99021 (2i) can drive mES cell move to a ground state. Surprisingly, we find that ablation of Setdb1 does not impair its pluripotency and inhibit trophectoderm differentiation in 2i ground state . To find out the different regulatory effect of Setdb1 plays in serum naïve and 2i ground pluripotent state, we make profiles of Setdb1, H3K9me2, H3K9me3 and H3K27me3's binding at different pluripotent state. Moreover, we hypothesis that there is a stronger safeguard rather than Setdb1 in 2i ground pluripotent state.

F-2176

MITOPHAGY REGULATION OF PLURIPOTENCY

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Although much is known about transcriptional networks that control embryonic stem cell (ESC) self-renewal and differentiation, the metabolic regulation of ESC is less clear. Autophagy is a catabolic process that is activated under both stress and normal conditions to degrade damaged organelles and aggregated proteins, and thus plays pivotal roles in somatic and adult stem cell function. However, if and how ESCs harness autophagy to regulate stemness remains largely unknown. Recently, we have defined that high autophagic flux as an intrinsic mechanism to maintain ESC identity through regulation of mitochondrial homeostasis. ATG3-dependent autophagy is an executor for both mitochondrial remodeling during somatic cell reprogramming and mitochondrial homeostasis regulation in ESCs. Dysfunctional autophagy by Atg3 deletion inhibited mitochondrial removal in both pluripotency induction and stemness maintenance, resulting in decreased reprogramming efficiency and accumulation of abnormal mitochondria in ESCs. Further ongoing mechanistic studies are focusing on identification of the receptors responsible for mitophagy to regulate pluripotency.

PLURIPOTENT STEM CELL DIFFERENTIATION

F-3002

ALBUMIN-FREE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO ENUCLEATED RED BLOOD CELLS

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The production of cultured red blood cells (RBCs) from adult hematopoietic stem and progenitor cells (HSPCs) or human pluripotent stem cells (hPSC) has many potential practical applications including the production of reagent RBCS, the production of rare RBCs for transfusion application and the production of therapeutic drug carrier RBCs . Protocols to produce cultured RBCs have improved considerably during the last few years but most published methods rely on historical culture medium formulation and the requirement for many components had never been fully tested. In particular, all media reported so far contain serum, albumin and/or transferrin of either animal or human origin which is a major problem because the yield of cRBCs is highly dependent on the lots of albumin. We report here that we have developed chemically defined media and supplements that can be used to produce cRBCs from adult HSPCs and from iPSCs that are free of any albumin or animal transferrin and in which the number of components was reduced to a minimum. In addition to lot-to-lot variation in media composition, two problems have prevented the production of therapeutically useful cRBCs from hPSCs. The first one is that while cRBCs produced from adult HSPCs enucleate in culture at a high rate, RBCs produced from hPSCs exhibit low rates of enucleation in the 2-5% range. The second problem is that adult RBCs produced in vivo express >99% adult hemoglobin globin (HbA) and less than 1% fetal hemoglobin (Hb F) while iPSCs-derived cells generally expressed a mixture of embryonic and fetal hemoglobins. We report that elimination of animal or human albumin and transferrin and simplification of the medium composition led to an unexpected dramatic increase in the rate of enucleation to about 70% of iPSC-derived cRBCs and in the production of cRBCs with up to 15% adult Hb A therefore solving the enucleation problem almost completely and improving the hemoglobin composition of cRBCs. We also report that we have developed a novel embryoid body-free method to initiate hematopoietic differentiation of hPSCs simplifying scaling the procedure to large volumes. Together these novel methods result in a robust, inexpensive process to produce enucleated cRBCs in large amounts from hPSCs, an immortal genetically modifiable cell type and from adult HSPCs

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F-3004

DEVELOPMENT OF COMMERCIALLY VIABLE CGMP MANUFACTURING PROCESSES FOR THE PRODUCTION OF HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVATIVES FOR CLINICAL APPLICATIONS

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The generation of induced pluripotent stem cells (iPSCs) represents a significant breakthrough for the production of clinically-functional cells for cell-replacement therapies. While some therapies may require a few million cells, this can easily go up to over 10^9 cells per patient dose for clinical indications such as chronic heart failure. To meet the clinical and commercial demand for high quality iPSC-derived products, the production of these cells requires large-scale manufacturing of iPSCs and their derivatives through a robust and reproducible differentiation process conforming to current good manufacturing process (cGMP) guidelines. We have recently reported manufacturing of iPSC master cell banks under cGMP to facilitate pre-clinical and clinical applications. Here we summarize a commercially viable approach to address the technical and logistical challenges involved in the large-scale manufacturing of cGMP iPSC-derivatives. We focused on the expansion of iPSCs and their specific differentiation into cardiomyocytes. By using best practices in bioprocessing and computational fluid dynamics (CFD) modeling, we established a large-scale 3D bioreactor process. iPSCs were first expanded and differentiated into cardiomyocytes (CMs) in small (100 mL) spinner flasks through a stepwise differentiation process from iPSCs to cardiac mesoderm, cardiac progenitor cells and cardiomyocytes, and production of approximately 100% beating cardiospheres in 14 days. The manufactured CMs reached a final viable cell yield of $6.0x10^7$ in 14 days and expressed cardiac specific transcription factor Nkx2.5 and cardiac troponin (cTnT). Using CFD and control of hydrodynamics, we enabled scale-up of the process to 3 L computer-controlled stirred suspension bioreactors. Incorporation of proper process design considerations would facilitate the smooth transition of this process to manufacturing for pre-clinical and clinical studies. Most importantly, the methodology used to develop this iPSC-CM differentiation platform using a cGMP-compliant cell

source, combined with scale-up methods from CFD modeling follows best bioprocessing practices for the manufacturing of iPSC-derivatives at commercial scale for therapeutic applications.

F-3006

AN ANALYSIS OF EXPERIMENTAL APPROACHES FOR IN VITRO DEVELOPMENT OF MACROPHAGES FROM HUMAN AND MOUSE PLURIPOTENT STEM CELLS

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Macrophages (Mφs) are phagocytic immune cells that are not only important for host defence, but are also involved in tissue injury and disease. The different environments that these Mφs occupy, or are recruited into, impose phenotypic and functional differences. Mφ ontogeny is diverse and this may have relevance for the in vitro derivation of various tissue-resident populations from induced pluripotent stem cells (iPSCs), as well as common comparisons to blood-derived monocytes. For example, a tissue-resident Mφ in the central nervous system (CNS), such as Microglia, have broad regulatory roles that include immune homeostasis and synaptic pruning. In contrast, tissue-resident liver Mφ (Kupffer cells) are essential to hepatic function regulation. A deeper appreciation of the functional diversity of Mφs, including contextual responsiveness - is currently missing from stem cell differentiation studies. Here, we conduct a meta-analysis of in vitro methodology for deriving Mφs from human and mouse embryonic stems cells (ESCs) and iPSCs. We reviewed and reanalysed the data from these papers, focussing on the functional assessment of the derived Mφs, reflecting on how these cells were, and are, currently defined in the stem cell literature. Here, we highlight variation in methodologies used to derive Mφs from stem cells, and assess the impact that these methods have on function and identity of the cells. The study highlights minimal standards required for the functional analysis of Mφs in terms of generalizable myeloid phenotype, desired maturation status and tissue context.

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F-3008

NOVEL TRANSCRIPTOMICS TARGETS FOR FUNCTIONAL IMPROVEMENT OF HEPATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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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 do not fully recapitulate the complete functionality of *in vivo* mature hepatocytes yet, and they still express some fetal hepatocyte markers. In this study, we applied versatile bioinformatic algorithms, including functional annotation and pathway enrichment analyses, transcription factor binding site enrichment, similarity and correlation analysis, to datasets generated from samples collected from different developmental stages during hPSC-HEP differentiation, and compared these to fetal or/and adult human liver tissues. Our results demonstrate a high level of similarity between the in vitro differentiation of hPSC-HEP and in vivo hepatogenesis. The key finding is 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 also showed expression of many genes involved in drug absorption, distribution, metabolism, and excretion. Furthermore, specific differences in gene expression between hPSC-HEP and adult liver were observed, and regulatory factors, as well as affected pathways, were identified. This leads to new knowledge for future intervention of in vitro hepatic differentiation in order to generate cells more useful for industrial and medical applications. Taken together, on a transcriptional level, our results show stronger correlation and higher similarity of hPSC-HEP to adult than to fetal human liver tissue. In addition, potential targets for further functional improvement of hPSC-HEP were also identified.

F-3010

SUBSTRATE-DEPENDENT HUMAN IPS COLONY COMPACTION AND ECTODERM DIFFERENTIATION

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We have successfully induced multiple-cellular zones from human iPS cells using laminin511-E8 fragment (LN511E8), which highly mimics in vivo eye development (Hayashi R. et al, Nature). However, the developmental mechanism of the characteristic concentric multi-zonal structure (named SEAM; self-formed ectodermal autonomous multi-zone) is unclear. In this study, to elucidate the mechanism of SEAM formation, we examined the influence of substrates on ocular cell differentiation of iPS cells. Human iPS cells were seeded on LN332E8- and LN511E8-coated plates to analyze gene and protein expression during colony formation process and ocular cell differentiation. On LN511E8, the cell density was higher inside the iPS colony and the YAP, the mediator of the Hippo signaling pathway, was localized in the cytosol whereas the cell density was lower at the periphery of the colony, and YAP was localized in the nucleus. After differentiation culture, the central part of the iPS colony was differentiated into neuroectoderm and the periphery was differentiated into surface ectoderm. Neuroectodermal differentiation inside iPS colonies was promoted by increasing of concentration of LN511E8 and was inhibited by treatment with blebbistatin, a myosin heavy chain ATPase inhibitor. On the other hand, on LN332E8, the proportion of cells in which YAP was localized in the nucleus was higher compared to that on LN511E8, and surface ectodermal differentiation was promoted. These results suggested that adhesion of iPS cells to laminin, particularly to LN511E8, promotes the translocation of YAP to extranuclear region by increasing cell density in iPS colonies via actomyosin contraction, resulting into subsequent neuroectoderm differentiation. Therefore, it was suggested that substrate-dependent cell density gradient in iPS colonies is important for induction of multi-zonal structure in SEAM.

F-3012

OPTIMIZING IPSC DERIVATION INTO HUMAN PANCREATIC PRECURSOR CELLS

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Diabetes is known to affect 422 million people worldwide 90% of who is diagnosed with Type-2 Diabetes (T2D). This chronic metabolic disease is characterized by the acquired insulin- independence and annually leads to 1.6 million deaths. T2D GWAS studies have identified 90 loci that influence disease risk. Considering the proportion of genetic information in the non-coding regions of the genome it is challenging to identify the target regions of the T2D risk signals and indeed until recently current genetic approaches were able to explain only 10% of the heritability of T2D leaving vast majority of the genetic contribution to the T2D pathogenesis unexplored. Functional validation of causal T2D risk variants requires the use of appropriate experimental models using relevant cell types. Previously, our lab has generated the iPSC Collection for Omic Research (iPSCORE) recourse of human induced pluripotent stem cell (hiPSC) lines from 222 distinct individuals. This cohort is comprised of 82 singletons and 140 individuals from 41 families including 8 monozygotic twin pairs. Whole Genome Sequencing (50X) was performed on DNA from the blood samples of all individuals in iPSCORE. 24 of the 222 individuals have been diagnosed with T2D. Our aim is to elucidate genetic variants contributing in the T2D development using iPSC-derived pancreatic progenitor (PDX1+ NKX6.1+) cells. Current protocols for derivation of pancreatic progenitors from iPSCs require significant line-to-line optimization and are often inefficient when compared to ESCs. We modulated the levels of activation and subsequent inactivation of WNT pathway in order to develop a robust protocol which can be applied to large number of iPSC lines. Next, we will apply this protocol to lines from 100 different individuals including the 24 hiPSC lines from T2D patients, to derive pancreatic progenitors and generate Hi-C chromatin conformation, scRNA-seq, ATAC-seq, H3K27ac ChIP-seq, and DNA methylation profiles. These data will be combined with datasets available from human islets to annotate molecular QTLs at T1D-risk variants, as well as to evaluate potential genetic contributions to disease etiology in the 24 T2D patients. These analyses will provide insight as to how T2D-risk variants alter local chromatin states and gene regulation in islets and progenitors cells.

F-3014

ENRICHMENT OF MATURE HUMAN IPS CELL-DERIVED HEPATOCYTE-LIKE CELLS FOR PHARMACEUTICAL RESEARCH USING GENOME EDITING TECHNOLOGY

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Human iPS cell-derived hepatocyte-like cells are expected to be utilized in pharmaceutical research such as drug screening. However, the purity of high-functioning hepatocyte-like cells is not high enough. In particular, the purity of cytochrome P450 3A4 (CYP3A4), which is a representative hepatic drug-metabolizing enzyme, positive cells is quite low (approximately 20%). To address this problem, we generated high-purity and high-functioning human ES/iPS cell-derived hepatocyte-like cells for pharmaceutical research. Therefore, we tried to establish the CYP3A4-NeoR transgenic reporter human ES/iPS cell line (CYP3A4-NeoR iPS cells) by using genome editing technology. However, in spite of use of CRISPR/Cas9, the efficiency of genetic engineering of human ES/iPS cells in transcriptionally inactive genes is extremely low, unlike that in transcriptionally active genes. To enhance the homologous recombination efficiency in human ES/iPS cells, we performed screenings of accessorial genes and compounds. We found that RAD51 overexpression and valproic acid treatment could enhance biallelic-targeting efficiency in human ES/iPS cells regardless of the transcriptional activity of the targeted locus. By using RAD51 and valproic acid, we succeeded in establishing CYP3A4-NeoR iPS cell line. The CYP3A4-NeoR iPS cells were differentiated into hepatocyte-like cells according to our hepatocyte differentiation protocol established previously. Then, the hepatocyte-like cells were treated with Neomycin to concentrate the hepatocyte-like cells which strongly express CYP3A4. After the Neomycin treatment, the percentage of CYP3A4-positive cells was higher than 80%. The gene expression levels of various drug-metabolizing enzymes, transporters, and hepatic transcription factors were significantly enhanced by Neomycin treatment. In addition, the CYP1A2, 2C19, 2D6, and 3A4 activities and biliary excretion capacities were also significantly increased by Neomycin treatment. We succeeded in obtaining human iPS cell-derived hepatocyte-like cells that highly express CYP3A4 at high purity. Our high-purity and high-functioning hepatocyte-like cells would be used to evaluate the risk of drug candidates more accurately than ever before.

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F-3016

ROLE OF POLYCOMB GROUP GENES IN STABILITY OF UNDIFFERENTIATED STATE OF HUMAN PLURIPOTENT STEM CELLS

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Maintenance of undifferentiated state is a critical requirement for regenerative medicine using pluripotent stem cells. The undifferentiated state of pluripotent stem cells is achieved by the actions of pluripotency master regulators such as Oct4, Sox2 and Nanog, and maintained by epigenetic factors such as polycomb group genes. However, although pluripotent stem cells including ES cells and iPS cells are well known to differentiate spontaneously, the differentiation mechanism in this natural culture condition is poorly understood. In this study, we created the spontaneous differentiation by removing MEF cells, and then analyzed global changes of gene expression during the spontaneous differentiation (collapse of undifferentiated state) using DNA microarray. As results of the microarray analysis using four human pluripotent stem cell lines, human ES cell: khES1 (Kyoto), khES2 (Kyoto), H1 (Wisconsin) and human iPS cell: 253G1 (Kyoto), we found that some polycomb group genes were commonly up- regulated during the collapse of undifferentiated state. These findings are surprising because polycomb group proteins are well known to function in the undifferentiated state. Then we analyzed function of the spontaneous differentiation-induced polycomb group genes in pluripotent stem cells by CRISPR-Cas9 mediated knockout. These knockout cells showed tendency to differentiate more frequently than control. Therefore these polycomb group proteins may function as defense against the spontaneous differentiation. To evaluate the proposed function, we are now analyzing targets of polycomb group proteins during the spontaneous differentiation by ChIP-seq.

F-3018

GENERATION OF FLUORESCENT AND ENZYMATIC HUMAN EMBRYONIC H9 STEM CELL REPORTER LINES FOR MICROGLIAL DIFFERENTIATION

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In the past 18 months, multiple methods of microglial differentiation from induced pluripotent stem cells (iPSCs) have been reported. These methods commonly use PCR along with immunolabelling and/or immunoblotting to show the presence of key developmental genes including PU.1, IRF8, CX3CR1, TREM2 and TMEM119. Although these studies report the presence of microglia in cultures, these cultures represent mixed populations of cells containing varying proportions of microglia. To reduce the heterogeneity of cultures we have used CRISPR-Cas9 on a parental H9 human embryonic stem cell line to create individual fluorescent and enzymatic knock-in reporter lines targeting five key microglial markers of early and late differentiation. Thus the following plasmids were designed: pDTA-TMEM119-2A-GFP-2A-AMP-FneoF; pDTA-TREM2 cDNA-IRES-Crimson-2A-lacZ-FneoF; pDTA-CX3CR1-IRES-tdTomato-T2A-NanoLuc-FneoF; pDTA-PU.1-GFP-AMP-CBG-FRT-neomycin-FRT; pDTA-IRF8-mCherry-lacZ-CBR-FRT-neomycin-FRT. PCR analysis indicates correct targeting of all five clones. So far expansion of the CX3CR1 clone shows marked red fluorescence following microglial differentiation. We anticipate confirmation of targeting by Southern blot and differentiation of the other lines to reveal fluorescence. The next stage of this work is to determine microglia responses to purinergic receptor activation as well as their effect upon neurons in culture. Targeting both early and late genes in microglial differentiation with spectrally distinct fluorescent reporters allows for cross targeting and the identification/isolation of microglia at different stages of development. The addition of the enzymatic reporter is, we believe, useful for programs of drug discovery and design. We believe that these tagged microglial cells will enable a consistent and rational approach to studies of microglial-neuron interactivity, in vitro.

F-3020

TRANSGENIC SCL CONTRIBUTES TO GABAERGIC NEURON DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Pluripotenthuman embryonic stem cells (hESC) can be induced to differentiate to several neuronal types by stimulation with growth factors or small molecules. Such cell commitment is triggered and consolidated by transcription factors. Stem Cell Leukemia (SCL/TAL1) is a bHLH transcription factor that has been implicated in hematopoietic differentiation. However, SCL is also expressed transiently in discrete regions of the Central Nervous System (CNS). In rodents, it plays an essential role in the GABAergic interneuron differentiation in the midbrain and the hindbrain, as well as in the ventral region of the developing spinal cord, where it also represses motor neuron development; little is known about SCL function in the development of human neurons. In this study, using the directed differentiation of pluripotent cells to GABAergic neurons of a SCL-overexpressing human ESC line, we explored the role of this transcription factor during neuronal induction. SCL overexpression accelerate the emergence of GABAergic neurons. At the end of the differentiation protocol, overexpressing cells generate a higher proportion of GABAergic neurons, which have a greater GABA content, compared to differentiated neurons from control hESC. In agreement, SCL-overexpressing cells has higher expression of GAD1 detected by RT-qPCR. We conclude that SCL potentiates GABAergic neuron differentiation of human pluripotent stem cells.

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F-3022

LAMININ PROMOTES THE DIFFERENTIATION OF RAT EMBRYONIC STEM CELLS INTO CARDIOMYOCYTES VIA ACTIVATING THE INTEGRIN/FAK/PI3KP85 SIGNAL AXIS

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The generation of germline competent rESCs opened a new window to explore rat lineage commitment such as cadiomyocyte differentiation. However, the potency of the in vitro differentiation still need to be improved for mimic early development of heart. In the present study, we captured up the high efficiency of cardiomyocyte differentiation from rESCs based on a suitable medium, in which laminin interacts with membrane Integrin, and promotes the phosphorylation of PI3K p85 and FAK, respectively. Meanwhile, the GATA4 expression levels was also upregulated. While, blocking Integrin pathway using inhibitor Cilengitide, the effect of laminin on cardiomyocyte differentiation was abolished with a down-regulation of phosphorylation level in PI3K p85, and corresponding inhibtion of Gata4 expression as well. Take together, laminin as a crucial component contributes to cardiomyocyte linerge in rESC differentiation medium through increasing rESCs proliferation via interacting with integrin. These results provide potential molecular mechanisms governing communication during cardiomyocyte differentiation from rESCs based on extracellular matirx laminin.

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F-3024

MATERUATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES USING NANOTOPOGRAPHY

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide abundant cell sources for disease modeling, drug screening and cell therapy. However, these cells are phenotypically immature. One possible reason is that current culture substrates fail to mimic the in vivo microenvironment. In the present study, we fabricated complex micro/nano-topographies called monolayer binary colloidal crystals (BCCs) to systematically investigate the cardiogenesis of hiPSCs and improve the maturation. Firstly, hiPSCs formed spheroid-like structure on SiPM surfaces but relatively flattened colonies on SiPS and flat surfaces. Real-time PCR illustrated that pluoripotent markers, Oct-4, Nanog and Sox-2, were significantly higher on SiPM surfaces. Next, hiPSCs were chemically differentiated into cardiomyocytes. The myocardial structural of cardiomyocytes was well-developed and the electrophysiological function performed better on BCCs. The expression of cardiogenic markers (MEF2c, KX2.5, GATA4) was significantly increased on the 5SiPM surface. N-Cadherin of hiPSC-CMs on 5SiPM surface was also dramatically higher than other surfaces indicating that cell-cell interaction play a critical role during cardiogenesis. In this study we proposed that the surface of BCC displays complex chemical, topographical, and mechanical properties which can mimic native cardiac microenvironment. Second, BCCs enhanced cell-cell interaction of hiPSCs and then improved cardiogenesis. Our findings highlight the importance of microenvironment for hiPSCs culture and BCCs have potential to become next generation cell expansion tool.

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F-3026

CHARACTERISTICS OF MOUSE LIVER PROGENITOR CELLS DERIVED FROM STEM CELLS

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The shortage of donor livers makes cell therapy an attractive option for treating various liver diseases such as congenital metabolic disorders, end stage liver failure and hepatocellular carcinoma. In particular, cell

therapy using liver progenitor cells (LPCs) holds great promise due to their greater proliferative potential and robustness compared to hepatocytes. Hepatocytes also have a fixed lifespan and there is evidence that their effectiveness diminishes with time following transplantation. LPC therapy is hampered by the lack of a cost effective protocol to generate them and the scarcity of LPCs in healthy liver makes it difficult to obtain sufficient numbers. To address this problem, we have established a novel approach that can generate large numbers of LPC-like cells from embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). These LPC-like cells express LPC markers including A6, CK19, CD24 and E-Cadherin. Although there is variation between isolates, RNASeq analyses show they cluster more closely to LPCs than to the stem cells from which they were derived. Similar to LPCs, LPC-like cells can be differentiated to express hepatocyte markers such as HNF4a, TAT and OTC. To be therapeutically useful LPC-like cells need to be effective in mouse models of liver disease. LPC-like cells derived from iPSCs would minimise immune rejection when transplanted back into the organism from which they were derived. The repopulation efficiency to achieve disease correction may vary with the pathology. In a mouse model for the congenital metabolic OTC deficiency disorder, we have shown that restoration of ~5% of wild-type OTC activity was sufficient to prevent hyperammonemia, the major clinical problem associated with the disorder. We are presently optimising the re-population efficiency of LPC-like cells in a mouse model of chronic liver injury and assessing the effectiveness of utilising progenitor cells for therapy. In summary we describe a method to generate LPCs from ESCs and iPSCs for use in cell therapy to treat liver disease. Approaches to maximise their engraftment and their efficacy is evaluated to assess their applicability for cell therapy.

F-3028

BILLION-SCALE PRODUCTION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS BY USING ROTARY CELL CULTURE SYSTEM

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Human hepatocytes play important roles, including drug metabolism and detoxification. In the field of drug screening, human hepatocytes are widely used to predict drug metabolism and drug-induced hepatotoxicity. In the field of regenerative medicine, Human hepatocytes are transplanted into patients with liver disease. For both applications mentioned above, a large number of human hepatocytes have to be prepared. In particular, more than one billion (109) human hepatocytes are required for hepatocyte transplantation. However, it is difficult to prepare human hepatocytes on that scale because of a shortage of donors. To overcome this problem, human induced pluripotent stem cell-derived hepatocyte-like cells (human iPS-HLCs) are expected to become alternative cell sources. However, to the best of our knowledge, there is no report on a successful large-scale production (more than a billion scale) of human iPS-HLCs. Therefore, in this study, we tried to establish a method to mass-produce human iPS-HLCs using a three-dimensional (3D) cell culture bioreactor called the Rotary Cell Culture System (RCCS). RCCS can perform large-scale (>1,000 ml) 3D suspension cultures by rotating cell culture vessels without propeller stirring. As a result of 3D suspension culture using RCCS, we obtained 3D-cultured hepatocyte-like cell (3D-HLC) spheroids that were approximately 200 μm in diameter. The total number of 3D-HLC cells was 1.5 billion per 1000 ml medium. The gene expression levels of some hepatocyte markers (alpha-1 antitrypsin, cytochrome (CYP) 1A2, CYP2D6, and hepatocyte nuclear factor 4 alpha) were higher in 3D-HLCs than in 2D-cultured hepatocyte-like cells. The percentage of TUNEL-positive cells, apoptotic cells, in 3D-HLC spheroids was less than 10%. These results suggest that RCCS could provide suitable conditions for hepatocyte maturation than the conventional 2D cell culture conditions. In addition, more than 90% of hepatocyte-like cells were positive for albumin and could uptake low-density lipoprotein in the culture medium. We succeeded in the billion-scale production of homogenous and functional hepatocyte-like cells from human iPS cells. This technology will be useful in drug screening and regenerative medicine.

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F-3030

ANEUPLOIDY CAUSES ENDOPLASMIC RETICULUM STRESS AND DYSFUNCTIONAL SECRETOME

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Aneuploidy often leads to severe developmental defects and is recognized as a hallmark of cancer cells. Recent studies have shown that aneuploidy promotes neoplastic progression in embryonic stem (ES) cells by impairing their differentiation. However, the underlying mechanism of differentiation defects caused by aneuploidy remains elusive. Here, we found that the extra copy of chromosome is actively expressed and produces excess proteins in aneuploid ES cells, which can be tolerated during cell self-renewal. However, upon stem cell differentiation, proteasome-mediated protein degradation activity cannot be efficiently stimulated to adapt the cell-fate change, which burdens the protein quality-control system and leads to endoplasmic reticulum (ER) stress. Accordingly, treatment with an ER stress inhibitor or proteasome activator rescues the differentiation defects in aneuploid cells. Furthermore, mass spectrometry-based profiling revealed that secreted proteins were widely changed during aneuploid cell differentiation. Also, supplementation with down-regulated extracellular factors partially rescues the differentiation defects of aneuploid ES cells. Thus, disturbed ER proteostasis and dysfunctional secretome underlie the impaired differentiation capacity in aneuploid ES cells, which could have important implications for tumorigenesis.

F-3032

GENERATION OF NOTO-GFP REPORTER IN HUMAN EMBRYONIC STEM CELL (HESC) LINE FOR NOTOCHORD-LIKE CELLS DIFFERENTIATION

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The low back pain is mainly caused by the intervertebral discs degeneration (IDD), which severelyaffects the living qualify of over 85% people in the world. However, traditional treatments, like surgery and anti-inflammatory medications, cannot cure this disease. Nucleus pulposus (NP), the core of intervertebral disc, contains notochord-like cells (NCCs), which can potentially differentiate into NP cells and repair the damaged inervertebral discs. NCCs differentiation from human embryonic stem cells (hESCs) allows shedding light on the mechanistic studies of IDD pathogenesis and developing cell replacement therapies. Unfortunately, the lack of specific surface marker for NCC has limited us to identify and isolate NCC-like population from differentiating hESCs. Through genetic-mapping, it has been recognized that homeobox protein notochord (NOTO) is a candidate for NCC identity marker. To establish a NOTO-GFP reporter in hESCs to identify and purify NCC, here we employed CRISPR/Cas9 technology and 2A-mediated cleavage system to precisely knock in an enhanced fluorescent protein (GFP) to the loci of Noto gene in hESCs. The results of Sanger sequencing indicated that no off-target and mutation existed in this Noto-GPF reporter, which did not affect the regular cell differentiation to form three germ layers in vitro. Importantly, immunostaining and real time PCR indicated the co-expression of Noto and GFP during the NCC differentiation. In summary, the temporal and spatial expression of Noto gene can be effectively monitored with this Noto-GFP reporter, which allows serving as a powerful tool for human NCCs differentiation, purification and further therapy development.

F-3034

DIRECT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO HEPATOCYTE-LIKE CELLS ON MODIFIED COLLAGEN-LIKE MATRIX

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Human pluripotent stem cells (hPSCs) have the potential to differentiate into all embryonic germ layers and thus promise to be an unlimited cell resource for basic and translational science. However, the yield of mixed cell types and immature phenotypes achieved are significant hurdles to be overcome. This study focuses on the directed differentiation of hPSCs to hepatocytes. Published protocols of hepatocyte differentiation use culture media and substrates that are not only expensive but also, in many cases, undefined. To address this issue, we designed a defined differentiation process using highly tunable collagen-like protein substrates to provide biochemical and structural support to differentiating hepatocytes. The hepatocyte-like cells were produced using a protocol based on previously published work. Three different constructs of non-animal modified collagen-like proteins were tested for hepatocyte differentiation. We demonstrated hepatocyte specification on these novel biomaterials by the expression of alpha-fetoprotein, albumin, and alpha-1-antitrypsin. We believe that modified collagen-like proteins have the potential for not only promoting hepatocyte differentiation but also purifying the cell population via differential adhesion.

PLURIPOTENT STEM CELL: DISEASE MODELING

F-3036

GENERATION OF PLAKOGLOBIN EDITED PLURIPOTENT STEM CELLS BY CRISPR/CAS9 FOR CARDIOMYOPATHY MODELLING

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The arrhythmogenic cardiomyopathy (ACM) is a genetic disease that affects mainly the left ventricle due to progressive replacement of myocardium by adipose tissue. Several mutations are reported to cause this cardiac condition, most of them related to desmosomal genes. The aim of this work was to analyze the expression of 5 desmosomal genes [desmoplakin, plakoglobin (Pkg), plakophilin 2, desmoglein 2 and desmocolin 2] during cardiomyocite differentiation from iPSCs cells and to generate a Pkg knockout iPSCs line by CRISPR/Cas9 in order to model the disease. The differentiation protocol consisted on a monolayer protocol in a serum-free system by temporal modulation of regulators of canonical Wnt signaling. RNA and protein samples were taken on day 0, 3.5, 7 and 21 of differentiation, from 3 independent experiments. We observed cell contractility by day 8, and after qPCR we observed a significant decrease in the expression of pluripotency genes (Oct4 and Nanog) and an increase of the expression of mesoderm-cardiac genes (Brachyury, NKX2.5 and cTnT), as the protocol advanced. A tendency to increase the expression of the desmosomal genes by day 7 and 21 was also observed, with no statistical differences though. However, a higher expression of PKG was assessed by western blot by day 7 of differentiation. In order to generate an early stop codon in the PKG gene, we designed 2 RNA guides (gRNA 1 and gRNA 2) to the exon 1 of the gene and a single strand oligo DNA (ssODN) of 70 bp containing the desired mutation and a silent mutation for a restriction site of BamH1. 1 ug of the CRISPR system and 1 ug of the ssODN were co-transfected to 200000 iPSCs. After puromycin selection, the cells were clonally expanded and evaluated for the ssODN incorporation. To achieve this, the gene was amplified by PCR and the amplicon was then digested with BamH1. We obtained different efficiencies of ssODN incorporation depending on the gRNA used: 15.4% (n=2/13) and 71.4% (n=10/14) for the gRNA1 and gRNA2, respectively. In summary, we were able to characterize the expression of the desmosomal genes during the cardiac differentiation protocol and to generate mutated iPSCs lines for Pkg with high efficiency. Now we are planning to differentiate the mutated lines to cardiomyocites in order to study their phenotype and to determine if we can model the ACM by this strategy.

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F-3038

PATIENT-DERIVED iPSC-BASED DRUG DISCOVERY PLATFORM HIGHLIGHTS RAPAMYCIN AS A DRUG CANDIDATE FOR FIBRODYSPLASIA OSSIFICANS PROGRESSIVA (FOP)

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Fibrodysplasia ossificans progressiva (FOP) is a rare and intractable disease characterized by extraskeletal bone formation through endochondral ossification and there is no treatment for FOP. FOP patients harbor point mutations in ACVR1 (also known as ALK2), a type I receptor for BMPs. Although mutated ACVR1 (FOP-ACVR1) has been shown to render ligand-independent constitutive activity and ligand-dependent hyper activity in BMP signaling, recently, we and Hatsell et al. discovered a novel mechanism of heterotopic

ossification (HO) by utilizing FOP patient-derived iPSCs (FOP-iPSCs) and FOP-ACVR1 conditional-on knock-in mice. These studies revealed that mutated FOP-ACVR1 abnormally transduces BMP signaling in response to Activin-A, a molecule that normally transduces TGF-β signaling but not BMP signaling, and Activin-A evoked enhanced chondrogenesis of induced mesenchymal stromal cells derived from FOP-iPSCs (FOP-iMSCs) via BMP and TGF-β signaling. By utilizing FOP-iPSCs and Activin-A, we developed a robust protocol to show accelerated chondrogenesis of FOP-iMSCs in vitro and in vivo. Taking advantage of these results, here, we developed a high-throughput screening (HTS) system for FOP and identified mTOR inhibitors as drug candidates by screening a drug repositioning-focused library. Particularly, rapamycin showed potent therapeutic effect on HO in two different in vivo HO models: FOP model mice expressing FOP-ACVR1 and a FOP-iPSC-based HO model in which ectopic bones derived from FOP patient-derived cells are formed in mice. Moreover, we identified ENPP2 as a linker of FOP-ACVR1 and enhanced mTOR signaling in chondrogenesis. These results uncovered rapamycin as a promising drug candidate for FOP, and a clinical trial has been started on October 2017 in Japan.

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F-3040

A PATHOLOGICAL STUDY OF FACIO-SCAPULO-HUMERAL MUSCULAR DYSTROPHY (FSHD) WITH PATIENT-DERIVED IPSCS

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Facio-scapulo-humeral muscular dystrophy (FSHD) is a genetically inherited disease causing progressive weakness of skeletal muscle with unique clinical features such as asymmetrically affected patterns and large varieties of disease progression among patients. As these aspects cannot be simply explained by the causative mutation, the involvement of environmental factors should be considered. The genetic backgrounds for both FSHD type 1 (FSHD1) and FSHD type 2 (FSHD2) lead to chromatin relaxation of disease-associated genomic locus, resulting in aberrant gene expression of DUX4, a key factor of FSHD pathogenesis by exerting toxicity against muscle cells. How DUX4 expression is regulated in FSHD remains unclear. Thus, we hypothesized that environmental factors may modulate DUX4 expression and focused on oxidative stress, a common stress in skeletal muscle. To investigate our hypothesis, firstly we established FSHD myocyte models by generating iPSCs derived from one healthy control, FSHD1 and FSHD2 patients and also isogenic control iPSC clones from FSHD2 by correcting its causative SMCHD1 mutation. All those clones efficiently differentiated into myocytes. Importantly, FSHD-derived myocytes, and not healthy control-derived myocytes, showed robust gene expression of DUX4 and its direct downstream targets, which were significantly suppressed in isogenic control-derived myocytes. Then, by stimulating those FSHD and non-FSHD myocytes with hydrogen peroxide, we confirmed that DUX4 expression was increased by oxidative stress, mediated by DNA damage response (DDR), and suppressed by inhibition of ATM, a DDR regulator kinase. Our study provides a new model for FSHD and indicates that oxidative stress and other genotoxic stresses can be risk factors against FSHD patients through toxic DUX4 expression.

F-3042

TYPE-2 DIABETES DISEASE MODEL WITH CRISPR-EDITED STEM CELL-DERIVED BETA CELLS AND EPIGENETIC PROFILING OF GENETIC VARIANTS

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Type 2 diabetes (T2D) is a long-term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. Considerable progress towards an understanding of T2D has been made in recent years due to the identification of genetic variants associated with T2D by genome-wide association studies. However, despite the identification of 128 lead SNPs associated with T2D and/or fasting blood glucose levels, little is known about how these variants affect cell function and cause disease. Here we prioritized five putative causal variants located in non-coding regulatory regions of the KCNQ1 gene by a combination of genomic and epigenomic analysis, and validate their function by gene editing in a hESC-based beta cell differentiation system.

Funding Source: NIH/NIDDK U01DK015541 Ren, Sander, Frazer (Multi-PI)

F-3044

AN IN VITRO MODEL OF MYOTONIC DYSTROPHY TYPE 1 USING HUMAN EMBRYONIC STEM CELL-DERIVED SKELETAL MUSCLE

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Myotonic Dystrophy Type 1 (DM1) is the most common form of adult-stage onset muscular dystrophy with a prevalence of 1/8,000 worldwide. Patients affected by DM1 display a range of symptoms (e.g. muscle wasting, intellectual disability, and infertility). DM1 is an autosomal-dominant, multisystemic genetic disorder characterized by CTG triplet repeat expansion in the 3' untranslated region of the dystrophia myotonica protein kinase (DMPK) gene, with larger repeat size directly correlating to higher disease severity. The CTG repeat expansion leads to sequestration of splicing regulators (such as Muscleblind Like Splicing Regulator 1 [MBNL1]) in nuclear foci containing DMPK mRNA, and subsequent splicing defects (e.g. exon inclusions and exclusions). In this work we differentiated unaffected control and DM1-affected (Genea067 [1626/1773 CTG], Genea157 [1060 CTG], Genea158 [180 CTG]) human embryonic stem cell lines to skeletal muscle (i.e. myoblasts and myotubes) using Genea Biocells' published uniquely robust and efficient protocol. In this study, we investigated these cells phenotype with the aim to establish a clinically relevant stem cell-based disease model of DM1. We first examined lineage commitment and differentiation kinetics of Genea067, Genea157, and Genea158 myoblasts to terminally differentiated myotubes and found that affected cells display aberrant myotube formation previously reported in animal models and the infant-onset form of DM1. At a molecular level, we used PCR and Nanostring gene expression analysis of known DM1 splicing defects to characterize differences in mRNA transcripts and observed evidence for impaired splicing. We further investigated cellular metabolism and energetics using Seahorse Technologies' XF Cell Mito Stress test to understand ATP production, basal and maximal respiration. We found an inverse relationship between disease severity, i.e. number of CTG repeats, and mitochondrial respiration. Together, this work demonstrates the usefulness of DM1-affected human pluripotent stem cell-derived skeletal muscle to accurately model DM1 in vitro, recapitulating known phenotypes that correlate with disease severity as well as tissue specific mechanisms. This model represents a new and attractive tool to develop and test muscle-targeted therapeutics for DM1.

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F-3046

DISEASE MODELING OF PRIMARY CILIARY DYSKINESIA USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Primary ciliary dyskinesia (PCD) is a predominantly autosomal recessive disorder of dysfunctional ciliary motility with reduced muco-ciliary clearance. Patients suffer from repeating respiratory infections and sometimes result in the irreversible respiratory failure. In general, definitive diagnosis of PCD is not easy, because clinical symptoms are heterogeneous, significant portion of the patients have normal ciliary structure by electron microscopy, and causative genetic mutations are so diverse that genetic testing is imperfect. Then, it has been difficult to elucidate the human pathology of PCD, due to limited source of clinical samples and difficulty of culturing patient-derived multi-ciliated airway cells (MCACs). In the previous study, we established the stepwise method of inducing functional MCACs in airway organoids derived from human induced pluripotent stem cells (hiPSCs), recapitulating the developmental stages. In the hope of applying our method to disease modeling, we generated genetic knockout hiPSCs of PCD causative genes (RSPH4A and DNAH11) by using a CRISPR-Cas9 system. And we differentiated these knockout iPSCs into MCACs and attempted to recapitulate the pathology of PCD in vitro. RSPH4A-deficient hiPSCs-derived MCACs showed a decrease in ciliary beating frequency by high-speed camera analysis and central microtubular abnormalities by electron microscopic study. On the other hand, DNAH11-deficient MCACs represented an increase in ciliary beat frequency in spite of their normal ciliary ultrastructure. Additionally, these newly generated hiPSC-derived MCACs demonstrated abnormal pattern of ciliary movement and showed reduced muco-ciliary transport by analyzing fluorescent beads flow placed on their epithelial cell sheets. These results were consistent with the previous reports of PCD patients with genetic abnormality of RSPH4A or DNAH11, respectively. In conclusion, disease modeling of PCD in vitro by using

hiPSCs would be beneficial for understanding the human pathology of PCD and be a potential platform for making a diagnosis and finding therapeutic targets in the future.

F-3048

DRUG DISCOVERY AND TARGET VALIDATION FOR RARE NEUROLOGICAL DISEASES USING IPSCS AND CRISPRS IN LAB COURSES AND RESEARCH LABS AT PRIMARILY UNDERGRADUATE INSTITUTIONS

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Rare and ultra-rare neurological diseases often carry similar cellular pathologies to more common or sporadic human neurological diseases. For example, 14 clinically distinct neurodegenerative diseases share the common pathology of abnormal intracellular accumulation of the microtubule associated protein tau (MAPT gene), however the affected cell type (neuron, oligodendrocyte, astrocyte, etc), predominant tau isoform, region of the brain, and whether this accumulation is considered a primary or secondary pathology varies widely between each of these diseases. Our goal is to understand how human neurons and astrocytes utilize the autophagy and ubiquitin proteasome pathways to survive cellular injury associated with toxic accumulation of proteins, in the case of proteinopathies, or of other lipids, polysaccharides, or nucleic acids, in the case of rare neurological diseases. Niemann-Pick disease types C1 and C2 are rare lysosomal storage diseases that result from mutations in the NPC1 or NPC2 cholesterol transporter genes, respectively, and are both secondary tauopathies. Prior work from our group indicates that failure to clear autophagic vacuoles results in the neurotoxic accumulation of depolarized mitochondria fragments in neurons derived from NPC1 patient induced pluripotent stem cells (iPSCs). We recently performed a drug screen against mitochondria membrane polarization in neurons derived from an NPC1 patient iPSC line with nearly complete loss of NPC1 gene function. Our on-going research program employs inducible CRISPR interference/activation (iCRISPRi/a) tools in human iPSC models of NPC1 and NPC2 diseases to dissect the molecular mechanisms of these drugs on the autophagy pathway, mitochondria homeostasis, neuronal viability, cholesterol mobilization, expression and post-translational regulation of NPC1, NPC2, and NPC1L1 gene products in neurons. Using CRISPR/Cas9-based genome engineering, our students are generating endogenously *mCherry*- or GFP- tagged NPC1, NPC2, or NPC1L1 HEK293t and human iPSC lines that are coupled with our iCRISPRi/a tools for high resolution analysis of the molecular pathogenesis of NPC1 and NPC2 disease in human neurons.

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F-3050

FUNCTIONAL ANALYSIS OF CARDIAC-SPECIFIC REGULATORY ELEMENTS INVOLVED IN HEART DEVELOPMENT AND DISEASE

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In Australia, more than 2000 children are born with heart malformations every year, characterised as Congenital Heart Disease (CHD). However, the majority of causes are still unknown. There is growing evidence that mutations not only in protein-coding DNA but also in non-coding DNA, functioning as cis-regulatory elements (CREs) controlling gene expression, can cause malformations. Consequently, we hypothesise that heart defects result from disrupted cardiac gene expression, which are triggered by changes in the cis-regulation of these genes. To address this, we sought to identify CREs essential for human cardiac function. First, we identified the binding locations of the cardiac transcription factor NKX2-5 genome-wide, using chromatin immunoprecipitation followed by deep-sequencing (ChIP-seq) in cardiomyocytes derived from human embryonic stem cells. This experiment provided an initial set of cardiac CREs, that we mined using an in-house bioinformatics comparative genomics pipeline to provide an extensive atlas of regulatory regions potentially controlling gene expression in the human heart. In order to validate the cardiac activity of these CREs in vivo, we performed transgenesis assays in medaka (Oryzias latipes). In this study, we demonstrate that the function of human cardiac-specific regulatory elements can be systematically and rapidly characterised in vivo in medaka. Subsequently, the most promising candidates will be functionally tested in vivo using the CRISPR/Cas9 system and in vitro using cardiomyocytes derived from human embryonic stem cells. By deciphering the conserved cis-regulatory landscape driving cardiogenesis and functionally analysing them in human and fish, we hope to determine the cis-regulatory elements vital in the formation of the heart and therefore heart disease.

F-3052

STEM CELL-DERIVED HUMAN NEURONAL MODELS TO STUDY NEUROTROPHIC VIRUSES

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Neurotrophic viruses present a huge challenge to human wellbeing, and are an increasing cause of morbidity and mortality worldwide. These viruses have greater impact compared to other pathogens, mainly because they target neuronal tissue, resulting in high rates of mortality and when patients survive, these viruses leave severe and long lasting damage in the nervous system. For example Lyssavirus, including Rabies, causes highly fatal encephalitis with no effective cure once clinical symptoms appear. A particular challenge in studying these viruses is the necessity to use host and cell-specific in-vitro models. Using embryonic and pluripotent stem cells, we have developed human forebrain-type neurons to study the pathogenesis of existing and emerging neurotrophic viruses. Using human forebrain-type neurons generated from stem cells, we aim to i) use specialised microfluidic chambers that separate the axons and cell body of neurons to assess the ability of Rabies virus entry into neuronal axons at the neuromuscular junction and transmit along synaptically connected neuronal axons ii) investigate the pathogenesis of Hendra virus, that causes acute respiratory illness and encephalitis to understand the cellular mechanisms of Hendra virus assembly, budding and transmission in human neurons, iii) transcriptionally profile small non-coding RNAs secreted by the stem cell human neuronal cultures infected by Rabies, Hendra and West Nile virus. These stem cell systems will reveal fundamental insights of viral pathogenesis and identification of novel biomarker signatures specific to each of these viruses will lead to the development of cutting edge next-generation diagnostic devices for the early detection of infectious diseases before clinical symptoms can be observed.

F-3054

REGENERATIVE POTENTIAL OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM PATIENTS UNDERGOING HEMODIALYSIS

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Recently, we developed a strategy to eliminate native nephron progenitor cells (NPCs) from the nephrogenic area, and replace them with allogeneic and xenogeneic exogenous NPCs. By using chronic kidney disease (CKD) patient-derived NPCs differentiated from induced pluripotent stem cells (iPSCs) as the cell source for this strategy, we aimed to generate CKD patient-derived kidneys while circumventing current problems such as organ shortages and immune rejection. Although CKD impairs stem cell functions, including proliferation, differentiation, and angiogenesis, iPSCs are expected to be unaffected by CKD. Hence, we generated iPSCs from patients undergoing hemodialysis (HD-iPSCs), as well as from age- and sex-matched healthy controls (HC-iPSCs). By examining the different biological properties of iPSCs and iPSC-derived products (including NPCs and nephrons), we assessed the potential of HD-iPSCs as a source of cells for kidney regeneration. HC- and HD-iPSCs were differentiated into spheres, including NPCs, using an embryoid body-mediated differentiation protocol. Gene expression, immunostaining, and flow cytometry analyses of these spheres showed that HD-iPSCs could differentiate into NPCs as efficiently as HC-iPSCs. Glial-cell derived neurotrophic factor (Gdnf) secreted from NPCs induces branching of the ureteric bud, while signals from this bud induce differentiation of NPCs into nephrons. Thus, Gdnf is a key signal for kidney development. We observed no significant difference in Gdnf expression between isolated HC- and HD-iPSC-derived NPCs. Next, we assessed the differentiation ability of iPSC-derived NPCs and the angiogenic function of iPSC-derived nephrons, and compared cells from patients on hemodialysis with healthy controls. Morphological and immunological analyses showed that HD-iPSC-derived NPCs differentiated into nephrons and attracted blood vessels as efficiently as HC-iPSC-derived NPCs. Overall, HD-iPSC-derived NPCs may be a suitable cell source for our strategy in terms of Gdnf expression, differentiation ability, and angiogenic function. This

study suggests not only the usefulness of HD-iPSCs and HD-iPSC-derived NPCs for our kidney regeneration strategy, but also for other strategies, and paves the way for patient-derived kidney regeneration.

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F-3056

HUMAN PLURIPOTENT STEM CELL-DERIVED CELLULAR MODELS REVEAL INSIGHTS INTO ZIKA VIRUS PATHOGENESIS

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Pluripotent stem cell-derived progenies represent an alternative cellular model to primary cells for the studies of viral pathogenesis. We have developed three distinct lineages of PSC-derived cells: monocytes/macrophages, neuronal cells and blood brain barrier (BBB) endothelial cells, for the study of Zika virus (ZIKV) infection. We first produced human cortical neural progenitors, the cells responsible for the development of human cortex, the part of the brain that is underdeveloped in microcephalic newborns and exposed these cells to ZIKV, followed by determination of infection rate and cell cycle analysis. We show that ZIKV infection of NPCs results in a pronounced S-phage arrest as a result of DNA damage checkpoint activation induced by viral replication. Related flaviviruses such as West Nile virus or dengue virus (DENV) did not arrest NPCs despite similar infection efficiency. Importantly, S-phase arrest also selectively enhanced ZIKV replication. Next, we investigated differential cellular responses to ZIKV and DENV by PSC-derived macrophages, which are developmentally related to York-sac derived tissue macrophages. ZIKV and DENV both efficiently infected macrophages derived from either embryonic or induced PSCs, but again the cellular responses including the induction of inflammatory cytokines and chemokines, to these two viruses are drastically different. Overall our macrophage experiments demonstrate the utility of PSC-derived macrophages for arboviruses and suggest that distinct impact of ZIKV and DENV on macrophage function underlie different pathogenesis of the two viruses. Finally, we have established PSC-derived BBB endothelial cells investigate the abilities of neurotropic versus non-neurotropic flaviviruses to infect these cells and to aid in the development of co-culture models that incorporate ECs, astrocytes, and neurons.

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F-3058

USING IPSCs TO DETERMINE THE VALIDITY OF GENETIC VARIANTS ASSOCIATED WITH SENSITIVITY TO ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN CHILDHOOD CANCER

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Following anthracycline exposure up to 20 % of children being treated for cancer develop the adverse drug reaction of cardiotoxicity. Anthracycline-induced cardiotoxicity (ACT) has variable severity ranging from asymptomatic echocardiographic findings of cardiac dysfunction to the development of congestive heart failure. ACT can have an early-onset, defined as developing within 1 year of treatment, or a late-onset, with increasing cumulative incidence up to 30 years post-treatment. Susceptibility to the development of ACT is known to have a genetic basis. Currently, predicting which patients will develop ACT is not possible. A retrospective cohort of 286 paediatric cancer patients exposed to anthracyclines have been recruited and 56 patients had evidence of ACT upon echocardiogram at follow-up. Peripheral blood samples were collected and iPSCs were generated from patients with severe cases of ACT (N=16). Case-control matched iPSCs have been differentiated into cardiomyocytes and whole exome sequencing (WES) has identified several novel candidate genetic variants enriched amongst patients sensitive to ACT compared to those who were resistant. Patient specific iPSC derived cardiomyocytes have been demonstrated to replicate, at a cellular level, the clinical presentation of patients. We plan to use a suite of functional assays, including cell viability and DNA damage, to establish if these novel genetic variants underlie the pathogenesis of ACT using iPSC derived cardiomyocytes as a model.

F-3060

MODELING CARDIAC DYSFUCNTION OF FRIEDREICH'S ATAXIA USING VENTRICULAR SHEETS, TISSUES AND CHAMBERS ENGINEERED FROM HUMAN PLURIPOTENT STEM CELLS

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Friedreich's ataxia (FRDA) is an autosomal recessive inherited disease that causes progressive damage to the nervous system by a mutation in the frataxin (FXN) gene. FXN, a mitochondrial protein involved in the biosynthesis of iron-sulfur proteins, is essential for oxidative metabolism. Hence, due to its high energy consumption, the heart is one of the first organs presenting pathological symptoms of FRDA. Indeed, cardiac dysfunction is the leading cause of death in FRDA patients. Although transgenic mouse models of FRDA have been previously created, the genotype, severity and disease phenotypes of human patients are not reproduced. To overcome this limitation, human ventricular cardiac anisotropic sheets (hvCAS) and tissue strips (hvCTS) were generated from human embryonic stem cell (hESC)- and induced pluripotent stem cell (hiPSC)-derived ventricular cardiomyocytes (VCMs) for modeling FRDA's electrophysiological and contractile defects, respectively. Lentivirus (LV)-mediated shRNA transduction of hESC- and iPSC-VCMs, as well as reprogrammed FRDA-specific iPSC-VCMs, displayed significantly suppressed FXN transcript and protein levels compared to controls. High-resolution optical mapping of hvCAS revealed such electrophysiological defects of LV-shRNA-transduced hESC and FRDA-iPSC preparations as reduced maximum capture frequency (MCF) and prolonged action potential duration consistent with a T-wave inversion observed in patient electrocardiograms. In the hvCTS assay, developed force at 1-Hz pacing was consistently suppressed (by 55-80%) in LV-shRNA and FRDA groups vs. control, displaying a strong positive correlation with FXN expression. Finally, rescue experiments were performed via forced FXN expression in LV-shRNA-hESC- and FRDA-hiPSC-hvCTS, reversing the reduction in developed force in both FRDA models. As further validation, cardiac organoid chambers (hvCOC; our "human heart-in-a-jar" model), are being tested so that clinically relevant and physiologically complex parameters such as ejection fraction, cardiac output, and pressure-volume loops could be obtained for thorough analysis. We conclude that these human-based FRDA models provide a biomimetic platform suitable to facilitate the studies of disease pathogenesis and pharmaceutical testing.

F-3062

INDUCED PLURIPOTENT STEM CELLS WITH KIT D816V MUTATION FOR MODELLING MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Myeloproliferative neoplasms (MPN) are chronic malignancies of the myeloid lineage, which are associated with specific point mutations in key signalling pathways, including the stem cell factor (SCF) receptor tyrosine kinase KIT. KIT D816V is a constitutively active version of KIT and causes a MPN phenotype in the mast cell compartment. 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 therapy, rendering this a fatal disease. We have generated a panel of induced pluripotent stem cells (iPS cells) of KIT D816V MPN 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. The KIT D816V progenitor cells obtained showed characteristic expression of CD31, CD43, CD45 and KIT (CD117). Importantly, the KIT D816V mutation impacted on specific hematopoietic progenitor populations, which are currently being analyzed for KIT downstream signalling and screened for novel inhibitors. As expected KIT D816V progenitors showed characteristics of constitutive KIT signalling. KIT D816V progenitors and unmutated progenitors were also differentiated into mast cells, which are currently being studied. In summary, we established iPS cell based disease model for KIT D816V malignancy that is most suitable for molecular studies and for screening of novel therapeutics. Our current activities focus on automation of iPS cell production and CRISPR/Cas genome editing to generate further human disease models.

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REPROGRAMMING

F-3066

DIRECT CONVERSION OF HUMAN PLURIPOTENT STEM CELLS TO SENSORY NEURONS BY TRANSCRIPTIONAL REPROGRAMMING

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The in vitro production of peripheral sensory neurons from pluripotent stem cells has been traditionally accomplished by complex induction protocols, often time-consuming or ineffective. To overcome these obstacles, we developed a simplified system to rapidly and efficiently generate human induced sensory neurons. First, we engineered a commonly used human pluripotent stem cell (hPSC) line to harbor a set of sensory neuron developmental transcription factors under the control of a doxycycline-inducible promoter. In the absence of doxycycline, expression of the transgene cassette was virtually undetectable, and engineered hPSCs retained pluripotent characteristics, amenable to passaging and cryopreservation. Upon exposure to doxycycline, the transgenes were highly activated, concomitant with a rapid morphological change in the hPSCs. Within seven days, >90% of cells adopted neuronal morphology and expressed both pan-neuronal and sensory-specific markers. We observed widespread expression of peripherin in the converted cells - a neurofilament exclusive to peripheral sensory neurons - which was undetectable in hPSC-derived cortical neurons. Further, we detected mRNA transcripts for a variety of receptor genes that bestow specific sensory functions in peripheral neurons, including TRPM8, TRPA1, P2RX3, and PIEZO2, the latter being a known mediator of touch sensation in humans. In response to mechanical stimulation, we measured robust PIEZO2-mediated currents in the converted cells, a hallmark of mechanosensory neurons in vivo. This streamlined differentiation paradigm may prove valuable in understanding human sensory neuron development and regeneration, studying disorders of the peripheral nervous system, or high-throughput screening of therapeutics.

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F-3068

UTILIZING A WHOLE GENOME CRISPR-CAS9 SCREEN TO IDENTIFY GENETIC MECHANISMS INVOLVED IN MOUSE PLURIPOTENT STEM CELL INDUCTION

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Understanding the mechanistic details of reprogramming is key to safely and efficiently harnessing the potential of reprogramming somatic cells to a pluripotent state for regenerative medicine applications and for modelling various diseases for drug discovery. We have profiled the transcriptomic changes that occur during reprogramming and identified three distinct phases of reprogramming: initiation, maturation, and stabilization. In addition, RNAi screening showed key roles for mesenchymal to epithelial transition and alteration of the splicing program during the early initiation phase. However, due to the potentially low efficiency of RNAi screens, there may remain a plethora of as yet unidentified genes that regulate somatic cell reprogramming during the different phases. Here, I am conducting an unbiased whole genome CRISPR-cas9 knockout screen on reprogramming MEFs using a lentiviral sgRNA-Cas9 whole-genome library with the goal of identifying novel regulators of reprogramming. By utilizing specific sgRNAs as individual barcodes, it will be possible to assess the temporal changes in clonal populations in parallel with monitoring guides that selectivity drop in or out of the population. This leads to a robust detection of sgRNA enrichments at the three phases of reprogramming and ultimately will define regulators that act as potential repressors at each stage of reprogramming. The newly identified regulators of reprogramming via CRISPR screening will not only shed new light on the reprogramming process, but also provide new avenues to manipulate the reprogramming process.

F-3070

OPTIMISING THE DERIVATION OF MATURE CORTICAL NEURONS FROM ADULT HUMAN INDUCED NEURAL PRECURSOR CELLS USING IN VITRO AND EX VIVO SYSTEMS

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The study and treatment of neurological disorders has been hampered by a lack of access to live human neurons. The advent of cell reprogramming technologies offers new and exciting avenues for the generation of live human neurons. While all strategies come with many advantages, each is limited primarily by the inability to produce high yields of authentic neurons while presenting safety concerns associated with the use of integrating gene delivery systems. Our laboratory has developed an efficient chemically modified mRNA (cmRNA)-based system to directly reprogram adult human dermal fibroblasts (HDFs) to human induced neural precursor (hiNPs) cells by transient ectopic expression of two neural genes, SOX2 and PAX6. Using our direct-to-iNP technology, this study optimised the derivation of cortical neurons from cmRNA-hiNPs using in vitro and ex vivo systems. The generation of cortical neurons from cmRNA-hiNPs was first optimised by investigation of a variety of reprogramming and differentiation techniques, which culminated in the development of a BrainPhys™ with REPSOX-reprogramming protocol combined with mixed cortical differentiation in astrocyte-conditioned medium (ACM). BrainPhys™ with the addition of REPSOX as the base medium for reprogramming promoted a consistent and large up-regulation of the glutamatergic precursor marker TBR2. The use of ACM as the base medium for cortical differentiation induced a greater expression of NSE and VGLUT1 compared to NBA-based differentiation media and significantly increased the proportion of TUJ1+ cells from ~9% to ~48%, while reducing the percentage of S100ß+ astrocytes from ~80% to ~20%. ACM rather than NBA supported the derivation of cortical glutamatergic neurons with ~79% VGLUT1+/TUJ1+ cells. We also confirmed the ability of cmRNA-hiNPs to differentiate into TUJ1+ neurons with long neurite extensions in a 3D matrix system using a photo-initiated gelatin-methacryloyl hydrogel. Lastly, cmRNA-hiNPs transplanted onto an established AMPA-treated rat ex vivo organotypic slice culture system gave rise to a population of TUJ1+ and MAP2+ neurons. Although further functional work is warranted, this study demonstrates that cmRNA-hiNPs can differentiate into dorsal forebrain glutamatergic neuronal-like cells both in vitro and ex vivo.

F-3072

KLF4-DOSE DEPENDENT METABOLIC SHIFT THROUGH TCL1 DURING IPSC GENERATION

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Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) is accompanied by morphological, functional, and metabolic alterations before acquisition of full pluripotency. Although the genome-wide effects of the reprogramming factors on gene expression are well documented, precise mechanisms by which gene expression changes evoke phenotypic responses remain to be determined. We used a Sendai virus-based iPSC generation system that permits reprogramming to progress in a strictly Klf4-dose dependent manner to screen for Klf4 target genes that are critical for the progression of reprogramming. The screening identified Tcl1 as a critical target gene that directs the metabolic shift from oxidative phosphorylation to glycolysis. High-dose Klf4-induced Tcl1 employs a two-pronged mechanism, whereby Tcl1 activates AKT to enhance glycolysis and counteracts PnPase to diminish oxidative phosphorylation. Moreover, an additional overexpression of Tcl1 could show quick and efficient reprogramming. Thus, these regulatory mechanisms highlight a central role for a reprogramming factor in orchestrating the metabolic shift toward the acquisition of pluripotency during iPSC generation. We will also discuss Klf4-dose dependent transcriptional regulation of Tcl1.

F-3074

DIRECT REPROGRAMMING OF MOUSE FIBROBLASTS INTO ANTIGEN-PRESENTING DENDRITIC CELLS

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Cell fate reprogramming of adult cells towards pluripotency or unrelated somatic cell-types has been demonstrated and explored in the context of regenerative medicine and cell replacement therapy. Dendritic cells (DCs) are professional antigen presenting cells (APCs) that initiate adaptive immunity by recognizing, processing and presenting antigens to T-cells. We hypothesized that the unique properties of DCs could be induced by cell reprogramming allowing the direct control of immune responses. Here, the requirements to impose DC-fate in unrelated cell-types

were investigated using combinatorial overexpression of Transcription Factors (TFs) in Clec9a-tdTomato mouse fibroblasts. This reporter system specifically marks the conventional DC lineage. We have identified Pu.1, Irf8 and Batf3 (PIB) as sufficient and necessary to induce reporter activation and establish DC morphology. The activation of the reporter occurs within 48 hours without cell division and its efficiency is increased by including additional TFs. Induced DCs (iDCs) express cDC1 markers and MHC-II and the co-stimulatory molecules CD40, CD80 and CD86 at the cell surface, essential for antigen presentation. Overexpression of PIB activates a genome-wide conventional DC type 1 (cDC1) transcriptional program. Pseudo-time reconstruction of the reprogramming trajectory with single cell transcriptomes revealed an iDC state showing interferon-induced maturation traits. Functionally, iDCs engulf particles, proteins and dead cells and upon stimulation of toll-like receptors, secrete inflammatory cytokines. iDCs capture, process and present antigens to CD4+ T-cells, inducing their proliferation and activation. Remarkably, iDCs have established the competence for cross-presentation, a hallmark of DCs, eliciting antigen-specific CD8+ T-cell responses. Hence, we provide evidence that antigen presentation and cross-presentation can be dynamically programmed by a small combination of TFs, opening avenues for understanding and inducing immunity with direct cell reprogramming. These findings provide insights into DC developmental specification and a platform for future development of patient-specific DCs for immunotherapy.

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F-3076

REPROGRAMMING OF PRIMARY SOMATIC CELLS DERIVED FROM BRAIN CANCER PATIENTS USING MRNA TOOL

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The ability to generate patient's induced pluripotent stem cells (iPSCs) could provide tremendous promises for regenerative medicine. It has reported that choice of reprogramming tools is essential for the safety and the increasing efficiency of reprogramming process and transduction. Non-integrating techniques as like mRNA based reprogramming protocols are available for the production of genetically stable iPSCs while avoiding the risks of genomic integration. In this study, we generate human iPSCs derived from brain cancer patient's fibroblast by mRNA's reprogramming tool. We obtained patient's somatic tissues at brain tumor surgery that informed consent obtained according to institutionally-approved protocols. Patient's primary somatic cells were isolated, and then used as the cell source for iPSCs generation under xeno-free conditions. Reprogramming produced by non-integration methods utilized the combination of reprogramming mRNAs (OSKMNL) with evasion mRNAs (EKB) (Stemgent) during four days. Reprogrammed cells showed the colonies like iPSCs after six days of transduction, and the colonies were expanded until pick-up at day 14. These colonies were stained for pluripotency-associated genes using TRA-1-60 and TRA-1-81 by immunocytochemistry. Established colonies were also expanded without feeder condition and stained with Alkaline Phosphatase. In the further study, generated patient's iPSCs will be required to investigate their availability of the clinical application under xeno-free conditions which could provide a path to GMP applicability that should facilitate the clinical implementation of patient- or disease-specific iPSCs therapies.

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F-3078

TRANSCRIPTOMIC AND METABOLIC ANALYSES REVEAL REPROGRAMMING CONDITIONS IMPACT THE HUMAN IPSC RESPONSE TO OXYGEN AND IDENTIFY RETENTION OF SOMATIC CELL METABOLIC MEMORY

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Oxygen is a key regulator of stem cell metabolism and fate. Induced pluripotent stem cells (iPSC) are routinely derived and cultured in atmospheric (~20%) oxygen, despite evidence that physiological (~5%) oxygen improving embryonic stem cell (ESC) establishment, metabolism and pluripotency. Reprogramming to pluripotency invokes profound metabolic changes, yet it is not known how modulating metabolism during reprogramming may affect the phenotype of resultant iPSC. This study characterised and compared the transcriptomic and metabolic phenotypes of human iPSC lines reprogrammed under 20% or 5% oxygen and subsequently challenged with 5% or 20% oxygen in culture. Neonatal human dermal fibroblasts (NHDF) were reprogrammed with Sendai virus containing OSKM under either 20% or 5% oxygen. Resultant iPSC were maintained in their respective oxygen conditions or challenged with the opposing oxygen concentration. Differentially expressed genes (DEGs) were identified by RNAseq analysis. The carbohydrate utilisation of NHDF and iPSC at 20% and 5% oxygen was measured. RNAseq revealed that at 5% oxygen, genes encoding

glycolysis, histone demethylation and stem cell naïvety were significantly (FDR adjusted p < 0.05) upregulated. For iPSC reprogrammed at 20% oxygen, 1,050 genes were differentially expressed due to oxygen in culture, while iPSC reprogrammed at 5% oxygen had 287 DEGs. 206 DEGs were shared between cells reprogrammed at each oxygen condition. Lactate production and glucose to lactate flux in both iPSC and NHDF were increased at 5% oxygen (p < 0.05), while in contrast to ESC, glucose consumption was not altered. These data highlight the key role of oxygen in regulating and modulating the transcriptomic and metabolic profiles of iPSC. In addition, these data indicate that metabolic modulation by oxygen during reprogramming impacts how resultant iPSC respond to oxygen in culture. Metabolic differences with ESC, and similarities with NHDF, suggest that iPSC metabolic reprogramming is incomplete, resulting in a retention of somatic cell metabolic memory and a perturbed regulation of iPSC physiology. These perturbations may plausibly persist in iPSC derived differentiated populations, and impact downstream applications for disease modelling and cell therapy.

F-3080

AN OPTIMIZED APPROACH FOR THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD DERIVED ERYTHROID PROGENITORS.

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Generation of human induced pluripotent stem cells (iPSCs) from cultured erythroid precursors is considered to be highly suitable for clinical applications due to their higher genome stability. However, several studies have shown that generation of iPSCs from these cells is highly inefficient. We hypothesized that this may be due to the continuous differentiation of erythroid cells while they are being reprogrammed and lack of use of the most suitable reprogramming factors that can increase the reprogramming efficiency. We incorporated two modifications in the protocol for reprogramming of erythroid cells; one, culture conditions that favor the robust expansion of early erythroid precursors without terminal differentiation, and, two, using alternative combinations of reprogramming factors. Using a xeno-free medium established in our laboratory we could selectively expand up to 5x106 erythroid precursors from 1x107 peripheral blood mononuclear cells in 8 days of culture. Flow cytometry analysis showed CD71 (81.2 3 8.8) and CD235a (48 3 12.7) expression, with the negligible expression of lymphocyte markers, CD3 and CD19. No significant difference in the differentiation of erythroid cells was observed till day 14. Previous studies have shown that a combination of plasmids to express OCT3/4, SOX2, KLF4, L-MYC, LIN28 and a p53 shRNA (Y4 combination) has the highest efficiency for reprogramming of many cell types, but it had an extremely low efficiency in reprogramming of erythroid cells. We compared the efficiency of Y4 with that of Y2 combination that expresses OCT3/4, SOX2, KLF4, C-MYC, LIN28, NANOG and p53 shRNA. Earlier reports had shown that the Y2 combination had an extremely low efficiency in all the tested cell types. We found that Y2 is able to provide 6-10 fold higher efficiency than Y4 in the reprogramming of erythroid cells and this combination showed colony formation from day 6 after nucleofection. All the iPSC clones generated with Y2 and Y4 combinations exhibited all the properties of pluripotency. Overall, this study demonstrates an optimized approach to obtain early-stage erythroid precursor cells and the Y2 combination of episomal plasmids provide high reprogramming efficiency erythroid cells.

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F-3082

GENERATION OF HUMAN NEPHRON PROGENITORS USING INDUCIBLE PIGGYBAC TRANSPOSON EXPRESSION OF SNAI2-EYA1-SIX1

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Cellular reprogramming holds great promise for the development of desperately needed novel treatment options for chronic kidney disease (CKD). The functional units of the kidney, known as nephrons, arise from a population of embryonic nephron progenitors (NPs). However, this population is depleted near birth in a final wave of nephron formation that renders the mature kidney unable to form new nephrons regardless of damage or disease in later life. Recreation of NPs may allow regeneration of entire nephrons, making the NP population an ideal target cell for cellular reprogramming approaches to generate alternate CKD treatment options. Using a lentiviral screening approach, we previously identified 6 transcription factors (SIX1, SIX2,

HOXA11, OSR1, EYA1 and SNAI2) sufficient to re-impose a NP-like state when co-expressed in adult human kidney epithelial (HK2) cells (Hendry et al., JASN, 2013). To improve this reprogramming and allow transferability to in vivo models of kidney disease, we have now developed an inducible, multicistronic piggyBac transposon construct and demonstrate efficient reprogramming of human cells to iNPs with just three transcription factors (SNAI2, EYA1 and SIX1). Coupled with maintenance in culture conditions supportive of NPs, the resulting population not only demonstrates phenotypic characteristics of endogenous NP cells, but can contribute to the formation of new nephrons in vitro, ex vivo and in vivo, as well as contribute to the nephron epithelium after acute kidney injury in mice. These results not only demonstrate the feasibility of transposon-based direct reprogramming, but also bring us closer to realizing patient-specific reprogramming to NPs for cellular therapies, bioengineering applications and nephrotoxicity screening.

F-3084

MANUFACTURING OF cGMP HUMAN STEM CELLS FOR CLINICAL TRANSLATION

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High quality clinical-grade cells are a critical requirement for the clinical translation of induced pluripotent cells (iPSCs) and their derivatives. However, the availability of such cells remains a major bottleneck for the advancement of iPSC-based cell therapies. Toward fulfilling this gap, Allele Biotechnology has developed a state-of-art current Good Manufacturing Practice (cGMP) stem cell manufacturing facility, with clean room working area up to ISO 5 specifications. At the core of our mission is the Research and Development division, which has already developed over twenty different stem cell differentiation platforms, which can be used for drug screening or transferred through our pipeline for cGMP manufacturing for cell therapy. Before transferring the procedures to the Manufacturing division, we follow a complete suite of risk analyses, in-process controls, and pre-validation steps. By adhering strictly to United States Pharmacopeia (USP) guidance, we have developed a rigorous Quality System to consistently produce and control the manufacture of clinically relevant cells. In the current presentation, we will provide an overview of our cGMP manufacturing pipeline. We will focus mainly on the manufacturing of iPSC and its derivatives by discussing the equipment, materials, personnel, documentation processes, and quality control measures involved before releasing them to the clinic. Finally, we will provide examples of the cGMP cell lines manufactured in our facility.

F-3086

A HYBRID CELL STATE IS REQUIRED IN THE EARLY STAGE OF CHEMICAL REPROGRAMMING TO PLURIPOTENCY

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The generation of chemically-induced pluripotent stem cells (CiPSCs) provides a new path in somatic reprogramming and a new paradigm to study the mechanism of cell fate reprogramming and determination. Although we have uncovered an extraembryonic endoderm (XEN)-like state that mediates chemical reprogramming from fibroblasts to CiPSCs, it is still unclear how the chemical compound combinations determine the XEN-like cell fates in the initial stage of chemical reprogramming. Here, by using both bulk and single-cell RNA sequencing for time-series samples in the early stage of chemical reprogramming from fibroblasts to XEN cells, we found the down-regulation of fibroblast genes and the up-regulation of XEN markers, such as Sox7, Sox17 and Sall4, within the initial 4 days of chemical reprogramming. Unexpectedly, we also found the up-regulation of many neuronal genes, as well as many other lineage-specific transcription factors such as Shox2, Maf, Ascl1, Sst, Zic1, Hnf1b, Nkx6-1, Gata2 and Gata3. Interestingly, single cell RNA sequencing analysis revealed a continuous reprogramming trajectory that undergoes a “hybrid cells” state, which mainly co-express XEN master genes and neuronal genes, around day 4 to day 8. Whereafter, the expression of neuronal genes decreased and the co-expression of XEN genes was getting to be more frequent until the formation of XEN-like cells at day 12. Moreover, knock down of Ascl1 or Zic1 hampered the generation of XEN, suggesting that the hybrid cell states are required for early chemical reprogramming. In summary, our findings suggest a

"diversify and specify" model rather than "hierarchical model" in the early stage of chemical reprogramming. The hybrid cell state uncovered in our study could also provide new opportunities to induce the cell fate reprogramming from fibroblasts into other cell lineages, or to improve the XEN-like cell generation for the further induction of CiPSCs.

Funding Source: Center for Life Sciences, Peking University

F-3088

OCT-3/4 AND KLF4 SYNERGISE TO TARGET CELL CYCLE REGULATORS DURING REPROGRAMMING

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The decision to replicate is controlled by checkpoints located in the G1 phase and leads to cell type specific cell cycle length that can vary from 30 hours for mouse embryonic fibroblasts (MEF) to ~12 hours for mouse embryonic stem cells (mES). Alterations in cell cycle time have been attributed to altered control of regulators of G1, including cyclins, cyclin dependant kinases (CDKs), CDK inhibitors, Retinoblastoma protein (pRb), Rb-related pocket proteins and other accessory factors. Reprogramming of MEFs to an induced pluripotent state offers an excellent system to study stage-specific cell cycle control. During the reprogramming process, two important early programs are altered. First, there is a dismantling of the controls that regulate the somatic cell cycle and a conversion to a shortened cell cycle with reduced gap phases, resulting in a rapid proliferation rate. Second, is a cell fate change involving the Mesenchymal to Epithelial transition (MET). In order to study the mechanisms that effect these changes, we designed an inducible reprogramming switch using a 4-hydroxy Tamoxifen (OHT) dependent Oct-3/4. Using this inducible system we find that Oct-3/4 and Klf4 can trigger the initial changes that lead to the speeding up of the cell cycle, a crucial step in the initial phase of reprogramming. We also show that Oct-3/4 and Klf4 acting in concert initiate MET by upregulating E-Cadherin and downregulating N-Cadherin, important epithelial and mesenchymal markers. These results suggest that Oct-3/4 and Klf4 are crucial for the initial phase of reprogramming and may also play a key role in inducing/maintaining the shortened cell cycle in mES.

LATE BREAKING ABSTRACTS

F-4002

MESENCHYMAL STEM CELLS AND NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS: A SHOW CASE OF MULTIMODAL APPROACHES TO INDUCE OSTEOSARCOMA CELL DEATH

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The success of Osteosarcoma (OS) treatment is still limited to a 70% 5-years survival rate due to failure in total tumor necrosis after chemotherapy and development of chemoresistance, leading to metastasis and recurrence. The future of an OS pharmacological cure may be moving away from targeted anti-oncogenic paradigms toward less toxic drug formulation, and more generalized oxidative-damage approaches. Nanotechnology-based drug delivery systems (DDS) represent a promising strategy for improving antineoplastic agents' efficacy, pharmacokinetics and selectivity to the target. A second level of targeted delivery is represented by human Mesenchymal Stem Cells (hMSCs), based on their specific tumor-tropism and their success in delivering several therapeutic agents as shown in many preclinical studies and a few ongoing clinical trials. The overall goal of our project is to engineer an hMSCs-DDS for OS treatment, using photodynamic therapy (PDT), chemotherapy

(Paclitaxel, PTX), or the combination of both. In this study we evaluated the in vitro efficacy of three different nanoformulations (NFs) in a 3D OS tumor model: NF I) PDT polimeric PtI@PMMA and PDT biodegradable Ce6@Ker NF II) Chemotherapy PTX@Ker NF III) Bimodal PTX-Ce6@Ker The NFs were generated based on their different drug release mechanism: PMMA displays low degradation rate therefore can be irradiated multiple times, whereas Keratin's biodegradable property bypasses issues related to the body clearance of PMMA after treatment and make it more suitable for controlled drug release. We isolated 10 bone marrow hMSCs lines and characterized their growth curve, multilineage potential, and immunophenotype. The internalization rate was >95% for all the NFs, and neither alteration of cell viability nor migratory capacity were detectable. In a 3D tumor model of MG63 OS cells co-cultured with hMSCs-DDS, the survival rate upon irradiation, measured by an ATP-based assay, was less than 5%. A massive cell necrosis in the whole spheroid mass was confirmed by CalceinAM/EthD staining and TEM imaging. Our result demonstrate the role of hMSCs-DDS as highly effective treatment in vitro, and encourage a possible application in clinic to provide a more efficient pharmacological treatment and increase the current overall survival rate for OS affected patients.

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F-4004

THE ROLE OF MESENCHYMAL STEM CELLS IN GASTROINTESTINAL CANCER PROGRESSION

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Recent studies have clearly demonstrated the significance of the tumor microenvironment which is composed of cancer-associated fibroblasts (CAFs), blood vessels, immune cells and the extracellular matrix. One of the key components of the tumor microenvironment is CAFs. CAFs are a heterogeneous cell population with different cell origins such as mesenchymal stem cells (MSCs) in the bone marrow and local fibroblasts and pericytes. However, the identification of marker proteins specific for CAFs and its origin MSCs has been limited so far, making it challenging to target CAFs for cancer therapy. Here, we have identified a novel candidate membrane protein marker for CAFs and MSCs using human gastrointestinal cancer tissues and genetically engineered mouse models. Genetic fate mapping has revealed that the stem cells give rise to CAFs, osteocytes, chondrocytes and adipocytes. This study will provide a novel insight into the cellular origin and heterogeneity of CAFs, which will help develop new therapeutic approaches to target CAFs.

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F-4006

INVESTIGATING POPULATION VARIABILITY USING HIGH THROUGHPUT ELECTROPHYSIOLOGICAL PHENOTYPING OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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The recent genomics revolution hopes to deliver an era of precision medicine. However, our ability to link primary genotypes to clinical phenotype is still limited. For example, Long QT syndrome (LQTS) type 2 is caused by mutations in KCNH2, which encodes the hERG protein that carries the rapid delayed rectifier K+ current (IKr) - a major component of cardiac repolarisation. Loss of function mutations in KCNH2 result in prolongation of the cardiac action potential and hence the QT interval on the surface electrocardiogram and increase risk of fatal arrhythmias. However, even in patients with the same primary mutation, phenotype can be highly variable. Similarly, in the acquired form of LQTS, which occurs as a result of drug block of hERG, proarrhythmic risk is highly variable across the population. One of the major factors that is thought to contribute to this phenomenon is the varied genetic background between individuals which alters the electrical context in which the primary insult must be considered. In this study we

have used a panel of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) combined with high throughput phenotyping to assess the role of variable genetic background in determining phenotype. RNA transcript levels of forty key ion channel genes, measured using Nanostring assays, varied between 1.1 and 22-fold across the six cell lines. Electrophysiology and calcium handling phenotypes were measured in the same cell lines using kinetic imaging cytometry highlighting significant differences. For example, for two normal cell lines, the action potential duration measured at 75 % repolarisation (APD75) was 440 +/-50 ms (SD; n=5400 cells) and 317 +/- 81 ms (SD; n = 800 cells). These results demonstrate that an approach incorporating genetically diverse iPSC lines and high throughout phenotyping provides a basis for quantitative analysis of the role of genetic variability in phenotypic presentation of arrhythmic cardiac electrical disorders.

F-4008

STABILIZING HETEROCHROMATIN BY DGCR8 ALLEVIATES SENESCENCE AND OSTEOARTHRITIS

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DiGeorge syndrome critical region 8 (DGCR8) has been well known as a crucial component of canonical microprocessor complex for microRNA biogenesis. However, the non-canonical functions of DGCR8 remain to be studied. Here, we demonstrate that DGCR8 plays an important role in maintaining heterochromatin organization and preventing aging. N-terminal-truncated DGCR8 (DR8dex2) accelerated senescence in human mesenchymal stem cells (hMSCs) independent of its C-terminal domain-mediated miRNA-processing activity. Further studies revealed that DGCR8 maintained heterochromatin organization via the interaction with nuclear envelop protein Lamin B1 and heterochromatin organization associated proteins KAP1 and HP1g, the overexpression of which in turn reversed the premature senescent phenotypes in DR8dex2 hMSCs. Finally, DGCR8 was downregulated in pathological and physiological aged hMSCs, whereas DGCR8 overexpression alleviated hMSC aging and mouse osteoarthritis. Taken together, our study uncovers a miRNA-independent novel role of DGCR8 in maintaining heterochromatin organization and preventing senescence, which may provide a new therapeutic target for alleviating human aging-related disorders.

F-4010

CLONAL ANALYSIS OF STEM CELL DYNAMICS DURING INJURY INDUCED REGENERATION OF MURINE BLADDER

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Tissue integrity is maintained through the highly regulated proliferation and differentiation of tissue-specific stem cells, and understanding of their regulation and dynamics during tissue homeostasis and repair is one of fundamental goals in stem cell biology. We previously identified epithelial stem cells, marked by the expression of Sonic Hedgehog (Shh), in the urinary bladder and found that these cells can regenerate the entire bladder epithelium upon injury. Cell and tissue dynamics of Shh-expressing urothelial stem cells during bladder regeneration, however, is poorly defined. Here, using in vivo stem cell marking and lineage tracing techniques and clonal analysis with multi-color mouse, we show that urothelial regeneration is achieved through the clonal expansion of small number of basal stem cells. The remarkable ability of a single urothelial stem cell and its progeny to colonize a major portion of the urothelium requires injury-induced autophagy as pharmacological inhibition of cellular autophagy whose activity increases during normal injury-repair processes impedes bladder regeneration. Autophagy-mediated urothelial regeneration, induced by either bacterial infection or chemical injury, is associated with heightened activity of stem cell proliferation and differentiation. Our finding explains some long-known but poorly understood phenomena, such as association of chronic injury with urothelial carcinoma and frequent recurrence of bladder cancer, which can be explained by clonal origin of the disease. Our work further provides a conceptual basis for injury induced tissue repair of epithelial tissues with implication for the development of autophagy-related therapeutic options in regenerative medicine such as bladder reconstruction following radical cystectomy.

F-4012

PUBLIC ATTITUDES TOWARD HUMAN-ANIMAL CHIMERA IN JAPAN

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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 a large-scale survey with 2,160 public responses and 1,115 responses from the member of the Japanese Society for Regenerative Medicine. Results showed that the public is more interested in the post-realization aspects of RM, such as cost of care, countermeasures for risks and accidents, and clarification of responsibility and liability, than in the scientific aspects; the latter is of greater interest only to scientists. In addition, we compared their attitudes toward researches on human-animal chimera. We found the gaps of acceptance for human-animal chimera between the public and scientists. And then, we conduced deeper analysis of the diversity of the public opinions to human-animal chimera focusing on effects of respondent's backgrounds.

F-4014

REGULATION OF SPERMATOGONIAL STEM CELLS BY H2K27 DEMETHYLASES

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Spermatogenesis is continuous process, because spermatogonial stem cells (SSCs) can maintain themselves as well as provide differentiated progenies. The maintenance of SSC compartment is supported by not only self-renewal of stem cells but also fragmentation of differentiating spermatogonia through abscission of intercellular bridges in a random and stochastic manner. The molecular mechanisms that regulate this reversible developmental lineage still remain unclear. We found that histone H3 lysine 27 (H3K27) demethylase, JMJD3 (KDM6B), has some roles in the regulation of SSC ccompartment. Although lack of JMJD3 in germ cells did not affect differentiation of spermatogonia, JMJD3 null mice have larger testes and sire offspring for a longer period compared to controls, likely secondary to increased and prolonged maintenance of the spermatogonial compartment. The absence of JMJD3 could induce frequent fragmentation of spermatogonial cysts by abscission of intercellular bridges. 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 fragmentation of spermatogonial cysts. Now we are analyzing other mouse models and expression profiles to elucidate the role of H3K27 demethylases in the regulation of SSC compartment. Our findings may be involved in maintenance of diverse stem cell niches.

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F-4016

GOING OUT ON A LIMB TO STUDY THE CELL POPULATIONS AND INTERACTIONS INVOLVED IN LONG BONE CATCH-UP GROWTH IN MICE

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Musculoskeletal disorders are the main cause of disability in modern societies, and a huge burden for healthcare systems. In most cases, the current treatments for bone growth defects involve surgical shortening or lengthening, with many side effects and slow recoveries. Thus, the overall aim of our lab is to unravel the mechanisms that compensate for developmental insults, with the long-term goal of harnessing these mechanisms into non-invasive therapies for growth disorders. As a model, we use the cartilage templates that drive long bone growth, which can recover the normal growth trajectory (i.e. catch-up) after an insult. This response has been proposed to be cell-autonomous, but some evidence does not support this model. Moreover, the contribution of external progenitors to bone growth and repair has not been resolved yet. To address the cell behaviours involved in catch-up growth in mice, we developed a genetic strategy to block proliferation in 50% of the cartilage chondrocytes that drive long bone elongation, specifically in the left hindlimbs, such that the right limb remains as an internal control. Strikingly, left-right symmetry was maintained, revealing the deployment of compensatory mechanisms. We observed a local response composed of three steps: First, extracellular matrix production is increased in response to reduced chondrocyte proliferation, allowing for bone growth to proceed at an almost normal rate, and leading to reduced cell density. Second, when cell density drops below certain threshold, compensatory proliferation is triggered in WT chondrocytes, in an extent proportional to the number of affected chondrocytes. Third, WT chondrocytes progressively replace the mutant ones, such that when the threshold cell density is recovered, compensatory proliferation stops, ensuring that overgrowth is not generated. In summary, our results reveal that long bone catch-up growth is mostly due to a cell non-autonomous response, representing a paradigm shift in the field that opens up new research avenues for basic and translational studies.

We are currently characterizing the cell populations and interactions involved in this process, including the potential participation of stem/progenitor cells from outside the cartilage.

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F-4018

MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES IMPROVE NEUROGENESIS AND ANGIOGENESIS IN A RAT STROKE MODEL VIA TRANSFER OF MIRNAS

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Extracellular vesicles (EVs) secreted from various types of cells contain proteins and nucleic acids, and are thought to regulate the microenvironment through cell to cell communications. Mesenchymal stem cells-derived EVs (MSC-EVs) exerts their therapeutic capability via paracrine actions of MSCs in infarcted tissue. But their therapeutic mode of action when administered to an animal stroke model have never been remains unclear. In this study, we investigated therapeutic efficacy and mode of action of MSC-EVs in a rat stroke model. MSC-EVs successfully enhanced neurogenesis and angiogenesis in a rat stroke model. As comparable to the MSC-treated group, the rats treated with MSC-EVs exhibited greater behavioral improvements than the control group ($p < 0.05$). In addition, those MSC-EVs were highly inclusive of various proteins and microRNAs (miRNA) associated with neurogenesis and/or angiogenesis compared to fibro-EVs. We further analyzed those miRNAs and found that miRNA-184 promote proliferation of NSCs by suppressing the expression of numbl, and miRNA-210 was key players in MSC-EVs to promote angiogenesis by suppressing the expression of ephrin-A3. In conclusion, this study demonstrated that MSC-EVs could be used for stroke treatment as an ideal alternative to MSCs. Also, our study showed that stroke induces changes in the expression profile of microRNAs in MSC-EVs, and the altered microRNAs actively play a role in the recovery of stroke.

F-4020

THE EFFECTS OF THE NOVEL HERBAL DRUG ON NEUROPATHIC PAIN IN MICE

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Neuropathic pain is caused by damage or disease in the nervous system. In neuropathic pain, inhibitory GABAergic neurons are destroyed, and excitato- ry neurons are hyperactivated. In this study, we showed that the novel herbal drug Gamijakyakgamchobuja-tang (KCHO-1) can effectively reduce neuro- pathic pain. In the spinal nerve ligation (SNL) model, neuropathic pain results from the loss of neurons utilizing γ-aminobutyric acid (GABA) and the genera- tion of reactive oxygen species (ROS) in the injured spinal cord. Treatment of SNL mice with KCHO-1 had an anti-oxidative effect, reducing ROS generation, and also induced the regeneration of GABAergic neurons in injured tissue. These data suggest that KCHO-1 may be a potential therapeutic drug for the treatment of neuropathic pain.

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F-4022

TWO-DIMENSIONAL EXPANSION OF GASTROINTESTINAL LGR5 STEM CELLS WITH A FOUR-COMPOUND COMBINATION

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Epithelial stem cells in the gastrointestinal tract marked by Lgr5, a receptor for R-spondin, are key factors in the homeostasis and regeneration of the injured tissues. Recent advances of three-dimensional culture methodology and understanding niche factors from Lgr5 stem cells isolated gastrointestinal tract have allowed the generation of epithelial organoids. In this study, we try to expand Lgr5 stem cells in a tow-dimensional culture to increase cost-effectiveness of organoid production. When mouse intestinal organoids derived from Lgr5-EGFP-IRES-CreERT2 transgenic mice were dissociated in single cells and seeded on to the Matrigel coated-surface and grown in the standard organoid medium including EGF, Noggin and R-spondin-1, Lgr5 stem cells lose stemness and were completely differentiated. To find enhancing compound for enhancing the stemness of Lgr5 stem cells in two-dimensional culture system, compound combinations with various compounds were tested using organoids from Lgr5-EGFP-IRES-CreERT2 transgenic mice. As a result, we found a chemical cocktail consisting of four-compounds to maintain Lgr5 positive stem cells in two-dimensional culture system. Furthermore, two-dimensional cultured Lgr5 stem cells with four-compounds were able to form normal functioning organoids again when transferred in three-

dimensional culture system without four-compounds. In this study, we developed a cost-effective tow-dimensional culture system to produce large quantity of Lgr5 stem cells and intestinal organoids.

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F-4024

GENERATION OF INDUCED HEPATIC STEM CELLS FROM FIBROBLASTS BY DEFINED FACTORS

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The generation of hepatic stem cells (HSCs) has a great potential for cell-based therapy in liver diseases. Recent studies reported direct conversion of hepatocytes and HSC from fibroblasts by using combinations of transcription factors. Although the therapeutic effect of hepatocytes has been explored in acute liver injury, the role of HSC in chronic liver damage remains unclear. Here, we demonstrate that combination of the transcription factors and hepatic culture conditions are sufficient to convert fibroblasts into expandable induced hepatic stem cells (iHSCs). These iHSCs possess self-renewal and bipotency with the capability to differentiate into hepatocytes and cholangiocytes. Hepatocytes-derived from iHSCs (iHSC-HEPs) exhibit the typical morphology and hepatic functionality including glycogen storage, low-density lipoprotein (LDL) uptake, Indocyanine green (ICG) detoxification, drug metabolism, and albumin secretion. Cholangiocyte-derived from iHSCs (iHSC-CCs) express cholangiocyte-specific markers and develop apical-basal polarity of the cystic and tubular structures with secretory function in three-dimensional culture condition. Furthermore, iHSCs show anti-inflammatory and anti-fibrotic effects in a carbon tetrachloride (CCl4)-induced chronic liver damage model. This study demonstrates that iHSCs show typical hepatic functionality in vitro and a therapeutic effect in liver fibrosis model. Therefore, directly converting HSC from somatic cells might facilitate the development of patient-specific cell therapy for chronic liver diseases.

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F-4026

LARGE-SCALE EXPANSION OF UMBILICAL CORD (UC)-DERIVED HUMAN MESENCHYMAL STROMAL CELLS (MSC) USING QUANTUM BIOREACTOR DOES NOT ALTER THEIR CHARACTERISTICS

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Human mesenchymal stromal cells (hMSCs) opened new perspectives for regenerative medicine due to their immunomodulatory properties and multilineage differentiation capacity. MSCs could be isolated from almost every human tissue of mesenchymal origin. A special interest should be given to MSCs derived from the umbilical cord (UC) tissue. A noninvasive procedure of derivation, minimal contact with the environment and fast self-renewal properties makes UC-derived MSCs attractive cells for regenerative medicine. The aim of this study was to establish a simple procedure for UC-hMSCs isolation, characterization and expansion, followed by a detailed comparison of UC-hMSCs properties harvested either on T-flasks or using a large scale bioreactor (Quantum Cell Expansion System; Terumo BCT, Denver, CO, USA). Human MSCs were isolated by tissue explant culture technique from UC Wharton's jelly, harvested after reaching at least 75% confluence and consequently cultured using 10 tissue culture flasks T-175, starting with 1 milion cells per flask (F-12/K, DMEM GlutaMax, FBS, 12 days). Obtained MSCs were harvested using TrypLE Express solution, tested and cryopreserved. Consequently, 25 milions of the cells were thawed, cultured in a bioreactor (F-12/K, DMEM GlutaMax, FBS, 7 days) and tested for changes in their properties. We observed comparable growth and immunomodulatory capacities of fresh and expanded UC-hMSCs. Large scale UC-hMSCs expansion did not alter their senescence, genetic stability or in vitro tumorigenicity. We found no differences in the ability

to differentiate toward adipogenic, osteogenic and chondrogenic lineages between classic and large scale hMSC expansion methods. We conclude that large-scale expansion of UC-hMSCs using Quantum Cell Expansion System does not alter "mesenchymal" features and quality of cells and represents therefore a suitable method for implementation in clinical application.

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F-4028

GENERATION OF HEMATOPOIETIC STEM/ PROGENITOR CELLS FROM HUMAN EMBRYONIC STEM CELLS VIA FEEDER-FREE, SERUM-FREE ES-SAC PROTOCOL

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Pluripotent stem cells possess the potential for limitless red blood cell production and hematopoietic stem/ progenitor cell (HSPC) derivation for transplantation purposes. However, there are no clinically relevant in vitro protocols for derivation of functional definitive HSPCs. ES-sacs (hemangioblast-like structures) concentrate phenotypic HSPCs (CD34+CD45+), and more definitive (dEP, CD235a-CD34+) and primitive (pEP, CD235a+CD34-) erythroid precursor cells, which can differentiate into β-globin expressing erythroid cells. However, there is a strict need for feeder cells, leading to variable results and limitations for clinical use. In our preliminary data, feeder-free ES-sac culture resulted in detachment of ES cells; therefore, we hypothesized that feeder cells are required for initial mesoderm differentiation, and embryoid body (EB) formation would allow for mesodermally differentiated cells to be used for ES-sac generation without feeder cells. First, to evaluate whether an EB protocol can be combined with original ES-sac protocol, we transferred EBs onto C3H10T1/2 feeder cells on various days (1 to 13 days) followed by serum-free ES-sac culture. After a 15-day ES-sac culture, ES-sac-like structures were noted among all conditions, and a 7-day EB transfer produced the greatest amounts of CD34+ cells (74.434.6%, $p<0.01$). Next, to adapt the EB-sac protocol for feeder-free conditions, 7-day EBs transferred to matrigel and/or gelatin coated wells. After 15-day ES-sac culture, ES-sac-like structures (EB-sacs) were observed in both matrigel and gelatin conditions. HSPC-like spherical cells were observed in as early as 5 days of differentiation, and 1.2330.11×104 spherical cells per EB transferred were derived at the end of differentiation (15 days). Surprisingly, feeder-free serum-free differentiation of EB-sacs resulted in a large population of HSPCs (16.534.3%), and greater amounts of dEPs (7.331.3%, $p<0.01$) than pEPs (3.430.2%). In summary, we developed a feeder-free, serum-free culture for hemangioblast-like EB-sac, resulting in a large population of phenotypically definitive HSPCs. The ability to derive HSPCs that give rise to red blood cells expressing globins associated with definitive hematopoiesis has prompted us to test engraftment of these HSPCs in immunodeficient mice.

F-4030

HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVED PANCREATIC "BETA-CELLS" FOR CELL REPLACEMENT THERAPY IN DIABETIC PATIENTS

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Type 1 diabetes is mostly managed by insulin injections and in certain cases by islet transplantation which can make a patient insulin injection free for about five years. However, the later procedure is limited by scarcity of donors as well as compromised quality of available islets. This necessitate the need of abundant in-vitro differentiated β-cells from Stem Cells. Orchestration of several signaling cascades play crucial role in the specification and differentiation of pancreatic progenitors including β-cells. Attempts have been made to produce β-cells in-vitro using combination of growth factors and small molecules. However, the available methods produce only "β-like" cells which fails to perform physiological glucose-stimulated insulin secretion (GSIS); a crucial characteristic of adult β-cells. Moreover, the available methods are highly inefficient and variable in reproducibility. Efficiency of differentiation ranges from 6-45% between different cell lines and labs. Additionally, glucose threshold level as well as gene profile group them closer to immature neonatal human β-cells. Portion of implanted pancreatic progenitors (PPs) under the kidney capsules mature in 3-4 months' time post-implantation by unexplained and poorly understood mechanism suggesting a lacuna in the understanding of underlying mechanism of β-cells maturation and insulin secretion. Developmental studies in rodents suggest crucial role of blood vessels in the development of pancreas. In adults, islet of Langerhans is richly vascularized and most β-cells are in direct contact with the blood vessels of the capillaries. In-vivo studies also suggest that the basement membrane secreted by endothelial cells might play crucial role in differentiation, growth, orientation as well as the function of β-cells. We aim to exploit in-vivo developmental cues in developing a robust, efficient and sustainable method for generating

"mature β-cells" with long term survival capacity. These differentiated β-cells will be tested in diabetic mouse model systems for further use in cell replacement therapies. Our preliminary data suggests >85% efficacy in pancreatic progenitor differentiation with significant number of cells (~40%) being insulin positive. Moreover, our differentiated β-cells are capable of GSIS in in-vitro assays.

Funding Source: Postdoctoral Four-Year Fellowship: John and Anne Chong Fellowship (Philanthropist)- Stem Cells To Treat Diabetes.

F-4034

DISEASE MODELING OF ALS WITH PATIENT SPECIFIC IPSC-DERIVED MOTOR NEURON CULTURES

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of upper and lower motor neurons in the brain and spinal cord, muscle degeneration, paralysis, and death. The underlying mechanisms of this disease remain unknown. While the majority of ALS cases are sporadic, several mutations in familial cases have been identified. Significant advances in stem cell biology and neuronal differentiations have provided a new platform to study ALS specific disease mechanisms in vitro. However, determining the consequences of the inherited genetic mutations on gene regulation and on phenotypic outcomes have been challenging due to the heterogeneity of the generated cells and the varying differentiation propensities between different lines. Here we describe the use of high-throughput single-cell RNA sequencing (scRNA-seq) to profile induced pluripotent stem cell (iPSC) derived motor neuron cultures from both healthy and ALS-afflicted patients. scRNA-seq analysis of cellular heterogeneity revealed these cultures to be highly enriched for multiple types of motor neurons, interneurons, glia, and neural progenitor cells, populations that are all found in vivo in the spinal cord and have been implicated in ALS disease progression. These data comprise a molecular atlas of ALS-associated cell states and associated "disease signatures" that could help empower in vitro disease modeling efforts and contribute to the development and validation of novel therapeutics.

F-4036

REPROGRAMMING ENRICHES FOR SOMATIC CELL CLONES WITH SMALL SCALE MUTATIONS IN CANCER-RELATED NUCLEAR AND MITOCHONDRIAL GENES

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Recent studies demonstrated that the observed high mutational load in induced pluripotent stem cells (iPSCs) is largely derived from their parental cells, but it is not known whether reprogramming may enrich for specific mutations that pre-exist in the mosaic parental cell population. We have derived 30 human iPSC lines from early passages of endothelial cells of neonatal and aged individuals under comparable conditions and sequenced the nuclear genomic (gDNA) as well as mitochondrial genomic DNA (mtDNA) of the iPSCs and their corresponding parental cell populations. We provide first evidence that individual small scale mutations in the nuclear and mitochondrial genome, present in small subpopulations of parental cells, become highly enriched among iPSC clones during reprogramming. This observation holds true especially for variants that confer a proliferation or survival advantage. Interestingly, enriched mtDNA mutations clustered in specific components of the respiratory chain that are frequently mutated in cancer and various, especially neurological, disorders. Furthermore, iPSCs from aged donors

contain significantly increased numbers of genetic variants that have been enriched during reprogramming and affect cancer-related genes. Here, a typically lower proliferation capacity and reprogramming efficiency of somatic cells from aged donors compared to their neonatal counterpart may support the enrichment for genetic variants that lead to a proliferation or survival advantage. The reprogramming associated enrichment of potentially pathogenic mutations may impact the functionality and clinical value of patient-derived iPSCs. Moreover, our data support the assumption that in particular iPSCs from elderly patients may be of lower biological quality calling into question their clinical usefulness.

F-4038

OSTEOCHONDRORETICULAR (OCR) STEM CELL THERAPY FOR OSTEOARTHRITIS: THE RIGHT CELLS FOR THE JOB

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Osteoarthritis (OA) is the most common form of arthritis worldwide, costing 1-2.5% of the gross national product in western countries. Prevalence of OA is estimated at 220 million people globally and central to the pathogenesis is the loss of articular cartilage. Although a multifactorial disorder, it should be considered at least in part, the failure of stem cells to adequately repair cartilage following typical challenges such as injury, obesity and ageing. Despite advances in OA treatment, we are still unable to adequately rescue or reverse disease progression. Newly discovered populations of skeletal stem cell in recent years have fuelled our interest in understanding stem cells in OA. Our research has investigated the role of traditional mesenchymal stem cells (MSCs), newly discovered skeletal stem cell populations and mature chondrocytes in injury-induced OA. Using lineage tracing we track the contribution of these different cell populations to cartilage and skeletal homeostasis during injury-induced OA. Our investigation further examines the role of these cells on both the genetic and functional level in hopes of uncovering the fundamentals of OA, offering future opportunities through translating basic cartilage biology into new clinical therapies.

Funding Source: National Health and Medical Research Council (NHMRC) and National Institute of Health (NIH)

F-4040

HAIR GROWTH PROMOTING EFFECTS OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS

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Human mesenchymal stem cells (hMSCs) including human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs), which have high proliferation capacity and tissue regenerative properties, are considered to be a good candidate for cell-based therapies. However, the therapeutic mechanisms involved are not well understood. Alopecia is a common disease for many people caused by many causes, such as aging or hormonal imbalance. In this study we investigated the hair growth-promoting effects of hUCB-MSCs treatment to determine whether hUCB-MSCs enhance the promotion of hair growth. hUCB-MSCs significantly increased the proliferation of human dermal papilla cells (DPCs) and keratinocytes (HaCaT) in vitro. Furthermore, we found that hUCB-MSCs increased the viability and up-regulated hair induction related proteins on hair loss induced by dexamethasone (Dex), a cetagen inducer. Moreover, we analyzed hair growth related-secretion factors of hUCB-MSCs by using human cytokine array. we found that hUCB-MSCs leads to significantly increased secretion of macrophage migration inhibitory factor (MIF), which was reported to strongly expressed on the proximal inner root sheath and matrix cells of anagen HFs and contributes to maintenance of hair follicle immune privilege. Treatment with MIF leads to increased hDPCs proliferation. And also, we examined the effects of MIF knockdown by treatment of hUCB-MSCs with siRNA. We found that MIF had positive effects in cell viability;

vascular endothelial growth factor (VEGF) secretion; expression of alkaline phosphatase (ALP). In addtion, the hUCB-MSC transplantation induced mouse hair loss model showed the therapeutic effects, whereas si-MIF abolished therapeutic effects of hUCB-MSCs. Taken together, these data suggest hUCB-MSCs can improve the efficacy of hair loss treatment and that MIF was a key regulator of hair growth.

F-4042

THE CHARACTERIZATION OF GENETICALLY ENGINEERED NATURAL KILLER (NK) CELLS USING NANOG-ENCODED ADENO-ASSOCIATED VIRUS (AAV)

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NK cells have been known to play a critical role in eliminating acute infections and providing immune response to tumor cells. Usually, gene delivery methods used in NK cells are viral transduction and transfection. In the case of viral transduction, lentivirus and adenovirus are typical. Their gene expression is stable, but NK cell viability may be compromised. Through transfection, it has high transduction efficiency without regulatory issues, but transient transgene expression may not be sufficient to induce long-term responses. To overcome the limitation of viral vector, AAV, which has been well known for lack of pathogenicity in most research was chosen in this research. AAV-4 derivative vector was developed by changing the amino acids on AAV-4 capsid surface. It has higher efficiency of transduction to NK-92 than other AAV serotypes. Moreover, a transcription factor in embryonic stem cells, such as NANOG gene has been thought to be a key factor in maintaining pluripotency. By using the novel AAV-4 derivative vector, NANOG gene effectively delivers into NK-92. The NANOG gene-modified NK-92 cells were characterized by gene expression, proliferation potential, and cytotoxic function. The novel AAV-4 derivative vector will contribute significant advancements into cancer immunotherapy using NK-92.

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F-4044

THE METABOLIC CHARACTERISTICS OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELL

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Human Mesenchymal Stem Cells (MSCs) isolated from various adult tissues are primary candidates in cell therapy and tissue regeneration. In MSCs-based therapy, studies of MSC metabolism have significant implication in optimizing bioprocessing conditions to obtain therapeutically competent MSC population for clinical application. Many studies on the metabolism of bone marrow and adipose derived MSCs have been reported, but studies in Umbilical Cord Blood derived MSCs (UCB-MSCs) are insufficient compared to their usefulness. We compared the metabolism of UCB-MSCs according to the culture method between traditional one and our proprietary growth and potentiation method. Metabolome analysis was performed in 14 samples in cultured cells using CE-TOFMS in two modes for cationic and anionic metabolites. We detected 225 metabolites (112 metabolites in Cation mode and 113 metabolites in Anion mode). PCA and HCA analysis showed a clear difference between the two groups. Also, the obtained metabolome data were superimposed and compared on the several metabolic pathway maps including central carbon, Urea cycle, lipid, amino acid, nucleotide and metabolism of coenzymes. Finally, the results of comparison analysis were conducted as categories on the basis of metabolic pathway (KEGG) and biological functions (HMDB) of candidate compounds. All these results may have important implications for the optimization and potentiation of MSCs and advance to new approaches for MSCs-based cell therapies.

F-4046

SURFACE-MODIFIED PCL SYSTEM FOR GENE-CELL THERAPY

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Cell-specific and local gene delivery systems are essential for gene-cell therapy. To develop this system, we used polycaprolactone (PCL), which is biodegradable and biocompatible polymer as a material, and adeno-associated virus (AAV), a gene carrier with various serotypes that has interaction with specific cells. Multi-AAV serotype receptor (AAVR) is utilized in this study to connect PCL and AAV. The complex was formed by attaching modified AAVR to surface-modified PCL materials. Instramuscular injection was performed to inject this system. The results show that local delivery is possible. Therefore, transferring specific genes to the target area of specific cells could also be possible using AAV bound to AAVR-PCL system. And, through the fact that AAVR and AAV are bound, we applied this system for AAV purification. Using this complex can overcome limitation of purification methods used in nowadays, such as cost problems and restricted use of serotypes. Based on the results of the experiment, this study will contribute to delivering gene to cell, and producing gene vector for gene-cell therapy.

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F-4048

ADENO ASSOCIATED VIRUS BASED POROUS COMPLEX FOR GENE CELL THERAPY

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This study introduces a simple and easy way to produce a novel patch with the adeno-associated virus (AAV) complex. The AAV based structure is constructed by connecting the 3,3'-Dithiobis(sulfosuccinimidyl propionate) (DTSSP) cross linkers to the AAV vectors which are well known for their moderate immunogenicity. Since the patch fabrication process occurs under water based condition, it seems to have advantages of easy attachment and high absorption to any organs and less side effects. Because of these advantages of the AAV patch system, the target gene can be efficiently delivered to the specific desired area. Especially, the porous structure characteristic of the AAV complex will help on intercellular interaction of stem cell. Also it is possible to regulate the size and the viral dose of complex. This will make the pellet more convenient to use. Based on this study, this system will be applicable to future research and treatment of various diseases.

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F-4050

ENDODERMAL DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS USING SIMPLE DIALYSIS CULTURE

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Endodermal differentiation of the human induced pluripotent stem cells (hiPSC) have a great potential for regenerative medicine and pharmaceutical application targeting to digestive system organs. To enbale these applications, efficient large-scale culture is necessary, but it needs very high costs because the differentiation process requires a variety of growth factors at high concentrations. Dialysis culture is one of the solutions to reduce the costs from the standpoint of cell culture engineering. A dialysis culture system allows continuous nutrient supplies and waste removals by the dialysis compartment, while holding both exogenous and endogenous growth factors in the cell compartments. The feasibility of such dialysis culture was already investigated in hiPSC expansion in the stirred-suspension bioreactor, but it has not yet been applied to hiPSC differentiation where the cost of growth factors is much higher. We therefore developed a simple and small-scale suspension dialysis culture system that is suitable to examine various cultural conditions. The dialysis membrane (MWCO, 3,000) was fixed at the bottom of a commercially-available culture insert and the cup was set into the deep well plate. The dialysis cup-loaded plates were put on a rotational shaker to enable suspension culture of iPSC aggregates in the dialysis cup. The cell culture compartment was dialyzed against the lower plates (dialysis culture medium compartment). The endodermal differentiation medium was prepared by adding growth factors larger than the molecular weight cut-off of dialysis membrane into the dialysis culture medium. The volume of dialysis culture medium was six times larger than that of differentiation medium. The morphology of differentiated aggregate after five days of culture had no difference between with and without dialysis. However, the aggregates cultured with dialysis showed reduced pluripotency and proceeded to the endodermal lineage by dialysis, according to their gene expression level. Although detailed analyses of the culture medium should be done, such dialysis culture surely accelerate endodermal differentiation, thus, leading to reduction of the cost. In addition, each differentiation step can be optimized using such small-scale suspension dialysis culture system.

F-4052

EFFECTS OF CARBONIC ANHYDRASE 9 (CA9) POSITIVE CANCER STEM CELLS TARGETED THERANOSTIC COMPOUND ON HUMAN BREAST CANCER

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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 AZ-BPS 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. AZ-BPS displayed enhanced 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, BPS

(an analogous agent without an acetazolamide unit). Considering all, our results strongly suggest that AZ-BPS could be a clinical applicable therapeutic agent to targeting CA9-overexpressing breast cancer stem cells.

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