POSTER ABSTRACT GUIDE

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23–27 JUNE 2020

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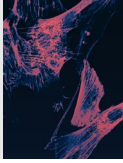


THURSDAY, JUNE 25, 2020

POSTER SESSION I
14:00 – 16:00

Theme: Cellular Identity

Theme: Clinical Applications

Theme: Modeling Development and Disease

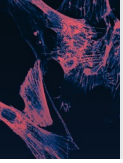
Theme: Tissue Stem Cells and Regeneration

POSTER SESSION II
22:00 – 23:59

Theme: Cellular Identity

Theme: Clinical Applications

Theme: Modeling Development and Disease



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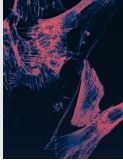


POSTER SESSION IV
14:00 – 16:00

Theme: Cellular Identity

Theme: Clinical Applications

Theme: Modeling Development and Disease

Theme: Tissue Stem Cells and Regeneration

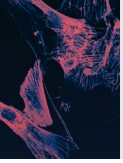
SATURDAY, JUNE 27, 2020

POSTER SESSION V
05:00 – 07:00

Theme: Cellular Identity

Theme: Clinical Applications

Theme: Modeling Development and Disease



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THURSDAY, JUNE 25, 2020

POSTER SESSION I
14:00 – 16:00

Theme: Cellular Identity

CARDIAC

CI319

PERTURBATION PANEL PROFILING IDENTIFIES TRANSCRIPTION FACTORS THAT ENHANCE DIRECTED CHANGES OF CELL IDENTITY

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It is extremely challenging to identify sets of genes whose expression or suppression can modulate cellular identities in a directed manner. Insightful prior work on identifying genes that enable directed cell identity changes relied conceptually on recapitulating normal cell type specification in development, inferring specifically active transcription factors, or conducting large-scale unbiased screens. However, the gene expression changes associated with cell type phenotypic stability or maintenance in the face of many different perturbations over time have remained largely unexplored. We hypothesized that gene expression responses to a wide array of perturbations during cell identity maintenance would allow us to identify genes that are useful in directed changes of cell identity. We developed Perturbation Panel Profiling (P3) as an experimental and analytical framework for perturbing cells in dozens of different conditions. We subsequently profile their gene expression changes transcriptome-wide and pick genes for use in directed changes of cell identity. We applied P3 to human cardiac myocytes and found that transcription factors important for cardiac myocyte development or for transdifferentiation to cardiac myocyte-like states were more frequently up-regulated following perturbations than other similarly highly expressed genes. Further, we found that this gene expression responsiveness to perturbation was more sensitive for identifying lineage-driving genes than tissue-specificity of expression. Lastly, we applied P3 to a second human cell type, dermal fibroblasts, and discovered several new factors that are barriers to fibroblast reprogramming to iPSCs in the set of genes that are highly perturbable in fibroblasts. Taken together, our data demonstrate that responsiveness to perturbation is a common feature of many key regulators of cellular identity.

Keywords: reprogramming, cell identity maintenance, perturbation

CI320

NOVEL THERAPEUTIC TARGET FOR ATRIAL FIBRILLATION USING HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED ATRIAL MYOCYTES

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Atrial fibrillation (AF) represents one of the most common arrhythmias seen clinically and is associated with a significant increase in morbidity and mortality, yet, current treatment paradigms have proven largely inadequate. One of the main contributors to the pathophysiology for the initiation, progression and persistence of AF is inflammation. Hence, reduction of inflammation associated atrial remodeling represents a novel therapeutic strategy for the treatment of AF. The cytochrome P450 products, epoxyeicosatrienoic acids (EETs), are anti-inflammatory metabolites of arachidonic acid with cardioprotective effects, however, EETs are rapidly metabolized by the enzyme soluble epoxide hydrolase (sEH). Our team has developed novel inhibitors of sEH (sEHIs) to prevent the catalysis of EETs, thereby enhancing their cardioprotective activity. To test the hypothesis that treatment with sEHIs will prevent adverse atrial remodeling associated with AF, human induced pluripotent stem cell-derived atrial myocytes (hiPSC-ACMs) were embedded in a novel 3D-Cell-in-Gel elastic matrix. An integrated approach using molecular biology, flow cytometry, and electrophysiology were used in hiPSC-ACMs and human atrial fibroblasts isolated from human atrial tissue.

Analyses of chamber-specific markers from differentiated hiPSC-ACMs showed a significantly higher expression of atrial markers MYL7, NR2F2, TBX5, NPPA, and CACNA1D and a low expression of ventricular markers MYH7, MYL2 and IRX4. HiPSC-ACMs in the 3D-Cell-in-Gel matrix made of a PVA hydrogel were treated with sEHI and real-time contraction and Ca2+ signals were recorded. Analysis of hiPSC-ACMs stimulated with TNF-α showed a significant increase in the phosphorylated ERK1/2 (pERK1/2) compared to the control. Treatment of hiPSC-ACMs with sEHI significantly decreased the pERK1/2 level and the reversal of the down-regulation of the transient outward K+ current compared to non-treated cells. Flow cytometric analyses of human atrial fibroblasts (Thy1.1+/ Lin−/ CD45−) showed an increase in proliferation and activation. Our findings not only provide important mechanistic insights into the roles of inflammation, fibrosis and electrical remodeling in AF but also represent a proof-of-concept study for a novel therapeutic target in the treatment of AF.

Funding source: AHA Career Development Award and Harold Geneen Foundation

Keywords: Atrial Fibrillation, stem cell-derived atrial myocytes, soluble epoxide hydrolase inhibitors

EARLY EMBRYO

CI132

DE NOVO HETEROCHROMATIN FORMATION DURING THE TRANSITION FROM TOTIPOTENT TO PLURIPOTENT STATE

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Heterochromatin formation is key to ensuring proper genome function during early development. However, the molecular mechanisms underlying de novo heterochromatin establishment remain largely unknown, mostly due to the minuscule amounts of material available during embryogenesis. Recently, exogenous overexpression of the transcription factor Dux was reported to be sufficient to induce early embryonic-like (2C-like) cells from pluripotent stem cells (PSCs). While PSCs have defined heterochromatic foci, 2C-like cells lack chromocenters. Therefore, the study of the exit from the 2C-like state back to pluripotency could help decipher the mechanisms of heterochromatin formation at the molecular level. Here, we performed DNA-mediated chromatin pull-down to identify a cluster of proteins that were selectively displaced from the 2C-like chromatome. Notably, this protein cluster was enriched in cell cycle regulators and DNA replication-related factors. Interestingly, compared to PSCs, 2C-like cells displayed a cell cycle profile characterized by a shorter S and a prolonged G2/M phases; this further supported the hypothesis of the absence of a chromatin-bound replication machinery. Additionally, cell cycle arrest induced by epigenetic inhibitors and cell cycle checkpoint modulators increased the fraction of 2C-like cells, suggesting a strong relationship between cell cycle regulation and the establishment of the 2C state. We are functionally validating the most promising candidates from the chromatin pull-down with a CRISPR-Cas9 loss-of-function screen to identify key contributing factors acting in the exit from the 2C-like state back to pluripotency. To summarize, our data suggests a fundamental role of cell cycle regulators and DNA replication in both 2C-like state emergence and heterochromatin formation. Importantly, our results could explain both in vitro and in vivo observations about mouse embryogenesis.

Funding source: This work is supported by the Generalitat de Catalunya, European Social Fund and the EU Horizon 2020 Programme (Grant Agreement No. 686637).

Keywords: 2C-like cells, Heterochromatin, DNA replication

CI140

IDENTIFICATION AND CHARACTERIZATION OF LIN28 MOLECULAR COMPLEXES REGULATING MRNA TRANSLATION IN MOUSE EMBRYONIC STEM CELLS

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RNA-binding proteins (RBPs) fulfill crucial roles in gene expression regulation of a multitude of cellular processes and their altered expression often leads to genetic diseases and cancer. The RBPs Lin28A and B control pluripotent stem cell differentiation as well as the establishment of induced pluripotent stem cells (iPSCs) through the reprogramming of somatic cells. Lin28 proteins can act through two main different mechanisms: miRNA-dependent and miRNA-independent. In biological contexts in which the let-7 miRNAs are expressed, Lin28 proteins mainly work to block the biogenesis of these miRNAs leading to the upregulation of let-7 targets. Lin28A triggers the destruction of let-7 precursors via recruitment of the redundant terminal uridyltransferases TUT4 and TUT7. However, the Lin28 effect seems to be context-dependent based on the availability of its targets. In the establishment of epiblast-like cells (EpiLCs) from embryonic stem cells (ESCs), where let-7 are poorly expressed, Lin28A can directly bind hundreds of mRNAs and positively and negatively regulates their translation through a miRNA-

independent mechanism. We have recently demonstrated that during establishment of EpiLCs, Lin28A enhances the translation of de novo DNA methyltransferase (Dnmt3a) and blocks that of the chromatin architectural factor Hmga2. These observations coupled with several papers from other groups strongly support our working hypothesis: Lin28 mediates the interaction of many mRNAs with different molecular machineries to enhance or block translation allowing the exit from the naïve state. How Lin28 fulfills this dual role remains to be determined. To address this point, we have used proteomic approaches and massive mass spectrometry coupled with functional screening in the model system of mouse ESC differentiation into EpiLCs. We have purified Lin28A-containing ribonucleoprotein complexes and we have identified many candidate partners of Lin28A by mass spectrometry. To analyze the functional interaction of Lin28A and its partners we have employed an RNA interference-based screening to find those partners that influence Lin28-dependent regulation of Dnmt3a mRNA. Our results demonstrate that Lin28A is part of an oligomeric machinery that finely regulates mRNA translation in the formation of EpiLCs.

Funding source: PRIN 2017 number 2017CH4RNP, MUR

Keywords: RNA binding protein, epiblast-like cells, RNA translation

CI176

MODULATION OF STI1 EXPRESSION IMPACTS STEMNESS AND PROTEOSTASIS IN MOUSE EMBRYONIC STEM CELLS

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Pluripotent stem cells have high rates of chaperones and co-chaperones synthesis which are critical to the integrity of the proteome and proper function of many regulatory proteins involved in stemness. Stress-Inducible Protein 1 (STI1), a co-chaperone essential for the formation of a functional complex between the heat shock proteins HSP70 and HSP90, plays a crucial role to proteostasis. Remarkably, complete depletion of STI1 in mouse is lethal, with early degeneration of the embryo, demonstrating a key unexplored function of this protein in initial stages of development. Considering these findings, mouse embryonic stem cells (mESCs) expressing different levels of STI1 were used as a system model to investigate the role of this co-chaperone in pluripotency maintenance. Our results showed that cells with decreased expression of STI1 have lower expression of pluripotency markers, such as alkaline phosphatase and the core transcription factors OCT4, SOX2 and NANOG. In addition, decreased levels of STI1 resulted in significant reduction in proliferation, and increased levels of DNA-damage and apoptosis markers. On the other hand, cells with higher STI1 levels show an enhanced expression of pluripotency factors and a substantial increase in proliferation rates, when compared to both wild-type and cells with reduced levels of STI1. A protective effect is observed in overexpressed STI1 cells since expression levels of apoptosis and DNA-damage markers were reduced. Furthermore, our data also demonstrate that STI1 may have an impact on the differentiation capacity of mESCs, since embryoid bodies expressing diminished STI1 levels have reduced diameter and volume. Together, these results suggest that STI1, a component of proteostasis network, plays a fundamental role in pluripotency maintenance in mESCs. This work contributes to the still recent understanding of posttranslational control to pluripotency, helping to clarify possible central players, such as STI1 and its partners, as masters post-genomic controllers of the pluripotent phenotype.

Funding source: Supported by CAPES, CNPq and FAPESP.

Keywords: Pluripotency, Proteostasis, Mouse Embryonic Stem Cells

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CI196

A MATURE SUBPOPULATION OF STEM CELL-DERIVED β CELLS IS MORE STABLE IN VITRO AND IN VIVO

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Stem cell-derive β (SC-β) cells could provide an unlimited source of human islet tissue for cell replacement therapies in type 1 diabetes. In order to achieve this potential we must learn more about the transcriptional programs underlying functional maturation of SC-β cells in vivo, after their transplantation into patients. To address this question we analyzed gene expression before and after transplantation of SC-β cells into immunodeficient mice during their acquisition of a mature glucose stimulated insulin secretion profile. We observed dramatic up-regulation of β cell secreted peptides other than insulin, including islet amyloid polypeptide (IAPP), correlated with functional maturation. Further analysis revealed that IAPP

is expressed in a small subpopulation of SC-β cells before transplantation, in vitro. To functionally characterize IAPP-expressing SC-β cells we generated a dual knock-in reporter line for INS and IAPP expression and examined the characteristics of this subpopulation. These studies revealed that IAPP marks a more mature subpopulation of SC-β cells in vitro defined by gene expression, insulin secretion profiles, and insulin secretion after transplantation. These IAPP-expressing SC-β cells may be the most clinically desirable differentiation product from pluripotent stem cells for cell-based therapy toward a cure for type 1 diabetes.

Funding source: This work was supported by grants from the National Institutes of Health and the National Institute of Diabetes and Digestive and Kidney Disease: UC4 DK104159 and UC4 DK104165

Keywords: SC-β cells, in vivo Maturation, Type 1 Diabetes

EPITHELIAL

CI205

LGR5+ TELOCYTES ARE A SIGNALING HUB AT THE MOUSE INTESTINAL VILLUS TIP

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Telocytes are large mesenchymal cells that have recently emerged as an important source of Wnt proteins, without which intestinal stem cells cannot proliferate and support epithelial renewal. Telocytes are heterogenous population of cells that is in contact with the entire epithelium. Here we combined single cell RNA sequencing with single molecule RNA fluorescence in-situ hybridization and mouse genetics to uncover a subpopulation of telocytes that surprisingly, express Lgr5, a hallmark of epithelial stem cells that unexpectedly located at the villus tip. Lgr5+ villus tip telocytes (VTT) in contrast to crypt base epithelial stem cells showed no proliferation capability and no active canonical Wnt signaling. Nevertheless, VTT express both the Lgr5 receptor and it's ligand R-Spondin3 together with the non-canonical Wnt ligand Wnt5a. To better understand the nature of VTT we ablated Lgr5+ cells using the Lgr5-DTR-EGFP mouse model in which Lgr5+ cells express diphtheria toxin receptor (DTR) and EGFP. 24 hours following diphtheria toxin administration both VTTs and crypt base stem cells were completely eliminated. RNA sequencing on the epithelial cells 24 hours following diphtheria toxin administration revealed that enterocyte genes normally zonated towards the bottom of the villus were induced upon VTT ablation whereas enterocyte genes zonated towards the tip were repressed. Long-term experiments, following intestinal tissue three weeks after VTT ablation demonstrate that VTT re-appear over a time scale of a few weeks. Importantly, at three week time frame some villi are still devoid of VTT and in those, epithelial tip expression programs remain down-regulated. Pointing at VTT, telocyte sub-population, as master regulators of enterocyte spatial expression programs along the villus axis. Our findings of comparable levels of Lgr5 at villus tip telocytes and the crypt base epithelial stem cells are essential for interpreting the physiological effects of perturbations driven by the Lgr5 promoter, such as lineage tracing, cell ablation and conditional knock-outs. More importantly, this work indicates that telocytes are master regulators of both stem cells and villus enterocytes. The concept suggests a new angle for studying spatial interactions between telocytes and epithelial cells in multiple organs.

Keywords: mesenchyme-epithelial communication, epithelial cell fate determination, intestine

CI207

CO-REPRESSORS MTG8 AND MTG16 REGULATE NICHE EXIT AND EARLY FATE DECISION OF INTESTINAL STEM CELLS

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Intestinal epithelium renews every five days, a process driven by the intestinal stem cells (ISC) located at the crypt base. ISCs divide and give rise to early progenitor populations at the +4/5 cell position, where lineage specifications take place. Notch signalling pathway is crucial in this process. Upon niche exit, lateral Notch inhibition between early progenitors at position +4/5 results in binary (secretory vs enterocyte) lineage specification. Transcription factor Atoh1, repressed by Notch in ISCs, specifies secretory lineage upon its de-repression in 'Notch-off' progenitors. It remains unclear what drives the ISC-to-progenitor transition, and how binary fate decision is established. Expression profile analysis of stem cells and progenitors identified the transcriptional co-repressors Mtg8 and Mtg16 that were strongly enriched in +4/5 early progenitors. We found that Mtg8 and Mtg16 were repressed by Notch signalling indirectly via Atoh1. Deletion of Mtg8 and Mtg16 induced crypt hyperproliferation and expansion of ISCs, while enterocyte differentiation was impaired. ChIP-seq analysis showed that Mtg16 bound to numerous stem cell-signature gene promoters (e.g. Lgr5, Ascl2) for their transcriptional repression. Importantly, the co-repressor also bound to many previously reported Atoh1-bound enhancer regions, including of delta-like (Dll) Notch ligands and other secretory-specific transcription factors. We propose that the co-repressors Mtg8 and Mtg16 play central roles in the earliest progenitors to repress the ISC programme for exit from the niche and control binary fate decision by repressing Atoh1 target genes. Unravelling stem cell fate decisions in the progenitor population will help to better understand ISC behaviour, not only in homeostasis but also in models of injury and cancer.

Keywords: Lineage specification, Notch pathway, Transcriptional regulation

HEMATOPOIETIC SYSTEM

CI217

A SINGLE CELL ATLAS OF MOUSE HEMATOPOIETIC ORGANS

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While the generation of hematopoietic progenitor cells from pluripotent stem cells (hiPSCs) can readily be accomplished in a dish, transgene free generation of long term reconstituting hematopoietic stem cells (HSCs) remains a major challenge to the field. In order to recapitulate definitive hematopoiesis in vitro, we need a better understanding of the developmental processes occurring during HSC emergence in vivo. Despite having identified the origin of HSCs to be specialized endothelium, termed hemogenic endothelium, we have yet to fully characterize all distinct cell types which may play a role during the process of endothelial-to-hematopoietic transition. Here, we have utilized single cell RNA sequencing to profile two major hematopoietic organs in the mouse. The aorta-gonad-mesonephros (AGM) region was analyzed on embryonic day (E)10.5, just prior to the first emergence of HSCs, and the placenta on E11.5, prior to its peak of HSC potential. In addition to capturing endothelial and nascent hematopoietic stem cells, we also included cells of the surrounding embryonic tissue, allowing us to computationally predict cell-cell interactions based on ligand and receptor expression. This approach validated previously described regulators of HSC emergence, i.e. the recently published surface antigen CD44, while also predicting additional signaling pathways, which have not been implicated in HSC development so far. Using a morpholino based knockdown screening approach in zebrafish, we tested the effect of the predicted target ligand and receptor interactions on the presence of runx1+/cmyb+ cells in the aorta. Following this approach, we were able to identify novel modulators of HSC emergence in the AGM. Strikingly, direct comparison between the AGM and placental dataset revealed a population of cells within the placenta with a transcriptional signature highly similar to AGM hemogenic endothelium. Functional validation of these cells will test their ability to acquire hemogenic potential after in vitro maturation in the presence of a supportive stromal layer. Implementation of the transcriptional and cell-cell communication findings will aid enhancement of hiPSC-derived hematopoietic differentiation protocols toward the production of functionally competent HSCs.

Funding source: Deutsche Forschungsgemeinschaft (DFG)

Keywords: Hemogenic endothelium, Endothelial-to-hematopoietic transition, cell-cell communication

IMMUNE SYSTEM

CI342

IDENTIFICATION OF AN ALTRUISTIC STEM CELL BASED INNATE DEFENSE MECHANISM AGAINST CORONAVIRUS INFECTION IN THE LUNG OF C57BL/6 MICE

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We speculate that similar to bacteria, stem cells may also exhibit the altruistic defense mechanism to protect their niches; this innate defense system may be exploited to develop novel vaccines against emerging pathogens such as COVID-19. We have identified the altruistic behavior of human embryonic stem cells, where the stem cells reprogram to a state of self-sufficiency, and sacrifice their own fitness to enhance group fitness against oxidative stress (PMID: 22689594). We speculate that this transient reprogramming mechanism of altruistic stem cells (ASC) may function as a putative innate defense system against invading pathogens as well as any factors that threaten the identity of the stem cells residing in their niches (PMID: 28884113). Here, we provide preliminary data on ASC-based defense against a mouse coronavirus MHV-1. In a mouse model of stem cell mediated M.tuberculosis (Mtb) dormancy, a mutant Mtb strain remains dormant intracellular to CD271+ MSCs in BM and lung of C57BL/6 mice (PMID: 23363977). Intranasal introduction of mouse coronavirus MHV-1 strain to these animals led to MHV-1 viral load in lung, which was 300-fold lower than the healthy control mice, suggesting the potential enhancement of an anti-MHV-1 defense by Mtb. To find out whether CD271+ MSCs underwent ASC reprograming and exhibited anti-MHV-1 defense, we performed the following. Briefly, MSCs were immunomagnetically sorted from 0-20 days infected mice and subjected to phenotypic analysis for ASCs as described previously ((PMID: 22689594). Potential anti-MHV-1 activity of the conditioned media of the sorted cells was assessed in the virus infected primary type II alveolar epithelial cells in vitro. We found that the Mtb + MHV-1 infected group versus MHV-1 alone group exhibited an 8-fold ($p<0.02$; n=4) higher ASC reprogramming and 5-fold ($p<0.001$; n=3, student t test) higher anti-viral activity. Moreover, the survival of the virus infected primary type II alveolar cells was increased in both groups significantly, although the Mtb-infected group showed 4-fold greater survival than the animals infected with MHV-1 alone. Conclusion: We suggest that CD271+MSC derived ASCs secreted factors that targeted MHV-1, and Mtb boosted this mechanism. Thus, we have identified a putative ASC based innate defense against coronavirus.

Funding source: Department of Biotechnology, India, and KaviKrishna USA Foundation, Lincoln, MA

Keywords: stem cell altruism, stem cell niche, COVID19

MUSCULOSKELETAL

CI231

A SINGLE-CELL TRANSCRIPTOMIC META-ANALYSIS OF MULTIPOTENT BONE MARROW STROMAL CELLS

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Multipotent bone marrow stromal cells (BMSCs) have been traditionally defined by their in vivo and in vitro trilineage potential. Although BMSCs have been the subject of intense investigation since their discovery, many fundamental questions have remained unanswered because of the lack of means to prospectively isolate and map BMSCs in vivo. Recently, several studies have sought to address this problem by determining the transcriptional profile of BMSCs using single-cell RNA-Seq (scRNAseq). Here, we performed a comprehensive meta-analysis of 16 scRNAseq murine data sets to identify universal and study-specific features of BMSC. We defined distinct populations in bone marrow stroma by unsupervised clustering and cross-study classification, and we inferred signaling pathways and transcriptional regulators that govern fate transitions. Despite the diverse origins, technologies, and means of BMSC isolation across the studies in our meta-analysis, we were able to uncover a substantial similarity in sub-populations, transition states, and regulators across data sets. Moreover, our analysis revealed a bi-directional dynamic transition between Cxcl12-abundant reticular (CAR) cells and osteoprogenitors (OsP). Our findings help to clarify the identity of multipotent BMSCs and will enable the improved isolation and experimental exploration of BMSCs in the future.

Funding source: R35GM124725 NIH/NIGMS

Keywords: Bone marrow stromal cell, Mesenchymal stem cell, Single-cell RNA sequencing

CI325

HELIX-LOOP-HELIX TRANSCRIPTION FACTOR ASCL4 IS A MYOGENIC REPROGRAMMING FACTOR FOR EMBRYONIC STEM CELLS

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The basic helix-loop-helix (bHLH) transcription factors play central roles in developmental processes including cell fate specification such as MyoD family for myogenesis and Achaete-scute complex-like 1 (Ascl1) for neurogenesis. Here, we found that all Ascl family (Ascl1-5) has the ability to induce myogenic program when overexpressed in embryonic stem cells (ESCs). Among them, we noticed that Ascl4 expression is detected in dermomyotome, a place for myogenic progenitor cells during mouse embryogenesis. In ESCs, overexpression of Ascl4 efficiently induces MyoD/Pax7-positive myogenic cells followed by myosin heavy chain-positive terminally differentiated myocytes when Ascl4 expression was withdrawn. Integrative analysis of RNA-seq and ChIP-seq data revealed that Ascl4 is able to induce MyoD expression through the binding of novel MyoD enhancer region located at 60 kb upstream of its start site. In transgenic mice, overexpression of Ascl4 is able to expand myogenic region during mouse embryogenesis. Together, these findings imply that Ascl4 may mediate activation of MyoD as a key regulator of somitic myogenesis.

Keywords: Myogenesis, Embryonic stem cell, MyoD

CI330

MIR-690 ENHANCES OSTEOGENIC DIFFERENTIATION IN MOUSE AND HUMAN EMBRYONIC STEM CELLS

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Osteogenesis is a complex and critical process for the proper development of vertebrates. The complexity of osteogenesis resides in the multitude of pathways that embryonic stem cells (ESCs) take to differentiate into osteoblasts and the intricate genetic regulatory network, which controls this lineage development. Adding to the complexity, osteogenic differentiation is induced from either mesoderm or neural crest cells; both of which can differentiate into mesenchymal cells and subsequently into osteoblasts. This overlap of differentiation into mesenchymal cells creates a convenient point at which to study osteogenesis, but reduces the focus on the intermediate stages of differentiation. Additionally, the role of microRNAs (miRNAs) in these stages appears to be crucial for the fate of specific progenitors. In this study we show that overexpression of a specific miRNA, miR-690, upregulates osteogenesis through the direct targeting of β-catenin. Additionally, the increased osteogenesis achieved from miR-690 overexpression during differentiation is time dependent, best achieved with a transfection during days 5-7 of differentiation. Furthermore, stable and transient transfection of miR-690 result in morphological differences as well as a shift in the calcification timeline possibly stemming from a shift in the overall cell population origin. Both the transient and stable transfection resulted in a change of the overall cell population origin producing more neural crest head mesenchyme/prechordal mesenchyme as well as lateral plate mesoderm, limb bud, and somatopleure. Additionally, these findings were mimicked in human ESCs yielding increased calcification and an alteration of cell fate origin. Together, our data indicate that miR-690 supports osteogenesis and plays a role in early cell fate decisions.

Funding source: Funding sources: Translational Centre for Regenerative Medicine Researcher Starter Grant to D. Kaniowska, and NIDCR R01 R01DE025330 to N.I. zur Nieden.

Keywords: mir-690, Stem Cell fate determination, osteogenesis

NEURAL

CI234

DOPAMINERGIC NEURONAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS: EXPRESSION PATTERNS OF CTCF, TRANSCRIPTOMICS AND CHARACTERIZATION OF ACTIVE ENHANCERS

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Embryonic stem cells are undifferentiated cells characterized by their ability for self-renewal and differentiation into all cell types from the three embryonic germ layers. Parkinson disease is characterized for the progressive degeneration of midbrain dopaminergic neurons. In vitro differentiation has allowed the study of several aspects of dopaminergic induction. High-order chromatin structures undergo remodeling during cell differentiation, altering nuclear topology and contact points between promoters and enhancers, which impact gene expression. These changes are mediated by a group of architectural proteins, such as CTCF. However, the role of CTCF and the possible enhancer activation during human dopaminergic neuron differentiation has not been evaluated yet. Therefore, the aim of this project is to use ATAC-seq and RNA-seq to determine putative enhancers, transcription factors and long no-coding RNAs (lncRNAs) orchestrating the differentiation of human embryonic stem cells to midbrain dopaminergic neurons, and correlate them with CTCF protein levels during this process. We found a decrease in CTCF protein levels during days 14 (neural precursors), 21 and 60 (neurons), with respect to day 0 (pluripotent stage); at day 28 there was a transient inecrease in CTCF levels. We also observed greater association of CTCF with heterochromatin at day 14 with respect to days 0 and 28. Interestingly, 65 of the top 100 differentially-expressed genes during the process where lncRNAs. Also, we found several open chromatin regions overlapping with active enhancer features. Combining TFs expression patterns and

binding motif analysis on ATAC-seq peaks, we identified many important enhancer binding sites for TFs, that might be important for this differentiation process.

Funding source: CONACyT, 272815 PAPIIT, IN213719

Keywords: Neural differentation, Epigenetic regulation, Non-coding elements

CI318

UNDERSTANDING CELL TYPE IDENTITY IN THE DEVELOPING HUMAN CORTEX AND GLIOBLASTOMA

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The human brain is composed of diverse cell types across brain regions that enable unique capabilities. In the cerebral cortex, the outer layer of the brain that provides for advanced cognitive function, these cell types emerge from an initially uniform neuroepithelia. Using single-cell sequencing, we have characterized the differentiation, maturation and area-specific trajectories of human cortical development. Interestingly, we identify outer radial glia-like cells in single-cell sequencing from primary glioblastoma tumor resections, and also validate these observations with time-lapse imaging. These findings suggest important preservation of developmental trajectories between normal development and cancer development. Experimental manipulation of these primary human cell populations will require in vitro models; we have compared cell types from primary developing human cortex to cerebral organoids. We observe that in vitro models of cortical development strongly recapitulate broader hierarchies of cell identity, but clearly do not possess identifiers of cell subtypes that exist in normal human development and up-regulate cellular stress pathways. Moreover, the contrast to organoid cell types indicates that broad cell type can be sufficiently directed in vitro, but that subtype specification at the earliest stages of development is regulated by conditions that are not present in the organoid.

Funding source: NIH K99NS111731

Keywords: Single-Cell RNA Sequencing, Human Cortical Development and Organoids, Glioblastoma

NEW TECHNOLOGIES

CI251

HIGH-THROUGHPUT SAMPLE PROCESSING FOR EXOSOME ISOLATION.

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Exosomes are extracellular vesicles (EVs) released by cells, which are present in almost all biological fluids. They carry proteins, small molecules, and genetic material, contributing to cell-to-cell communication and taking part in a wide range of in-vivo functions. Thus, exosomes are of particular interest in the study of biomarkers with high potential in therapeutic applications. To study exosomes, their purification is a critical step and multiple methods are already available on the market. However, these methods are generally low-throughput, with the ability to process only individual samples. This can become laborious when scale-up of exosome isolation is desired. In this poster, we are presenting a solution: Our latest size exclusion chromatography (SEC) based method, which can purify up to 96 samples simultaneously. Here we present valuable data including yield, purity, consistency, and reproducibility results using human blood (serum and plasma) samples demonstrate a scalable, reproducible, and flexible high-throughput innovative exosome isolation system. This product is convenient for the purification of exosomes from several samples at once without compromising reproducibility.

Keywords: High-throughput, Exosome isolation, Reproductibility

CI255

APPLICATION OF MITOCHONDRIAL TRANSFER TO GENERATE DESIGNER STEM CELLS

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Although mitochondrial DNA (mtDNA) mutations are associated with metabolic disorders, cancer and dramatically reduced health spans, no treatments exist due to the inability to correct detrimental mtDNA sequences. The replacement of mutant mtDNA with healthy mitochondria and mtDNA represents a potential path to overcome this roadblock. However, no transfer technologies can stably introduce mtDNA into primary cells. We developed a mitochondrial transfer approach to insert isolated mitochondria from individuals of various ages, genders, and ethnicities into neonatal dermal fibroblasts (NDFs) devoid of endogenous mtDNA (ρ0) to determine the parameters required for successful mitochondrial replacement. Cells that retain exogenous mitochondria were isolated in uridine-deficient, galactose media. Transfer of aged mitochondria into NDF ρ0 cells generated up to 30 colonies that stably retained exogenous mtDNA. Mitochondrial function in the engineered cells were characterized through the Seahorse extracellular flux analyzer, immunohistochemistry, and quantitative PCR. Furthermore, the engineered fibroblasts were reprogrammed into induced pluripotent stem cells to quantify the role of mtDNA sequence on fate transition. This work will provide further insights into the interactions between the nuclear and mitochondrial genomes and provide a potential avenue for generating engineered cell lines for cell-based therapies.

Keywords: Mitochondrial transfer, Reprogramming, mtDNA mutations

CI257

ANALYSIS OF CHROMATIN ACCESSIBILITY IN 152 IPSC LINES PROVIDES INSIGHTS INTO EFFECTS OF REGULATORY VARIANTS

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Chromatin accessibility identifies active regions of the genome, often at transcription factor (TF) binding sites, enhancers, and promoters which contain regulatory genetic variation. Here, we generated high quality ATAC-seq data for 152 iPSC lines (from 134 individuals) utilizing a step-wise experimental design (i.e. completed each experimental step for all samples before proceeding to the next) to minimize batch effects, and a modified protocol to minimize the number of mitochondrial reads. Additionally, to enrich for non-nucleosome spanning reads (less than 140 bp in length), we included a double size selection purification step. Using this protocol across all samples, a total of 5.5 billion reads (mean 41 million per iPSC line), were generated. We found that, compared to the original ATAC-seq protocol, our modified protocol resulted in an average of 7.5% less mitochondrial reads, 8.8% higher FRiP, 8.4% more active reads, and 41k more peaks per sample. We called over 1 million ATAC-seq peaks and showed that they displayed known accessible characteristics of regulatory elements by examining their overlap and consistency with higher order chromatin structure at low-resolution, chromatin states, and H3K27ac peaks. Using 50X WGS data for the 134 individuals we tested for associations between the height of the accessible site and all genetic variants within 100kb. We found 235k sites with an associated genetic variant, which is consistent with previous estimates of the fraction of accessibility that is explained by variation. We next examined the chromatin states enriched at these sites, and found an enrichment for non-promoter chromatin states, suggesting that genetically associated sites were more likely to be distal regulatory in nature than located at gene TSSes or flanking chromatin. Overall, these analyses identify sites whose total accessibility is genetically associated, and show that they are more likely to occur at iPSC distal-regulatory elements.

Funding source: California Institute for Regenerative Medicine (CIRM) grant GC1R-06673 and NIH grants HG008118-01, HL107442-05, DK105541-03 and DK112155-01

Keywords: human iPSC, ATAC-seq, variants, genetics, chromatin, co-accessibility

CI263

A MIRNA-BASED STRATEGY TO EXPAND THE DIFFERENTIATION POTENCY OF STEM CELLS

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Full differentiation potential along with self-renewal capacity is a major property of pluripotent stem cells (PSCs). However, the differentiation capacity frequently decreases during expansion of PSCs in vitro. We show here that transient exposure to a single microRNA, expressed at early stages during normal development, improves the differentiation capacity of already-established murine and human PSCs. Short exposure to miR-203 in PSCs (miPSCs) induces an early and transient expression of 2C-markers that later results in expanded differentiation potency to multiple lineages, as well as improved efficiency in stringent assays such as tetraploid complementation and human-mouse interspecies chimerism. Mechanistically, these effects are at least partially mediated by direct repression of de novo DNA methyltransferases Dnmt3a and Dnmt3b, leading to transient and reversible erasing of DNA methylation. These data support the use of transient exposure to miR-203 as a general and single method to reset the epigenetic memory in PSCs, and improve their effectiveness in regenerative medicine.

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Keywords: Stem cell differentiation, Epigenetic memory, Regenerative Medicine

CI281

CLONING, GENE EDITING AND EXPANSION OF HIGH-QUALITY HUMAN PLURIPOTENT STEM CELLS IMPROVED THROUGH MEDIUM OPTIMIZATION

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Specialized culture medium is required to maintain the self-renewal and pluripotent properties of human pluripotent stem cells (hPSCs). The majority of culture systems require time-consuming daily medium changes to replenish the levels of critical components and eliminate accumulated metabolic waste. mTeSR™ Plus, based on the mTeSR™1 formulation, was specifically developed to provide a supportive environment—enabling versatile feeding schedules—while maintaining quality and workflow compatibility of hPSC cultures. FGF2 levels are stabilized in mTeSR™ Plus over 72 hours at 37°C (83.6 ± 7.3% of fresh medium, n = 3), and buffering capacity is enhanced to maintain pH ≥ 7.0 for up to 72 hours without medium exchange. Together, this supports versatile feeding schedules with excellent cell quality and higher cell yields. Compared with hPSCs grown in mTeSR™1, hPSCs in mTeSR™ Plus have an increased plating efficiency (1.3 ± 0.2 fold increase, n = 5 cell lines), and similar cell division times (14.8 ± 1.9 and 14.3 ± 1.8 hours, respectively; n = 2 cell lines). Importantly, hPSCs maintained in mTeSR™ Plus with reduced feeding for ≥ 20 passages retained ideal morphology features, high expression of OCT4 and TRA-1-60 (average 98.2 ± 2.1% and 93.5 ± 3.1%, respectively), and were karyotypically normal by G-banding and showed no common chromosomal abnormalities during routine screening using the hPSC Genetic Analysis Kit (n = 4 cell lines). Cultures maintained in mTeSR™ Plus were also compatible with directed differentiation to 10 separate cell types across the 3 germ layers, as well as established cloning and gene editing workflows. hPSCs cultured in mTeSR™ Plus demonstrated 59.7 ± 6.2% gene knockout efficiency using the ArciTect™ CRISPR-Cas9 genome editing system (n = 3) and 26.3 ± 2.8% cloning efficiency when supplemented with CloneR™ (n = 4); these were similar to or better than results for hPSCs cultured in mTeSR™1. In summary, mTeSR™ Plus is an improved medium that promotes a more consistent cell culture environment, enabling versatile workflows while maintaining high-quality hPSCs that are fully compatible with established genome editing and differentiation protocols.

Keywords: Pluripotency, hPSC Expansion, hPSC Cloning and Gene Editing

CI282

GROWTH-RESTRICTING PATHWAYS AND THEIR RELATED MUTATIONS IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) hold a great promise for studying human development, disease modelling and regenerative medicine. These cells have the capacity to differentiate into all cell types and the ability of self-renewal. Although hPSCs can grow in feeder-free medium, we still lack sufficient knowledge about the different pathways that control their growth in culture. To this end, we performed a genome-wide loss-of-function screen to identify growth-restricting pathways, using CRISPR/Cas9-mediated mutagenesis targeting 18,000 genes with nearly 180,000 sgRNAs in haploid human embryonic stem cells (hESCs). In this screen, the mutated cell population was analyzed every 2 weeks for more than 100 days in order to identify the enriched pathways after long-term culturing of the cells. We could identify four pathways by gene-set enrichment analysis: the TP53 target pathways BAX/NOXA and IGF1/mTOR, the RHOA/ROCK pathway and the novel MAP3K7 pathway. We then validated that chemical inhibition of MAP3K7 indeed provides growth advantage to the cells, and showed that it reduces the rate of apoptosis in cultured cells. Since disruption of growth-restricting genes provides growth advantage to the cells, we wondered whether spontaneous mutations in these genes may already exist in culture-adapted hPSCs. We thus utilized a previously-published methodology (developed in our lab) to analyze RNA-sequencing data of two of the most commonly used hESC lines in a high-throughput manner in order to find point mutations in growth-restricting pathways. We identified RHOA/ROCK as the pathway with the highest number of mutations, while TP53 mutations exist in more samples. Genes belonging to other pathways are also mutated but to a lesser degree. We showed that these mutations are relatively pathogenic and that some of them have high allelic fractions. Lastly, we showed that the same genes mutated in hESCs are also mutated in iPSCs, and that both types of hPSCs have a higher average number of mutations per sample compared to mesenchymal stem cells, highlighting the relevance of these pathways to pluripotent

cells. Finally, we suggest that our analysis should enable finding medium supplements that will allow optimal growth of hPSCs and minimize their mutation rate.

Keywords: Human Pluripotent Stem Cells, Genetic Screening, Point Mutations

CI308

AN INDUCIBLE CRISPR PLATFORM IN HUMAN STEM CELLS IDENTIFIES NOVEL CELLULAR PROCESSES CONNECTED TO HERV-H

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The human genome project delivered an astonishing discovery that a remarkable portion (44%) of the mammalian genome consists of retrotransposons. Among these, approx. 10% of the human genome contains so-called “human endogenous retroviral elements” (HERVs). However, the functional roles of HERVs remain elusive. To this end, we generated an inducible CRISPRactivation (CRISPRa) and CRISPRinterference (CRISPRi) system in induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) to identify cellular processes that are actively regulated via long terminal repeats (LTRS) of the HERV-H family. We used the inducible CRISPRa/i system to transcriptionally activate and repress these LTRs in hESCs and hiPSCs, respectively. Afterwards, we analyzed the effects on gene regulation important for cellular processes using whole genome RNAseq. We observed regulation of gene networks involved in early developmental processes and cell metabolism upon HERV-H transcriptional activation or repression in hESCs as well as hiPSCs, respectively. However, we also identified several gene networks driving distinct cellular processes specific for either hiPSCs or hESCs. Taken together, our study identified novel cellular processes in hESCs as well as hiPSCs connected to HERV-H using an inducible CRISPR platforms combined with whole genome RNAseq.

Keywords: Human Endogenous Retroviruses-H, Inducible CRISPR activation and repression, Human Embryonic stem cells

CI321

LINEAGE ANALYSIS OF CELLULAR STATES PREDICTING REPROGRAMMING INTO HUMAN INDUCED PLURIPOTENT STEM CELLS

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The ability to generate induced pluripotent stem cells (iPSCs) from differentiated somatic cells via ectopic expression of OCT4, KLF4, SOX2, and MYC (OKSM) has enabled engineering of cellular states for disease modeling and regenerative medicine. However, only a small subset (<1%) of cells exposed to OKSM actually become iPSCs. This low efficiency is observed even when OKSM is integrated stably and clonally into the genome, suggesting that this variability must be due to heterogeneity at the cellular level instead of simply technical noise. Furthermore, we still do not definitively know whether anything is different about the rare cells that become iPSCs and when their ability to reprogram successfully is established. Here, we show that “primed” cellular states encoding for reprogramming success exist before OKSM exposure and are heritable across cell divisions when reprogramming human fibroblasts. These primed states have not yet been characterized because of their rarity as well as the conceptual and technical challenge of isolating them before OKSM exposure even with newer single cell approaches. To directly isolate and profile these rare primed cells we leverage a novel method utilizing barcoding, sequencing, imaging, and flow sorting called “Time Machine”. We show that Time Machine can effectively distinguish between primed and unprimed cells before OKSM exposure via unique and transcribed DNA barcodes. We also show preliminary evidence of potential markers of the primed states identified by Time Machine. These markers are significantly upregulated in primed cells that eventually become iPSCs and are associated with maintaining pluripotency during development. Finally, we show preliminary evidence that cells can be converted from unprimed states to primed states with the use of reprogramming boosters to increase overall reprogramming efficiency. This work is poised to answer longstanding questions about the existence and nature of rare cells primed for reprogramming. More broadly, it will help us identify new pathways to modulate reprogramming in predictable ways and reveal the molecular basis of plasticity in seemingly differentiated cells.

Keywords: reprogramming, cell state, cell identity

CI341

POYSEQ: THE CREATION OF A NEW POLYMER-BASED LABELING SYSTEM FOR RAPID HETEROGENEOUS MULTIPLEXING IN NEXT-GENERATION SEQUENCING APPLICATIONS

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Next-generation sequencing (NGS) provides a powerful tool for unparalleled investigative depth into transcriptomic and genomic profiles. Combined with single-cell techniques,

NGS has revolutionized experimental investigation within the biological sciences. Single-cell techniques offer the ability for high-resolution analysis of a heterogeneous sample. However, with the caveat of only one experimental condition per library preparation, elevating the costs to run multiple samples as the preparation of multiple libraries are therefore required. During preparation, conveniently, there exists an opportunity for sample-specific molecular barcoding. This barcoding complements single-cell experimental power by allowing for multiple samples to be mixed and prepared in parallel within a single library, thereby presenting a time and cost savings while simultaneously enhancing high-throughput potential. Here were report the development of a polymer-based molecular barcode labeling system, termed POLY-seq, synthesized with low cost, commercially available reagents capable of binding standard hashing oligos in 10 minutes. The POLYseq system achieved functional barcoding within one hour using standard hashing oligos, allowing for the correct identification of barcode labels in 90 % of cells derived from a heterogeneous pool of hepatic organoids prepared on the 10x Genomics single-cell RNA-seq platform.

Keywords: POLYseq, single-cell, multiplexing

PLACENTA AND UMBILICAL CORD DERIVED CELLS

CI292

NAIVE HUMAN EMBRYONIC STEM CELLS READILY TRANSDIFFERENTIATE TO TROPHOBLAST LINEAGE

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Human embryonic stem cells (hESCs) readily differentiate to somatic or germ lineages but have impaired ability to form extra-embryonic lineages such as placenta or yolk sac. Here we demonstrate that hESCs cultured in naïve media conditions can be readily converted into cells that exhibit the cellular and molecular phenotypes of human trophoblast stem cells (hTSCs) derived from human placenta or blastocyst. The resulting "transdifferentiated" hTSCs show reactivation of core placental genes and the ability to differentiate to extravillous trophoblasts and syncytiotrophoblasts, though some imprinted genes are dysregulated. Their ability to form trophoblast-like cells indicates that naïve hESCs have a broader differentiation capacity than conventionally cultured primed hESCs. These results also demonstrate a new way of generating hTSC lines and modeling human trophoblast specification.

Funding source: New Frontiers in Research Fund, Canadian Institutes of Health Research

Keywords: Pluripotency, Trophoblast, Placenta

Theme: Clinical Applications

CARDIAC

CA112

IN VITRO MATURED HESC-DERIVED CARDIOMYOCYTES FORM GRAFTS WITH ENHANCED STRUCTURE AND IMPROVED ELECTROMECHANICAL INTEGRATION IN INJURED HEARTS

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Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) have tremendous promise for application in cardiac repair, but their immature phenotype greatly limits their translational potential. The present study was designed to two hypotheses: 1) that previously reported methods to promote the maturation of hESC-CMs by culture on soft polydimethylsiloxane (PDMS) substrates can be upscaled to the quantities required for transplantation studies; and 2) that PDMS-matured hESC-CMs will stably engraft in injured hearts and form graft myocardium with enhanced structural and functional properties. First, we cultured hESC-CMs on either PDMS or tissue culture plastic (TCP) for 20 and 40 days, then phenotyped the resultant populations. All hESC-CMs were engineered to express the fluorescent voltage-sensitive protein ASAP1 to facilitate in vitro and in vivo electrophysiological studies. Relative to their counterparts on TCP, hESC-CMs on PDMS at both time-points exhibited increased cardiac gene expression as well as a more mature structural and electrophysiological phenotype in vitro. Single-cell transcriptomics confirmed enrichment of cardiac maturation

markers including gene pathways involved in cardiac contraction, extracellular matrix organization, sarcomerogenesis, and adult heart development in PDMS versus TCP cultures. Next, we transplanted day 20 or 40 TCP vs PDMS ASAP1+ hESC-CMs into injured guinea pig hearts. Recipient hearts were later analyzed by ex vivo optical voltage mapping studies and histology. While CMs from both substrates showed similar capacity for engraftment, grafts formed with PDMS-matured myocytes had more mature structural properties including enhanced alignment, sarcomere lengths and maturation marker expression. Most importantly, graft formed with PDMS-matured myocytes showed improved electrophysiological properties including better host-graft electromechanical integration and more rapid and uniform propagation. We conclude that large quantities of matured hESC-CMs can indeed be economically produced by these methods. Moreover, PDMS-matured myocytes form large intramyocardial grafts with enhanced cardiac structure and electrical function, thereby establishing that maturation prior to transplantation meaningfully improves outcomes in vivo.

Funding source: McEwen Centre for Regenerative Medicine, the Peter Munk Cardiac Centre and the University of Toronto's Medicine by Design/Canada First Research Excellence Fund initiative.

Keywords: hESC-derived cardiomyocytes maturation, Cardiac regeneration, Graft electromechanical integration

EARLY EMBRYO

CA265

REVEALING THE 'PRIMED' CELLULAR STATES UNDERLYING VARIABILITY IN FATES FOLLOWING DIRECTED DIFFERENTIATION

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Fifteen years after their discovery, induced pluripotent stem (iPS) cells have revolutionized biomedical research but remain far from clinical translation. One major concern is that differentiated cell populations are far from pure; that is, profound variability in cell fate exists even among clonal cells that "successfully" traverse the differentiation protocol. For instance, distinct populations of atrial and ventricular cells arise within a single well during cardiomyocyte differentiation. Could it be that there are differences in initial iPS cell states that "prime" isogenic cells to take on different fates during differentiation? If so, are these primed states, and their mapping to distinct cellular fates, shared between different differentiations? That is, what can we predict about the iPS cells that are primed to differentiate in one protocol given what we know about primed iPS cell states and their linked fates in another protocol? To answer these questions, we need to profile both the final and initial states of differentiating cells and link them together. To this end, we will use Rewind, a new method developed in our lab, to label individual initial cells with unique DNA barcodes that are transcribed. After a few cell divisions, we will randomly separate related cells carrying identical barcodes to create a "carbon-copy" for immediate single-cell RNA sequencing of the initial state, while their sibling cells are differentiated for profiling of the final state. Using single-cell RNA sequencing we can classify differentiated cells into their final fates and, because the barcodes are transcribed, determine which cellular lineages contribute to each fate. We will return to the initial state data and examine how these barcode-marked lineages clustered in iPS cell expression space, uncovering the expression states that predispose cells to traverse particular paths. Repeating this process, we can determine how those paths might compare between differentiation protocols. Successful completion of this work will significantly expand our knowledge regarding the processes that underlie cell type variability following differentiation, addressing longstanding questions of purity and bringing us one step closer to the dream of stem cell-based personalized medicine.

Funding source: NIH T32 DK007780, F30HG010822

Keywords: cellular priming, nongenetic variability, cellular state

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CA119

HEPG2-CONDITIONED MEDIUM IMPROVES HEPATIC SCAFFOLD RECELLULARIZATION

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Hepatic bioengineering technologies have been developed aiming the future of human organs transplantation. New tissue engineering tools have been designed to produce decellularized organs (i.e. scaffolds) which could be recellularized with human

cells. However, protocols for inducing better liver scaffolds recellularization need to be ameliorated. The aim of the present investigation is to improve the hepatic recellularization by coating liver scaffolds with HepG2-conditioned medium (HepG2-CM). Wistar rat livers were collected and cannulated by portal vein (PV) and vena cava (VC). They were decellularized by perfusion of 1% of Triton-X solution with 0.05% NaOH. The samples were analyzed by immunohistochemistry, scanning electronic microscopy (SEM), histology and proteomic assays. Human iPSCs and differentiated cells were characterized by immunofluorescence staining (IF), flow cytometry, RT-qPCR. The anatomical organization of hepatic extracellular matrices (ECM) was preserved after the decellularization procedure. The liver scaffolds had no nuclei and cellular residues. Proteomic analysis suggested that pre-coating liver scaffolds with HepG2-CM enriched acellular liver ECM. Pre-coated decellularized livers were recellularized with HepG2, hiPSCs-derived mesenchymal stem cells (hiMSCs) and human aortic endothelial cells (HAEC) for up to 5 weeks and an improved liver recellularization was observed. Further studies are still needed but our preliminary results suggest that pre-coating of livers-ECM significantly improved recellularization, revealing the positive effects of liver ECM and CM components association.

Funding source: Fapesp CNPq Ministry of Health

Keywords: Liver, Scaffold, Recellularization

CA124

OPTIMIZING PROCESS PARAMETERS FOR THE SCALABLE MANUFACTURE OF INSULIN PRODUCING CELL CLUSTERS

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Type 1 diabetes (T1D) is characterized by chronically elevated blood glucose levels as a result of beta-cell dysfunction and insulin deficiency. The efficacy of cell replacement therapy for the treatment of T1D has been demonstrated with islet transplantation using the Edmonton Protocol. However, its widespread implementation as a form of cell replacement therapy is limited by the scarcity of cadaveric donor islets and the necessity of chronic immune suppression. Stem cells are potentially an unlimited source of beta cells for the treatment of T1D. Using embryonic stem cells (ESCs), we have developed a differentiation protocol that generates pancreatic progenitor aggregates comprising of >80% NKX6.1+/PDX1+. With further differentiation, we make cell clusters capable of secreting insulin in response to the incretin mimetic, exendin-4, in the presence of 16.7 mM glucose. The current differentiation protocol is initiated in monolayer culture and then transitions into suspension cultures following the aggregation of pancreatic progenitors. In order to enhance cell yields, we are optimizing the scalability of the current process by addressing process parameters such as seeding density, aggregate size, vessel format, and agitation rate using ESCs. Various cell culture platforms with different geometries were evaluated for aggregate formation efficiency, growth kinetics and viability. PBS Mini, a vertical wheel bioreactor, reproducibly generated clusters with a narrow size distribution and >50% aggregation efficiency. The bioreactor agitation rate was identified as a critical process parameter that determines aggregate size. In spinner flask conditions, cluster size distribution was broader. Clusters generated using rollerbottles showed a wide range of aggregation efficiencies (8%-79%) and cluster size distribution. Nevertheless, the identity of the ESC aggregates was maintained based on gene expression and protein levels regardless of the cell culture vessel tested. Insights from these studies will help identify key critical process control parameters that will guide the process development design to minimize cell loss throughout the manufacturing process while maintaining the identity and quality of the stem cell-derived insulin-producing cells.

Keywords: Insulin-producing cells, Differentiation, Process parameter development

CA125

3D BIOPRINTING LIVER ORGANOID USING HEPATIC SPHEROIDS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS SUSTAIN LIVER FUNCTION AND VIABILITY IN VITRO

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The liver is responsible for many metabolic, endocrine and exocrine functions. Approximately 2 million deaths per year are associated with liver failure. Modern 3D bioprinting technologies combined with autologous induced pluripotent stem cells (iPS)-derived grafts could represent a relevant tissue engineering approach to treat end stage liver disease patients. However, protocols that accurately recapitulate liver's epithelial parenchyma through bioprinting are still underdeveloped. Here we evaluated the impacts of single cell dispersion (i.e. obtained from conventional bidimensional differentiation) of iPS-derived parenchymal (i.e. hepatocyte-like cells) as compared to iPS-derived hepatocyte-like cells spheroids (i.e. three-dimensional cell culture), both in combination with nonparenchymal cells (e.g. mesenchymal and endothelial cells), into final liver tissue functionality. Single cell constructs showed reduced cell survival and hepatic function as well as unbalanced protein/amino acid

metabolism when compared to spheroid printed constructs after 18 days in culture. In addition, single cell printed constructs revealed epithelial-mesenchymal transition, resulting in rapid loss of hepatocyte phenotype. These results supports the advantage of using spheroid-based bioprinting contributing to improve current liver bioprinting technology and liver physiology aiming future regenerative medicine applications.

Funding source: FAPESP (2015/14821-1); FAPESP (2019/18469-1)

Keywords: Bioprinting, Liver, iPS

EPITHELIAL

CA130

COLLAGEN CHITOSAN , BONE MARROW DERIVED MESENCHYMAL STEM CELLS AND EXTRACELLULAR VESICLES ENRICHED COLLAGEN CHITOSAN SCAFFOLDS IN THE HEALING OF AN INDUCED SKIN WOUND (A RAT MODEL)

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Over the past ten years, an alternative for maxillofacial reconstruction was offered through regenerative medicine. It provided a new scope for improving the reconstruction of the oral and maxillofacial structures whether they are hard tissues, which include the teeth and bone, or the soft tissues like the oral mucosa, skin, nerves and blood vessels. The aim of this study is to evaluate the effect of the collagen chitosan scaffold alone or supplemented with bone marrow derived mesenchyme stem cells (BM-MSCs) and their secreted extracellular vesicles (EVs), on the duration and quality of skin wound healing. A full thickness skin wound was induced to the back of thirty-two adult male Sprague-Dawley rats and then classified into control, scaffold, scaffold + MSCs and scaffold + EVs groups where the different treatment modalities were assessed on the duration and quality of skin wound healing. All treated group showed accelerated wound healing in comparison to the control group and with no significant difference between them. Quality of healed skin was significantly better in EVs, BM-MSCs and scaffold treated groups respectively. These results were attributed to the better collagen alignment and deposition and thus higher skin tensile strength in these groups. In conclusion, the collagen chitosan can be considered as an appropriate scaffold for MSCs or their EVs in wound healing.

Keywords: Collagen chitosan scaffold in wound healing, Mesenchymal stem cells, Extracellular vesicles

CA133

PRE-CLINICAL APPLICATION OF TISSUE-ENGINEERED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED AIRWAY GRAFTS

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Long-segment airway stenosis and disease require transplantation, which primarily fails due to epithelial dysfunction and epithelium-triggered immune rejection. We developed two approaches for producing functional grafts using human induced pluripotent stem cell (hiPSC)-derived airway progenitors (APs): 1) direct re-epithelialization of de-epithelialized allograft tracheae in a bioreactor; and 2) generation of fully differentiated biomaterials-based epithelial grafts in vitro for application in airway defects. We produced 65.6±2.4% P63+ APs and seeded 5-cm long de-epithelialized pig tracheae in a perfusion-based bioreactor for 3 days. A uniform cell monolayer formed along the tracheal lumen with P63-KRT5+KRT8+ expression, indicating an intermediate cell differentiation stage at day 3. The next step for these grafts is orthotopic pig tracheal transplantation. For our second approach, we developed a Silk Fibroin and Collagen Vitrigel Membrane (SF-CVM) biomaterial, which could be surgically manipulated and allowed differentiation of APs into 64.6±7.8% ciliated cells and 2.1±1.4% goblet cells in ALI. This differentiation of ciliated cells was physiologically relevant, being comparable to cell proportions present in human tracheae. We established a pig airway defect model for implanting these differentiated SF-CVM epithelial grafts to determine their integration and survival across 3 days. A 2x4 cm tracheal defect was created, pig mucosa was manually stripped and replaced with hiPSC-derived SF-CVM epithelial graft (labeled with CMAC dye) prior to defect closure. Ex-vivo evaluation of these grafts under ALI confirmed cell viability and maintenance of ciliated and goblet cells on day 3. In vivo studies demonstrated SF-CVM integration with surrounding tracheal tissue, with the CMAC-labeled epithelium being intact and alive 3 days post-operatively. This is the first pre-clinical application of biomaterials-based airway grafts generated

from hiPSCs. The development of non-immunogenic chimeric tracheal grafts with recipient-derived epithelium will significantly enhance the viability of tracheal transplants.

Funding source: 1. University of Toronto Medicine by Design Initiative, funded by the Canada First Research Excellence Fund 2. Henry White Kinnear Foundation

Keywords: Biomaterials, Bioreactor, in vivo

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

CA138

THE VIEWS OF PHYSICIAN SPECIALISTS AT U.S. ACADEMIC MEDICAL CENTERS TOWARDS UNPROVEN STEM CELL THERAPIES

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The unproven stem cell intervention (SCI) industry has been growing globally with the largest market perhaps existing in the U.S. This marketplace is replete with hype, misinformation, and emotional appeals driving demand for unproven SCIs. Although most patients seek information online, physicians constitute the first line of defense to counter misinformation surrounding unproven SCIs. However, little is known about physicians' familiarity with and views toward unproven SCIs. We present preliminary results of 25 interviews with physicians from U.S. academic medical centers across 5 specialties (cardiology, neurology, ophthalmology, orthopedics, and pulmonology) who had experience addressing patient questions about SCIs. Our analysis indicates that many physicians were moderately familiar with the marketplace. Their sources of information included conferences, media, clinical society statements and journal articles. Many physicians expressed strong views and considered the unproven market to be highly problematic, citing patient harms, deceptive marketing practices, lack of scientific justification for marketing, and high out-of-pocket costs. All physician specialists besides orthopedists deemed offering unproven SCIs outside a clinical study to be inappropriate; the majority voiced a desire for robust clinical trials and regulatory approval before offering SCIs therapeutically. Orthopedists expressed a desire for additional research, but some explained that unproven SCIs could be conditionally appropriate as long as patients are provided accurate information about the experimental nature and uncertain clinical outcomes of SCIs. A few participants expressed a willingness to consider real world evidence or compassionate use pathways as options for navigating solutions to unmet patient needs. Despite common views of overall hesitancy toward unproven SCIs, physicians expressed enthusiasm for the future of research in regenerative medicine. Our study shows that physicians at academic medical centers are excited about regenerative therapies, know about the unproven market, and have strong views against unproven SCIs, although some orthopedists reported it may be appropriate to offer regenerative interventions under certain conditions.

Keywords: physician perspectives, unproven stem cell interventions, translation

EYE AND RETINA

CA145

RESTORATION OF VISUAL FUNCTION IN MICE WITH END-STAGE RETINAL DEGENERATION BY TRANSPLANTATION OF HUMAN STEM CELL DERIVED CONE PHOTORECEPTORS

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Age related macular degeneration (AMD) and hereditary retinal diseases, which lead to photoreceptor loss, are major causes of irreversible blindness in the western world. Current treatments for macular degeneration require the presence of photoreceptors, leaving a group of patients, in whom the retinal degeneration is too advanced without any viable therapeutic option. Despite being challenging, restoration of functional connectivity following transplantation is a major goal for CNS repair. The macula is an ideal area to explore the potential of cell replacement as it is small and accessible. It is possible to envisage that few transplanted cone photoreceptor cells might be necessary to establish synapses with the host retina in order to achieve clinical benefits. To date no robust proof of functional synaptic connectivity between a pure population of human cone photoreceptors and host end-stage degenerated retinal has been shown. In this study human embryonic (E) SC-derived cones were transplanted into mice with advanced retina degeneration. Control sham transplants of human induced pluripotent stem cell derived cones, containing mutation in CNGB3 gene, were performed under the same conditions. The CNGB3 mutation does not impair the development of cone photoreceptors but is essential to phototransduction. This sham control allows exclusion of rescue of function by material transfer or neuroprotection of the few remaining host photoreceptors. Following transplantation, immunohistochemistry data showed that ESC-derived cones matured, developing outer segment-like structures, and interacted with the host retina, with host bipolar cells seeking out terminals of transplanted cones. Pre and post-synaptic markers were seen in close proximity. Establishment of synapses between transplanted cones and host retina were further supported by Multielectrode Array (MEA). MEA analysis

showed presence of a variety cone-like responses to light. Similar responses were absent in control retinas. Behavioural data corroborates these findings, as treated animals displayed different light-avoidance behaviour than control animals. This data suggests that it is possible to restore functional synaptic connectivity following transplantation, thereby rescuing visual function of severely degenerated retinas.

Keywords: Stem cell-derived cones, Cell Transplantation, Retina

GERMLINE

CA150

AMNIOTIC FLUID MESENCHYMAL STEM CELLS AND THEIR SECRETED EXTRACELLULAR VESICLES AS THERAPEUTIC TOOLS IN EXPERIMENTAL PREMATURE OVARIAN DYSFUNCTION: A POTENTIAL IMPLICATION OF MIRNA-21

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Chemotherapy induced premature ovarian dysfunction (POD) is a major concern for female cancer survivors and is considered irreversible once established. Chemotherapeutic agents damage highly dividing somatic granulosa cells that are also essential for survival of follicles. Amniotic fluid derived mesenchymal stem cells (AFMSCs) have the potential to rescue fertility; however the notion of stem cells regenerating into follicles is highly controversial due to the finite ovarian reserve pool theory. Novel underlying mechanisms remain to be unraveled.This study investigates whether the secretion of extracellular vesicles (EVs) rich in miRNA-21 from the amniotic fluid mesenchymal stem cells can treat premature ovarian dysfunction through the anti-apoptotic effects of the EVs on damaged granulosa cells. Ninety female rats were randomly divided into four groups. Cyclophosphamide (Cy) was administered once intraperitoneally to seventy two rats to simulate POD. These treated rats were further subdivided into an AFMSCs treated group (n=24), an EVs treated group (n=24) and a POD untreated group (n=24). The remaining 18 rats not treated with Cy were considered as healthy controls. Rats were sacrificed at 24 hours, 7 days, and 2 months post-treatment and assessed for serum Anti-Müllerian hormone (AMH) levels, caspase-3 and PTEN proteins levels in ovarian lysate, Haematoxylin and eosin staining was done to ovarian sections to determine the total follicular count (TFC). Functional assessment was performed as well by daily vaginal smears and mating trials.Treatment with AFMSCs and their EVs restored TFC, AMH levels, regular estrous cycles and fruitful conception. Our findings illustrate that miRNA-21 expression in AFMSCs-derived EVs has anti-apoptotic potential, and may serve as a novel therapeutic gene regulatory agent functioning through PTEN to prevent POD.

Keywords: Premature Ovarian Dysfunction, Amniotic fluid derived stem cells, Extracellular vesicles, miRNA-21

CA151

BANKING OF CLINICAL GRADE HUMAN PLURIPOTENT STEM CELLS: A VIEW FROM THE UK STEM CELL BANK

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The UK Stem Cell Bank (UKSCB) is a key partner of the UK regenerative medicine infrastructure, focused on procuring, banking, testing, and distributing human embryonic stem cell (hESC) lines as seed stocks for research and clinical applications. In addition, the UKSCB develops research focused on standardisation, quality and safety of stem cells and stem cell-derived products. The UKSCB repository comprises over 140 stem cell lines, of which 30 are currently available for distribution. More than 300 shipments of hESC lines, mouse fibroblasts and human induced pluripotent stem cells have been completed worldwide since 2007. Currently available UKSCB cell lines are primarily for research uses, but the UKSCB is actively working on increasing numbers of EUTCD-compliant cell lines (meeting the EU Tissue and Cell Directives (EUTCD) criteria, as set out in Human Tissue Authority (HTA) regulations), suitable for human applications. Much of our banking work has focused on the adaptation of the feeder-dependent hESC lines into feeder-free systems. This adaptation is associated with a considerable reduction in banking time (from thawing) and thus reduced usage of reagents, consumables, facilities and staff time. At present, 21 cell lines from four UK derivation centres have been successfully adapted to feeder-free conditions and are either under quality control or final review stages (http://www.nibsc.org/ukstemcellbank). UKSCB cell lines undergo a rigorous and thorough quality control process prior to release. In addition to viability, sterility, identity and genomic stability, we measure cellular parameters such as pluripotency and tri-lineage germ-layer differentiation. We are currently investigating the effects of processing variables such as derivation centre, cell-culture media and presence of feeder cells on these cellular characteristics, using multivariate analysis. Results will be informative both for optimisation of stem cell culture processes and selection of optimal cell lines by customers for a specific purpose. Through this programme of work, the UKSCB offers increasingly high-quality, deeply-characterised hESC lines, to support the regenerative medicine community in the development of quality and safety-assured cell therapies.

Keywords: Embryonic Stem Cells, Cell Therapies, Quality Control

HEMATOPOIETIC SYSTEM

CA157

ANALYSIS OF CD34+ SUB-POPULATIONS FROM BONE MARROW AND MOBILIZED PERIPHERAL BLOOD SHOW DIFFERENTIAL TRANSDUCTION AND ENGRAFTMENT POTENTIAL IN THE NSG MOUSE MODEL

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The success of ex vivo gene therapies depends on the efficient transduction and engraftment of long-term hematopoietic stem cells (LT-HSCs). Ex vivo gene therapy leverages lentiviral vector (LVV) transduction of CD34+ cells enriched from bone marrow (BM) or mobilized peripheral blood (mPB), both of which are a heterogenous mix of hematopoietic stem and progenitor cells (HSPCs). LVV integration across CD34+ cells is non-uniform, and the contribution of these differentially transduced subpopulations to engraftment is not fully understood. Here, to begin to address this question, we characterized the transduction efficiency and engraftment potential of different CD34+ subpopulations from BM and mPB.CD34+ HSCPCs from mPB were transduced with a GFP LVV, flow sorted on GFP expression levels, and then characterized in vitro by quantifying vector copy number (VCN) and colony-forming cells (CFC), and immunophenotyping (CyTOF). The GFP-high cells with the highest VCNs were biased toward a differentiated immunophenotype and had 2-fold lower CFC compared to the GFP-low cells. Moreover, NSG mice transplanted with GFP-high cells had >100-fold lower engraftment than mice transplanted with GFP-low cells. BM-derived CD34+ cells are known to have bimodal CD34 expression by flow cytometry: CD34dim cells can comprise up to 25% of total CD34+ in healthy donor (HD) BM and up to 50% in sickle cell disease (SCD) BM. Experiments evaluating HD BM found that CD34dim cells were more readily transduced (1.3-2.0 fold higher VCN) and had diminished CFC (2.9-6.6 fold lower) compared to donor-matched CD34bright cells. Similarly, CD34dim cells derived from SCD BM showed diminished CFC (19-140 fold-lower) and higher VCN (1.3-2.0 fold higher) compared to donor-matched CD34bright cells. Consistent with the impact on colony formation, transplant of CD34dim cells from a SCD donor into NSG mice resulted in a 33-fold lower engraftment compared to donor-matched CD34bright cells. Taken together, these data demonstrate the varying potential of different CD34+ subpopulations in achieving robust engraftment. Moreover, our data suggest that the most highly transducable CD34+ cells are not true LT-HSCs and do not contribute to long-term hematopoiesis in the NSG model.

Keywords: hematopoiesis, engraftment, CD34+ cells

IMMUNE SYSTEM

CA159

AN EPIGENETIC QPCR ASSAY FOR IMMUNE CELL IDENTITY AND PURITY TESTING OF CELLULAR THERAPIES

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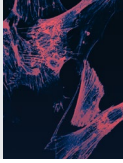
Cell therapy is a technology where the power of immune cells is harnessed to fight certain types of cancer. Development of cell therapies require thorough testing for product release during which contaminating byproducts may be present. Characterization of these biological products thus becomes an essential process which includes determination of biological activity, identity, purity and impurities. Identity and purity assays most commonly utilize cell surface markers or secreted molecules which are measured by flow cytometry. Some of the critical challenges experienced with flow cytometry is the lack of standardization due to user and technical variability as well as the need for live samples. PureQuant are cell specific epigenetic qPCR-based assays and offer an alternative to overcome the challenges with flow cytometry. Chimeric Antigen Receptor (CAR) T cells generated using a second generation (CD3ζ and 4-1BB) anti-CD19 CAR lentivirus were evaluated using PureQuant methylation assays specific for CD3+, CD4+, CD8+ T cells, and contaminating B cells and monocytes. Enriched B cells and monocytes were spiked in CAR-T cells of the same donor at known cell numbers and interrogated using flow cytometry and PureQuant assays to establish sensitivity and accuracy. Each assay includes internal controls to check for assay performance, assay-specific differences, and standards to deduce copy number. The study results between flow cytometry and PureQuant corroborate; however, PureQuant offers a direct result reported as percent cell type without the need for gating strategies and subjectivity.



Keywords: characterization, immunotherapy, PureQuant

CA162

GENERATION OF IMMUNE CELLS FROM HUMAN PLURIPOTENT STEM CELLS IN STROMA-FREE, SERUM-FREE CULTURES

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Human pluripotent stem cells (PSCs) offer a potentially unlimited source of immune cells for disease modeling and development of cell therapy applications. We developed two separate serum-free, stroma-free culture systems to generate monocytes and lymphocytes, respectively, from multiple PSC lines. To generate monocytes, PSC colonies were passaged as clumps in mTeSR1 medium. After one day the medium was replaced with a differentiation medium to induce mesoderm and then on days 3 and 7 to promote hematopoietic specification and differentiation into CD14$^+$ monocytes. Continued culture allowed for prolonged generation and repeated harvest of monocytes from the culture medium between days 14 and 23. The average peak frequency and yield of CD14$^+$ monocytes was 74% (range: 50 - 90%, n = 22 from WLS-1C, STiPS-M001, H1 and H9 cells) and 6.2x10^6 (range: 1.8x10^6 - 1.7x10^7) per 6-well plate. The PSC-derived monocytes also expressed CD11b, CD33, CD16, and CD68 and were further able to differentiate into M1 and M2 macrophages and dendritic cells. To generate T and NK cells, PSCs were aggregated in AggreWell plates and cultured for 10 days to induce mesoderm specification and hematoendothelial differentiation. Cell aggregates were then dissociated into single cells and CD34+ cells were isolated and cultured under either NK or T cell differentiation conditions for 4 weeks. NK cell differentiation cultures produced CD56+ cells with an average frequency of 86% (range: 57 - 98%, n = 24) and yield of 1.1x10^7 (range: 2.2x10^5 - 4.5x10^7) per 5x10^4 input CD34+ cells. NK cells also expressed NKp46 and CD16, when stimulated produced IFN-γ and were able to kill K562 cells in vitro. T cell cultures produced CD4+CD8+ double-positive (DP) T cells at an average frequency of 38% (range: 5 - 85%, n = 16) and yield of 2.7x10^6 (range: 7.3x10^3 -1.9x10^7) per 5x10^4 input CD34+ cells. On average, 15% of DP cells co-expressed CD3 and TCRαβ (range: 0 - 57%). Overall, we show that serum- and stroma-free culture conditions support differentiation of PSCs into monocytes and NK cells that are phenotypically and functionally similar to their counterparts from primary tissues. Current work is focused on differentiating DP T cells into mature functional T cells. Overall, these cultures systems will be valuable for basic and translational research on immune cells.

Keywords: T cell, NK cell, Monocyte

NEURAL

CA168

AN ADOPTIVE TRANSFER OF BONE-MARROW DERIVED MACROPHAGES AS POSSIBLE TREATMENT FOR NEONATAL HYPOXIA-ISCHEMIA IN MICE

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Hypoxia-ischemia (HI) remains a major cause of perinatal brain injury and long-term disabilities. While the primary cause of HI may be diverse and often elude diagnosis, inflammation is a common feature. Microglia and peripheral immune cells infiltrating the brain through a disrupted blood brain barrier play a pivotal role in this chain of events. In this study we investigated the effects of an adoptive transfer of bone-marrow derived macrophages after neonatal HI. Cx3cr1GFP/+Ccr2RFP/+ mice pups of both sexes were subjected to HI at P10 (postnatal day 10) via electrocoagulation of the right carotid artery and 60 minutes hypoxia. Either unpolarised (M0) or anti-inflammatory (M2) macrophages were administered i.p. at P15 and the behavioural activity evaluated three weeks post-HI with beam walk, open field and rotarod tests. Mice were then sacrificed and brain sections processed for immunohistochemical analysis. Our results showed that mice treated with M0 significantly worsened their performance in the beam walk test and had bigger brain injury. On the contrary mice treated with M2 showed a significant functional recovery in the beam walk test even though no alteration of the extent of the infarction size was detected. No differences were observed in the open field or rotarod tests. In conclusion, our results suggest that bone-marrow derived M2 macrophages might be a possible therapeutic strategy to treat neonatal HI. Further investigations are needed to verify the molecular mechanism involved and if other sources of macrophages offer similar protection.

Keywords: Neuroinflammation, Bone-marrow derived macrophages, Hypoxia-Ischemia

CA194

ACHIEVING CLINICAL SAFETY IN HUMAN ESC- OR IPSC-DERIVED DOPAMINERGIC CELL THERAPY FOR PARKINSON'S DISEASE

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Cell therapy shows promise as a therapeutic method for Parkinson's disease (PD) treatment. To ensure safety of human ESC- or iPSC-based cell therapy, some risks must be minimized including 1) assurance of karyotype and genomic integrity; 2) elimination of proliferating undifferentiated stem cells from the final product; 3) confirming absence of migration of implanted cells away from the graft. To address these concerns, we implemented a battery of tests to ensure a safety threshold appropriate to clinical use. First, we confirmed genomic integrity of iPSC-derived cells by karyotyping, whole exome sequencing, and whole genome sequencing, and then applied bioinformatics analyses to rule out presence of any known cancer-causing mutations. Second, to eliminate completely any stem cells remaining undifferentiated or bearing sub-clonal tumorigenic mutations with proliferative or neoplastic potential, we developed a chemical method using quercetin, a specific inhibitor of BIRC5 (encoding survivin) uniquely expressed in hPSC, that eliminates undifferentiated PSCs with >99.99% efficiency. Our theoretical calculations based on qRT-PCR analyses of OCT4 expression predicts fewer than 0.0017 undifferentiated cells per 10 million D28 dopaminergic progenitors, following this quercetin treatment. Since the spontaneous incidence rate for all types of glioma ranges from 4.67 to 5.73 per 100,000, risk of tumor formation would thus be comparable to the spontaneous incidence of gliomas in the target population. We next tested the in vivo safety of this method using D14 or D28 differentiated dopaminergic progenitors transplanted into striatum of immunodeficient NOD SCID mice. No teratoma formation was observed when D14 cells or D28 cells were transplanted. However, grafts in animals treated with D14 cells showed rosette formation while D28 cells did not, suggesting that D28 cells may be safer. Finally, we tested the safety of D28 cells by assessing their bio-distribution. At 6 months following transplantation of D28 cells into the striatum, genomic qPCR detected no human DNA sequences in any central nervous system regions other than the graft site, or in any peripheral organs. Taken together, our study provides strong evidence of the clinical safety of this product for potential future therapeutic use.

Funding source: This work was supported by NIH grants (NS070577, NS084869, and OD024622) and the Parkinson's Cell Therapy Research Fund at McLean Hospital.

Keywords: Parkinson's Disease, Cell therapy, Safety

NEW TECHNOLOGIES

CA198

A NOVEL THREE DIMENSIONAL HUMAN SKIN MODEL FOR THE STUDY OF CELL-CELL INTERACTION AND EXTRACELLULAR MATRIX SECRETION

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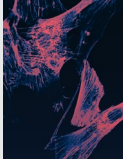
Three-dimensional (3D) tissue models of human skin harboring primary stem progenitor cells have been used to better understand disease phenotypes and to screen potential drugs. However several factors have limited the value of these in vitro 3D skin tissues in their utility as a testing platform. These include: 1- The use of non-human, animal-sourced collagen in the production of the extracellular matrix (ECM), 2- the relatively low tissue throughput formats, and 3-the need for greater cellular complexity. As a result, while current 3D tissue models incorporate stem progenitors that create normal epithelial tissue architecture, they do not fully recapitulate the cell-cell interactions and ECM production typical of human skin phenotypes seen in vivo. In this study, we present an approach for the fabrication of 3D skin-like tissues that addresses these limitations. Our study reveals that adult and neonatal fibroblasts deposit an endogenous ECM de novo that serves as an effective stroma to support full differentiation of epithelial stem cells and complete epithelial tissue development. Immunohistochemical staining for Type 1 and Type 3 collagens showed the presence of immature and mature collagen fibers, and staining for K10 and Ki67 demonstrated a proliferative and differentiating epithelium. We have successfully miniaturized these tissues to a 24-well format for higher throughput analysis. In addition, we have shown that blood monocytes can be incorporated into this model and differentiate into tissue macrophages to increase tissue complexity. Our humanized 3D- skin model constructed with patient derived stem progenitors decreases dependency on animal-derived products, thus increasing reliability of tissue



fabrication, while increasing tissue throughput and complexity in ways that enable the study of inflammatory responses in skin related diseases. This skin model has the potential to be a reference point to assess the differentiation of iPSC derived keratinocytes and fibroblasts, their cross-talk, and their ability to recreate the structure of human skin.

Funding source: National Institute of Arthritis and Musculoskeletal and Skin Diseases 2R44AR072170-02 National Institute of Diabetes and Digestive and Kidney Diseases 5U24DK115255-02

Keywords: 3D tissue model, epithelial tissue differentiation, Patient derived adult stem cells

CA201

ENGINEERING A DRUG-INDUCIBLE CASPASE-9 SYSTEM IN HUMAN EMBRYONIC STEM CELLS TO IMPROVE THE SAFETY OF CELL THERAPIES

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Human pluripotent cell lines and their derivatives have great potential as cell-based therapies to treat multiple diseases, including type 1 diabetes. In attempt to add an additional layer of safety, we have introduced a dual inducible-caspase-9/luciferase transgene at the AAVS1 locus of H1 human embryonic stem cells. In the event of possible unfavourable outcomes following cell administration to a host, the inducible-caspase-9 can initiate implant-specific apoptosis when exposed to the AP20187 pro-drug. The luciferase can be used to monitor cell survival post-implant into live animals and to visualize the efficiency of induced cell death. Preliminary in vitro studies have been performed to functionally test this system in two successfully targeted hemizygous clones. The presence of luciferase was confirmed by addition of D-luciferin and an AP20187 time course experiment revealed that cell death could be detected within the first hour of treatment, and over 99% of the cells were eliminated within 24 hours. Notably, we observed several rare genetic events in a small population of cells that prevented them from responding to the pro-drug treatment. Differentiation to pancreatic progenitors followed by the administration of AP20187 revealed that the safety-switch still functioned in this cell type. Implantation studies in mice are planned to test the functionality of the safety-switch in vivo in teratomas and in a cell therapy model for diabetes. Future iterations of this transgene design are currently under investigation to target both AAVS1 alleles for functional redundancy and to target a region further upstream within the locus that we predict will be less amenable to potential silencing.

Funding source: Funding for this research was provided by the Canadian Institutes of Health Research, Juvenile Diabetes Research Foundation, National Institutes of Health, and Canada Foundation for Innovation.

Keywords: gene editing, cell therapy, safety

CA207

DESIRABILITY PROFILING TO RANK MESENCHYMAL STROMAL CELL 3D AGGREGATES ACCORDING TO MULTIMODAL MECHANISMS

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Mesenchymal stromal cells (MSCs) are being investigated for treatment of osteoarthritis due to their chondroprotective, anti-inflammatory, angiogenic, and anti-fibrotic properties. However, insufficient potency and deficient knowledge on therapeutic mechanisms can limit MSC treatment efficacy. The Viswanathan lab has pioneered a novel, non-genetic, and proprietary strategy to augment MSC anti-inflammatory functions through culture of MSCs in 3D cell aggregates. Hypoxic culture has also been shown to enhance MSC paracrine functions and chondrogenesis. Our objective is to systematically characterize 3D and hypoxic MSCs in terms of their chondrogenic, anti-inflammatory, angiogenic, and anti-fibrotic properties, and to apply desirability profiling to empirically rank the potency of the MSCs according to these different properties. We expanded human adipose tissue-derived MSCs using serum-free medium and performed 3D and hypoxic (5% O2) culture for 24 h. 2D normoxic MSCs served as controls. RT-qPCR and a NanoString 50-gene panel were used to measure MSC gene expression, while microRNAs (miRs) were examined by next generation sequencing. 3D MSCs showed greater expression of immunomodulatory genes (e.g. TSG6, IDO, with up to 10-fold increased expression vs. control) while hypoxic MSCs had higher expression of angiogenic genes (e.g. VEGF with 2-fold increased expression vs. control) by qPCR. Investigation into miR expression revealed that 3D AT-MSCs had a differential miR profile vs. controls with greater expression of immunomodulatory and chondrogeneic miRs. In ongoing work, these data are being analyzed using desirability profiling, and additional functional read-outs will be performed using MSC co-cultures with human chondrocytes, monocytes, endothelial cells, or fibroblasts. In conclusion, MSCs act through multimodal mechanisms that can be enhanced through 3D or hypoxic cultures. Statistical modeling will be used to systematically rank the MSC culture methods in terms of their chondroprotective, anti-inflammatory, angiogenic, and anti-fibrotic functions in vitro, and select methods will be validated within osteoarthritis mouse models. Ultimately, this work will lead to methods for improved MSC products with therapeutic profiles that are “fit-for-purpose.”

Keywords: mesenchymal stromal cell, statistical modelling, microRNA-sequencing

CA220

OPTIMIZED ANIMAL COMPONENT-FREE MEDIUM AND WORKFLOW FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN SUSPENSION CULTURES

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Suspension culture enables efficient scale-up of human pluripotent stem cell (hPSC) manufacturing. However, the use of media optimized for 2D adherent culture can result in low volumetric productivity and laborious workflows when applied to 3D culture. To overcome these limitations, we developed TeSR™-E8™3D, an animal component-free kit optimized for suspension culture of hPSCs as aggregates; the kit consists of a seed medium and a fed-batch feed supplement. During media development, 3 human embryonic (H1, H7, H9) and 2 induced pluripotent stem cell lines were maintained as aggregates for up to 35 days. Aggregates were passaged either by dissociation into small clumps using Gentle Cell Dissociation Reagent or by enzymatic dissociation into single cells, then re-seeded into fresh medium with 10 µM Y-27632. Cultures were passaged with 3 day cycles, 4 day cycles, or alternating 3 / 4 day cycles. Cultures were fed daily using optimized fed-batch feed supplement, with a 50% media exchange on day 3 of 4 day cycles. The culture seed medium and fed-batch feed supplement were optimized using a novel iterative scheme that improved the base media formulation to maintain target nutrient levels, increase growth rates and retain cell quality. Screening experiments identified components that affected cell growth and quality; multifactorial experiments were conducted to determine the optimal formulations. The feed supplement formulation was designed to minimize volume changes following feeding cycles, which disrupts mixing during cell culture. Results demonstrate that all passaging schedules had equivalent growth and cell quality; however, passaging as single cells increased the risk of karyotype abnormalities. The optimized media and workflow resulted in >1.4-fold expansion per day, >90% viability, >90% expression of OCT4 and TRA-1-60, functional pluripotency as measured by in vitro trilineage differentiation, and normal karyotypes (n=15). Suspension culture-optimized TeSR™-E8™3D fed-batch media enables the manufacture of hPSCs as aggregates with an efficient workflow, high cell quality, and improved volumetric productivity.

Keywords: Fed-batch suspension culture, hPSC culture scale-up, Media optimization

CA224

A VERSATILE POLYPHARMACOLOGY PLATFORM PROMOTES CYTOPROTECTION AND VIABILITY OF HUMAN PLURIPOTENT AND DIFFERENTIATED CELLS

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Clinical translation of human pluripotent stem cells (hPSCs) requires advanced strategies that ensure safe and robust long-term growth and functional differentiation. Pluripotent cells are capable of extensive self-renewal, yet remain highly sensitive to environmental perturbations in vitro, posing challenges to their therapeutic use. Here, we deployed innovative high-throughput screening strategies to identify a small molecule cocktail that dramatically improves viability of hPSCs and their differentiated progeny. We discovered that the combination of Chroman 1, Emricasan, Polyamines, and Trans-ISRIB (CEPT) enhanced cell survival of genetically stable hPSCs by simultaneously blocking several stress mechanisms that otherwise compromise cell structure and function. In proof-of-principle experiments we then demonstrated the strong improvements that CEPT provided for several key applications in stem cell research, including routine cell passaging, cryopreservation of pluripotent and differentiated cells, embryoid body and organoid formation, single-cell cloning, genome editing, and new iPSC line generation. Thus, CEPT represents a unique polypharmacology strategy for comprehensive cytoprotection, providing a new rationale for efficient and safe utilization of hPSCs. Conferring cell fitness by multi-target drug combinations may become a common approach in cryobiology, drug development, and regenerative medicine.

Funding source: NIH Common Fund, NCATS Intramural Research

Keywords: Cell survival, Single cell cloning, High-throughput screening

CA233

BIOSILK - A NEW 3D BIOMATERIAL THAT COMBINES THE BIOLOGICAL INSTRUCTIVE CUES FROM ECM LAMININS WITH THE MECHANICAL AND TOPOLOGICAL SUPPORT FROM SILK, ALLOWING, CONTROLLABLE AND TUNABLE ORGANOIDS

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Cells in tissues are in contact with other cells and are embedded and anchorage to ECM proteins, thereby receiving biological instructive cues that regulate cell behavior. To be able to culture authentic cells from which safe and therapeutically relevant cells can be generated or real biological questions can be answered, the cell culture environment must be designed to finely replicate the architecture of the native tissue, where the extracellular matrix (ECM) framework let the cells to adhere, spread, proliferate, differentiate, and maturate, similarly to what they do in vivo. Biosilk is a new biomaterial made of recombinant silk, which is functionalized with human recombinant laminin protein (Biolaminin™). Laminin proteins are a family of unique heterotrimeric glycoproteins naturally present in all tissues in the body. Laminins are tissue-specific and are essential for maintaining the natural environment for all cell types, influencing cell adhesion, differentiation, migration, phenotypic stability, and cell functionality. The Biosilk biomaterial supports the integration and proliferation of human pluripotent stem cells in vitro and serves as a viable base for the development of subsequent lineage-specific differentiation in a 3D format. Human PSCs (2 hESC and 2 hiPSC lines) seeded in the Biosilk scaffold integrate, migrate, and form small colonies at day 1 post-seeding. Human PSCs in the Biosilk scaffold express pluripotent markers, generate all three tissues germ layers upon spontaneous differentiation, and can be directly differentiated into different cell fates. Furthermore, the Biosilk can be used to for organoid culture, without the need of Matrigel. We also show how Biosilk scaffolds reproducibly guide self-organization and maturation of forebrain, midbrain and glia organoids with the expression of correct regional specific markers, cell morphology, and layers. Moreover, compared to Matrigel organoid structures, the internal microscale of the Biosilk scaffold gives more perfused organoid structures and better preserves the organoid viability. In sum, Biosilk is a xeno-free and defined, biorelevant 3D system that is biocompatible and biodegradable and is thus an ideal system for more authentic cell culturing and many other biomedical applications.

Keywords: Brain organoids, 3D culture, extracellular matrix

CA237

A VERSATILE MICROENCAPSULATION PLATFORM FOR DURABLE CELL ENCAPSULATION OR FOR A SLOW-RELEASE CELL DELIVERY SYSTEM

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Cells as medicine to repair or replace tissue is a rapidly expanding field with broad potential utility in the treatment of diseases such as diabetes, cancer, heart disease and immune disorders. However, the delivery of the cells has been a challenge with IV administration leading to thrombosis and first-pass effects of the lungs. Localized delivery of cells can be best accomplished by first encapsulating the cells in hydrogels. While traditional alginate microspheres suffer from poor biocompatibility, microencapsulation using advanced hydrogels is challenging due to their slow gelation rates. We developed a non-cytotoxic, non-emulsion-based technology that can produce microspheres from a wide variety of biocompatible hydrogel materials, Core Shell Spherification™ (CSS). The purpose of this study was to compare microparticles created using CSS from different starting hydrogels that use either UV or chemical crosslinking: polyethylene glycol diacrylate (PEGDA), methacrylated hyaluronic acid (MeHA) or thiolated hyaluronic acid (ThHA). Microspheres of MeHA had the greatest swelling ratio, the largest average diameter, and the lowest diffusion barrier. In contrast, PEGDA microspheres had the smallest diameters, the lowest swelling ratio, and the highest diffusion barrier, while microspheres of ThHA had characteristics that were in between the other two groups. To test the ability of the hydrogels to protect cells while preserving function, diabetic NOD mice received intraperitoneal injections of PEGDA or MeHA microencapsulated canine islets. PEGDA microspheres reversed diabetes for the length of the study (up to 16 weeks). In contrast, islets encapsulated in MeHA microspheres at the same dose restored normoglycemia, but only transiently (3-4 weeks). Non-encapsulated canine islet transplanted at the same dose did not restore normoglycemia for any length of time. In conclusion, CSS provides a non-toxic microencapsulation process compatible with various hydrogel types.

Keywords: Diabetes, Encapsulation, Arthritis

CA239

INVESTIGATING THE EXTRACELLULAR MICROENVIRONMENT AS A POTENTIAL TARGET TO MONITOR AND CONTROL LARGE-SCALE DIFFERENTIATION BIOPROCESSES IN STEM CELL-DERIVED THERAPIES

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Allogeneic stem cell-derived therapies are being investigated for a number of rare and common indications and in many cases require large cell numbers per dose. This has driven manufacturing away from planar flask technologies towards bioreactor systems, which can better support scale and automation. Unlike traditional, pharmaceutical products, cell therapies are inherently more prone to variability, increased risk of batch failure and costs if processes are not sufficiently controlled. This is exacerbated with pluripotent stem cells, where lengthy culture periods and regular manipulation are common. In-process analytics can help maintain control and reduce risks of failure. However, it is difficult to apply analytics that are used for monitoring expansion and differentiation in planar flasks to bioreactor systems with the most common analytical method in use being microscopy. The extracellular microenvironment provides a potential target to determine the impact of processing conditions on process outcome with the myriad of molecules consumed and produced by cells during differentiation alluding to cell state and potential cell fate. By harnessing spent media from bioreactors, we can utilise process analytical technologies (PAT) for the monitoring and control of

large-scale cell differentiation bioprocesses and enable the control of critical processing parameters to maintain product critical quality attributes without requiring cell-based analysis to be conducted. Using two exemplar differentiation processes, going from induced pluripotent stem cells to, either, definitive endoderm or cardiomyocytes, we have characterised the cell secretome with respect to metabolites and secreted proteins, and performed multi-parametric analysis to determine potential novel biomarkers for monitoring future large-scale differentiation processes without the need to harvest cell, therefore using cost- and time-effective PAT alternatives.

Keywords: secretome, microenvironment, process analytical technologies

CA263

APPLICABILITY OF EX-VIVO MSC THERAPY FOR SEVERE COVID-19

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Coronavirus disease 19 (COVID-19), caused by SARS-CoV-2 was declared a pandemic in March 2020 and remains without any approved treatments. After entering the cells, the virus begins to replicate and viral antigen is presented to antigen presenting cells (APCs), the cells that stimulate the body's normal anti-viral immune response. In severe cases however, this immune reaction becomes dysregulated as evidence by high levels of certain cytokines and chemokines in the blood, a reaction known as cytokine storm. This results in a systemic uncontrolled inflammatory state that triggers a violent attack by the immune system to the body, causes acute respiratory distress syndrome (ARDS) and multiple organ failure, leading to death. Mesenchymal stromal cells (MSCs) are a unique source of secreted factors that modulate an inflammatory response and enhance the repair of injured tissue. MSCs have been extensively studied in ARDS and other acute organ injuries. Sentien has created a novel delivery approach to enable sustained exposure to MSCs and their secreted factors, overcoming limits of cell transplantation/infusion while preserving their broad acting and dynamically responsive properties. Our lead product, SBI-101, contains allogeneic human MSCs inoculated into a hollow-fiber hemofilter, which enables communication with patient blood via the semi-permeable membrane, while maintaining MSC viability. Through this interplay, SBI-101 aims to restore balance to the immune system by reprogramming the molecular and cellular components of blood in patients with severe inflammation and organ injury. Sentien's Phase I/II clinical study of SBI-101 in critically ill patients with Dialysis-Requiring Acute Kidney Injury (AKI-D) has produced data to support the therapeutic hypothesis of SBI-101 as a potent immunotherapy. Consistent with MSC biology, inflammatory markers, such TNFa and IFNg, were shown to be modulated, suggestive of a shift from a pro- to an anti- inflammatory state in treated patients. Data obtained in our AKI-D trial showed modulation of many biological molecules and immune populations that are significantly correlated with severe COVID-19 immunopathology. Here we make the case, using our existing AKI-D trial data, that SBI-101 may be of therapeutic benefit to severe cases of COVID-19.

Keywords: MSC, COVID-19, AKI

PLACENTA AND UMBILICAL CORD DERIVED CELLS

CA241

A NATIONWIDE AUTOLOGOUS UMBILICAL CORD BLOOD BANK SUPPORTING CLINICAL TRIALS IN CONGENITAL HEART DISEASE IS FEASIBLE AND HAS A HIGH UTILIZATION RATE

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HLHS is a congenital heart disease (CHD) affecting about 1,000 US babies yearly. Treatment is a series of three surgeries making the right ventricle into the primary pumping chamber. Many patients go on to right ventricular heart dysfunction and heart transplant. We have created an autologous umbilical cord blood (UCB) banking program to support phase 1 and 2 trials of intracardiac injection of autologous UCB mononuclear cells (UCBMNC) during surgery for HLHS. After diagnosis of HLHS, a UCB collection kit is mailed to the family, allowing the UCB to be collected at any hospital. The mother requests her OB provider to collect UCB after delivery. After UCB collection the UCB is shipped to the manufacturing center via courier service. UCBMNC are isolated by density gradient and frozen for administration during the patient's scheduled Glenn procedure, the 2nd stage surgery for HLHS, which occurs at about 5 months of age. Patients receive their UCBMNC at one of the participating consortium hospitals. One day prior to the planned surgery frozen UCBMNC is transported on liquid nitrogen to the participating site by study staff. The vial is thawed in the OR and administered after the surgical procedure. Injection is into the right ventricle with a 27 ga. needle at 0.1 mL/Kg. Total dose is 1-3 million nucleated cells per Kg. We have shipped >230 kits to expectant mothers. 80% of kits were shipped at least 1 week prior to the baby's birth. Extra kits are maintained at consortium sites to be used when labor begins before a kit is shipped. 209 UCB have been collected and manufactured. UCB have come from 32 states, Germany, Lithuania and Canada. 46% and 90% of UCB were manufactured within 24 and 36 hours of collection,

respectively. 82% of products met all release criteria for purity, potency and safety. Low cell count attributed to low cord blood volume was the primary reason for failure. 51 patients have been treated on the phase 1 and a follow on phase 2 study. This represents a utilization rate of approximately 30% of collected UCB. An autologous UCB bank targeted for HLHS is feasible, with a high utilization rate compared to both public and private cord blood banks. Following a phase 1 pilot study we are finishing a phase 2 study and actively planning a phase 3 trial of UCB for HLHS. We are also expanding the indication for UCB banking to include CHD. The clinical trial ID (clinicaltrials.gov): NCT03779711; IND 15343 administered by the US FDA.

Keywords: Umbilical Cord Blood, Hypoplastic Left Heart Syndrome, Congenital Heart Disease

Theme: Modeling Development and Disease

ADIPOSE AND CONNECTIVE TISSUE

MDD101

A HUMAN STEM CELL MODEL TO CONFRONT THE HAPLOINSUFFICIENCY AND DOMINANT NEGATIVE MOLECULAR MECHANISMS OF MARFAN SYNDROME

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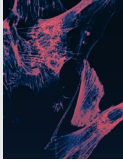
The FBN1 gene encodes the extracellular matrix (ECM) protein fibrillin-1, the major structural component of the microfibrils that form the elastic fibers. Mutations in this gene were related to the occurrence of Marfan Syndrome (MFS), a pleiotropic autosomal dominant connective tissue disorder whose clinical manifestations include Dilated Cardiomyopathy (DCM), Thoracic Aortic Aneurysm (TAA), bone overgrowth and thoracic deformities. Although around 1,300 mutations have been described for the syndrome, there are no well-established genotype-phenotype correlations, with few exceptions, and two molecular mechanisms have been proposed and validated to explain its development: haploinsufficiency (HI – i.e. the lack of the regular amount of wild-type fibrillin-1) and dominant-negativity (DN – i.e. the disturbance of the ECM by an incorporated aberrant protein), and differences between groups of patients classified as HI or DN have been reported. Because patients have different genetic backgrounds, their derived human induced pluripotent stem cell (hiPSC) models may not satisfactorily address the questions about how HI and DN mutations lead to different phenotypic manifestations at the molecular level. Also, the lack of specific and KO validated anti-human fibrillin-1 antibodies hinders the correct classification of identified mutations as HI or DN, especially if mRNA levels are not affected. To overcome these issues, we have successfully edited a hiPSC line from a healthy donor using CRISPR/Cas9 to generate isogenic HI and DN mutants for FBN1. We validated the mutations by using homozygous (double KO or double DN) clones and showed the predicted consequences at the mRNA and protein levels for both groups of mutations. We also showed the specificity of two commercially available antibodies (for Western Blot and immunocytochemistry), by validating them using our double KO mutant. Therefore, we can now take advantage of the plasticity of these stem cell lines to better understand the pathogenesis of Marfan Syndrome in different cell types.

Funding source: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

Keywords: Marfan Syndrome, FBN1, hiPSCs



CARDIAC

MDD118

ANALYSIS OF GENE EXPRESSION OF NADPH OXIDASE IN HIPSC-DERIVED CARDIOMYOCYTE FROM NORMOTENSIVE AND HYPERTENSIVE SUBJECTS

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Hypertension is an important risk factor for various cardiovascular diseases, such as stroke, acute myocardial infarction and chronic kidney disease. It is a complex, multifactorial disease whose cellular mechanisms are not well understood. Recently several studies have focused on the role of oxidative stress regarding the development of hypertension. Increasing evidence over the last decades indicates an association between reactive oxygen species (ROS) and arterial hypertension. ROS are essential for cellular physiology, but in an unbalanced situation, an exacerbated production of ROS can damage cellular components and trigger pathological processes. Among the different ROS sources that are present in the heart, NADPH oxidases (NOXes) are particularly important because they are involved in many features of heart dysfunction. However, in humans the role of NOXes in cardiac pathologies caused by hypertension and the relation between NOX-derived ROS and hypertension are poorly understood, the NOX5 mainly, which does not express in the mouse. In this scenario, human induced pluripotent stem cells (hiPSC) can be a potent tool for understanding cellular mechanisms in response to oxidative stress in hypertension–induced cardiomyocyte dysfunction. In the present work, we propose to use hiPSC-derived cardiomyocytes from normotensive, responsive and resistant hypertensive patients as a source for the study of NOXes. We established a collection of hiPSC from normotensive, hypertensive responsive and hypertensive drug-resistant individuals. We show differential expression of 18 genes related with hypertension condition in undifferentiated cells. We also show that hiPSC-derived cardiomyocytes were able to express NOX genes, and present different patterns of NOX expression that can be binding to hypertension phenotype. Thus, this collection of hiPSCs from hypertension patients is a powerful tool to studies the role of oxidative stress as a molecular mechanism of the disease.

Funding source: FAPESP, CNPq, BNDS, Capes

Keywords: hiPSCs, Hypertension, NOX

MDD124

EXPLORING TISSUE SPECIFIC FUNCTIONS OF THE AT1 ANGIOTENSIN II RECEPTOR IN PRIMARY RAT NEONATAL AND HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Cardiovascular disease is one of the leading causes of morbidity and mortality in North America. Numerous drugs aimed at improving cardiovascular function are designed to modulate the activity of G protein-coupled receptors (GPCRs) such as β-blockers, ACE inhibitors and angiotensin II receptor blockers. As important therapeutic targets, GPCRs are conformationally labile proteins whose agonist-induced conformation are influenced by their tissue specific environment. Since adopted conformations control downstream function, there is a need to better understand how different cellular milieux affect conformational flexibility. As a primary readout, we are interested in exploring whether the conformation of a cardiac-relevant GPCR, the human angiotensin II type I, AT1 receptor (AT1R), behaves differently when expressed in different cardiac cell lineages. To assess GPCR conformation, we previously described the engineering of FlAsH BRET-based conformation-sensitive biosensors that report responses upon agonist stimulation or

due to allosteric interactors. We showed that biosensors built into different positions in the coding sequence of the receptor result in agonist-specific conformational signatures when expressed in HEK 293 cells. We are currently using our biosensors in rat primary neonatal cardiomyocytes as they better mimic the native AT1R environment. However, to understand more fully the effect of cellular context on receptor conformation and function, we are using inducible pluripotent stem cells (iPSCs) differentiated towards the mesodermal lineage that faithfully recapitulate human physiology with disease relevance. iPS cell lines can be transduced with our biosensors of interest and differentiated to give rise to cardiomyocytes, vascular smooth muscle cells, cardiac fibroblasts as well as endothelial cells as means to model the cardiovascular system. We predict that a deeper understanding of cell-type specific receptor function and signalling will advance rational drug design for the treatment of cardiovascular diseases.

Funding source: This project is supported by the Canadian Institutes of Health Research (CIHR).

Keywords: Cellular Signalling, Conformational Profiling, iPSC-Cardiomyocytes, GPCRs, hAT1R

MDD125

HUMAN CARDIOMYOCYTE COMMUNICATION IN APICAL 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. 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, hiPSC lines were generated from heart patients with a HCM associated ACTC1 mutation (E99K). Isogenic controls were created for each line by correcting 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 exosomes isolated and characterised. The pattern of release (size and quantity) of exosomes produced by diseased cardiomyocytes was significantly altered compared to isogenic control lines. Stressed conditioned medium, containing exosomes, 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 this causes activation of neighbouring cells to result in unexplained disease phenotypes such as fibrosis.

Keywords: Cardiomyopathy, hiPSCs, Exosome Signalling

MDD128

OPTIMIZATION OF CHRONIC PACING PROTOCOLS FOR FUNCTIONAL MATURATION OF HIPSC-CMS

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have advanced in vitro assays for disease modeling and evaluation of cardiac safety risk, yet remain an immature representation of human ventricular myocytes. Recent literature has demonstrated a variety of techniques, ranging from electrical pacing over chronic timescales to patterned substrates, to mature aspects of hiPSC-CM structure and function. Here, we characterize the contractile and electrophysiological maturation of hiPSC-CMs elicited through a range of chronic pacing protocols. The hiPSC-CMs were paced at 2Hz for 12 hours to 7 days. Electrical pacing was also compared to optogenetic pacing. Microelectrode array (MEA) technology was used to measure the cardiomyocyte action potential and excitation-contraction coupling following the chronic pacing protocols. The functional maturation, as measured by changes in repolarization timing and the development of a positive “force-frequency” relationship, was increased in a subset of wells within 24 hours of chronic pacing. However, 48 hours of pacing was required to observe changes in functional maturation consistently across wells, and no further improvement was observed for longer pacing protocols. Optogenetic pacing produced similar changes in repolarization and the “force-frequency” relationship as electrical pacing, but, notably, the optogenetic pacing caused a cessation in spontaneous beating following the pacing protocol. Using the optimized 48 hour electrical pacing protocol, the “matured” cardiomyocytes displayed shortened repolarization timing relative to measurements taken before chronic pacing (baseline: 423 +/- 21 ms; “matured”: 316 +/- 15 ms), without a significant change in beat period (baseline: 1255 +/- 40 ms; “matured”: 1314 +/- 84 ms). The contraction beat amplitude was sampled during spontaneous beating and in response to increasing pacing rates (1, 1.2, 1.5, 2, and 2.5 Hz). Before chronic pacing, the beat amplitude decreased as the pacing rate increased, whereas the same wells displayed an increase in beat amplitude as the pacing rate increased after the 48 hours of chronic pacing. In summary, this abstract demonstrates that some aspects of hiPSC-CM electrophysiology and contractile function can be matured after 48 hours of chronic pacing.

Keywords: Electrophysiology, Cardiomyocyte, Maturation

MDD132

SINGLE CELL RNA-SEQ ANALYSIS OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES TO STUDY MECHANISMS OF DRUG-INDUCED CARDIOTOXICITY

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The cardiotoxic side effects of the chemotherapeutic drug doxorubicin is well-documented and extensively studied. Nonetheless, a definitive picture of the cardiotoxic mechanisms is still lacking and an efficient prevention against the induced cardiotoxicity remains a challenge. Recent years advancements in single cell isolation and analysis has opened up for studies of genomics at a single cell resolution, including in-depth mechanistical investigations of cellular events. In this study, we investigated doxorubicin-induced cardiotoxicity in hPSC-derived cardiomyocytes, using single cell RNA-Seq. The cardiomyocytes were exposed to a low concentration of doxorubicin for 48 hours, followed by a 12 days wash-out period, with cell harvest directly after exposure and after the wash-out. Approximately 1,500 single cardiomyocytes were isolated and processed for sequencing using the SMARTer™ ICELL8® Single-Cell System (Takara Bio). More than 95% of the cells expressed cardiac specific genes, such as Actin, Myosin, and Troponins. When analyzing the different sample groups, we are able to identify subpopulations of cells that differ in transcriptional profiles. Marker gene analysis of the subpopulations from the sample groups, indicate that the clusters of cells differ in their cellular identity. The cluster analysis also reveals a separation between the different sample groups. Especially the doxorubicin-treated cells taken directly after exposure show a strong separation from the other groups. One cluster, that contains cells from all sample groups, show a significant upregulation of mitochondrial genes. In addition, the control sample groups have a distinct cluster that solely contains cells that are in G2M or S phase of the cell cycle. Notably, the doxorubicin-treated sample groups contain cells with a considerably lower percentage of cells in G2M or S phase. Further analysis of key cell cycle regulatory genes indicates that the exposed cells enter a stage of G1 arrest as a response to the treatment, with elevated levels of e.g. p21 expression. In conclusion, these results highlight the potential of using in vitro models derived from hPSC to study drug-induced toxicity, and together with single cell transcriptomics demonstrate the utility for hPSC-derived cardiomyocytes as a tool for mechanistic studies.

Funding source: This study was conducted under grants from the Knowledge Foundation [2016/0330] and The Swedish Fund for Research Without Animal Experiment [F2018-0007].

Keywords: Single cell transcriptomics, drug-induced cardiotoxicity, hiPSC-derived cardiomyocytes

MDD137

ADVANCING MATURATION OF IPSC-DERIVED CARDIOMYOCYTES IN CULTURE USING BIOENGINEERING APPROACHES IMPROVES THE PREDICTIVE POWER OF IN VITRO ASSAYS

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iPSC-derived cardiomyocytes (CMs) exhibit fetal phenotypes in culture. This immaturity is thought to be responsible for their failure to recapitulate critical aspects of human biology. For example, testing arrhythmogenic drugs on CMs in culture demonstrates the misclassification of several compounds of known effect. Considerable effort is directed at advancing the maturity of these cells by recapitulating in vivo stimuli that are known to be important in development, maturation, and function. Electrical pacing, extracellular matrix engineering, mechanical stretch, and other stimuli are known to advance structural and functional maturation. However, these strategies are challenging to perform on industry-standard assays and instrumentation platforms as building increasingly intricate artificial culture environments often drives decisions to trade off biological complexity with experimental throughput. Here, we utilized a combination of maturational stimuli to test whether they can synergistically advance the maturation of CMs in culture and improve assay predictivity. We used nanoscale fabrication techniques to make 2D anisotropic cardiac syncytia in SLAS/ANSI formats. We tested other maturation stimuli such as electrical pacing, addition of hormones, and regulation of the expression of key genes involved in CM maturation. We applied these stimuli across well-characterized commercial cell lines and found that this approach synergistically improves a variety of structural and functional metrics. Further, we tested the effect of these maturational stimuli with respect to the detection of the arrhythmogenicity of known cardiotoxicants. Our data show that these cues can impart these cells with the ability to properly classify previously misidentified compounds, demonstrating that cell maturity plays a critical role in assay fidelity. We conclude that bioengineering techniques can enhance the predictivity and maturity of CMs in culture without impacting throughput.

Funding source: NIH NHLBI

Keywords: Cardiomyocyte, Maturation, Bioengineering

MDD474

MODELING HUMAN TBX5 HAPLOINSUFFICIENCY PREDICTS REGULATORY NETWORKS FOR CONGENITAL HEART DISEASE

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Haploinsufficiency of transcriptional regulators causes human congenital heart disease (CHD). However, underlying CHD gene regulatory network (GRN) imbalances are unknown. Here, we define transcriptional consequences of reduced dosage of the CHD-linked transcription factor, TBX5, in individual cells during cardiomyocyte differentiation from human induced pluripotent stem cells (iPSCs). We discovered highly sensitive dysregulation of TBX5-dependent pathways— including lineage decisions and genes associated with cardiomyocyte function and CHD genetics—in discrete subpopulations of cardiomyocytes. GRN analysis identified vulnerable nodes enriched for CHD genes, indicating that cardiac network stability is sensitive to TBX5 dosage. A GRN-predicted genetic interaction between Tbx5 and Mef2c was validated in mouse, manifesting as ventricular septation defects. These results demonstrate exquisite sensitivity to TBX5 dosage by diverse transcriptional responses in heterogeneous subsets of iPSC-derived cardiomyocytes. This predicts candidate GRNs for human CHDs, with implications for quantitative transcriptional regulation in disease.

Keywords: Gene Regulatory Networks, Gene Dosage, Congenital Heart Disease

MDD480

ADAPTABLE PULSATILE FLOW GENERATED FROM STEM CELL-DERIVED CARDIOMYOCYTES USING QUANTITATIVE IMAGING-BASED SIGNAL TRANSDUCTION

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Endothelial cells (EC) in vivo are continuously exposed to a mechanical microenvironment from blood flow, and fluidic shear stress plays an important role in EC behavior. New approaches to generate physiologically and pathologically relevant pulsatile flows are needed to understand EC behavior under different shear stress regimes. Here, we demonstrate an adaptable pump (Adapt-Pump) platform for generating pulsatile flows from human pluripotent stem cell-derived cardiac spheroids (CS) via quantitative imaging-based signal transduction. Pulsatile flows generated from the Adapt-Pump system can recapitulate unique CS contraction characteristics, accurately model responses to clinically relevant drugs, and simulate CS contraction changes in response to fluidic mechanical stimulation. We discovered that ECs differentiated under a long QT syndrome derived pathological pulsatile flow exhibit abnormal EC monolayer organization. This Adapt-Pump platform provides a powerful tool for modeling the cardiovascular system and improving our understanding of EC behavior under different mechanical microenvironments.

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Keywords: pulsatile flow, cardiomyocytes, endothelial cell

MDD486

ENGINEERED PLATFORMS TO INVESTIGATE EFFECTS OF 3D ADHESIONS ON HIPSC-CM MATURITY

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Heart disease is a leading cause of death worldwide, yet basic processes of cell dysfunction or death due to disease remain unknown. Cell models are needed to study how structure and function are related in health and disease. Cardiomyocytes (CMs) underlie the mechanical pumping function of the heart. Due to the scarcity of primary human heart tissue, most of what we know about CM physiology comes from animal models. Essential differences between animal and human heart physiology have limited the translatability of these models. Human induced pluripotent stem cells (hiPSC) offer the potential to engineer human CMs in vitro to better understand basic cardiac function and disease progression. However, these models have been limited by immature structure and function. Adult human CMs have an elongated, cylindrical shape with average length-to-width ratio of approximately 7:1 and highly aligned sarcomeres. hiPSC-CMs are smaller and have an irregular shape and disorganized sarcomeres, similar to fetal human CMs. Many studies have investigated methods of inducing a mature phenotype in hiPSC-CMs, including 2D cell patterning devices to replicate cell-ECM adhesions. However, few studies have considered 3D microenvironments and their effect on hiPSC-CM maturity. In this work, we fabricated 3D microwells using ~10 kPa polyacrylamide hydrogels. We seeded microwells with hiPSC-CMs modified with a GFP fluorophore labeling α-actinin, a protein that facilitates actin crosslinking within the z-disc of the sarcomere. We imaged these hiPSC-CMs to assess sarcomere alignment, a key marker for CM maturity, and cell height, a missing feature in 2D-patterned hiPSC-CMs. We observed increased sarcomere alignment in hiPSC-CMs in microwells compared to those on 2D patterned substrates. We also noted an improvement in cell height when comparing cells seeded in 2D micropatterning systems and 3D microwells. The hiPSC-CMs achieved a consistent average height of approximately 10 µm throughout the cell in 3D microwell. By increasing hiPSC-CM maturity, we expand the potential for hiPSC-CMs as models of human heart function and disease. In future work, we will further evaluate maturity markers commonly measured in hiPSC-CMs to quantify the improvement of maturity between 3D microwells and 2D micropatterning technologies.

Keywords: hiPSC, cardiomyocytes, engineered

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

MDD159

AN AUTOMATED QUALITY CONTROL PIPELINE TO ENABLE KIDNEY ORGANOID PRODUCTION AT SCALE FOR DRUG DISCOVERY

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Chronic Kidney disease (CKD) affects 1 in 9 people worldwide. Since common therapies such as dialysis and organ transplantation carry substantial economic burden there exists an unmet need for small molecule therapies that extend and restore kidney function. Human iPSC-derived organoids are a powerful model to understand human kidney development and regeneration. To establish this model as a tool for drug target validation and discovery, we implemented an automated quality control (QC) pipeline to monitor gene expression during kidney organoid production at scale using single cell RNA sequencing (scRNAseq) and NanoString. ScRNAseq was assessed at eight timepoints in triplicates over a 28-day differentiation time course. In total, 204,748 single cells were profiled from 24 organoids. At the endpoint of differentiation, podocytes and other kidney cell types were recovered with only 6.7% off-target cells. Importantly differences in cell proportions between organoids processed on the same day was small (average Jensen–Shannon (J-S) divergence: 0.001 ± 0.001) compared to variation in cell proportions across timepoints (average J-S divergence: 0.26 ± 0.19). We further established NanoString as an assay to rapidly measure multiplexed gene expression. From our scRNAseq data 3,082 genes were automatically selected that were specifically expressed in a single cell cluster and developmental stage (z-score > 2). Based on this data we curated a panel of 228 NanoString probes to measure gene expression in organoids over the 28-day differentiation time course. NanoString gene expression was concordant with scRNAseq data, allowing

estimations of cell type proportions within organoids. Principal component analysis (PCA) of combined NanoString data showed more similarity between replicates than between timepoints indicating reproducibility among organoids. Our QC pipeline is embedded in a computational infrastructure that automatically processes raw data, archives files in an AWS data lake, with a customized visualization portal that allows interactive exploration of the data. In summary, we have implemented an automated QC pipeline that enables reproducible production and evaluation of organoids which will form the basis of our discovery of novel treatments for CKD.

Keywords: kidney organoid, drug discovery, scRNAseq

MDD165

BIOPRINTED PERFUSABLE HUMAN LIVER TISSUE FIBRES FOR TESTING DRUG INDUCED LIVER INJURY

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Drug-induced liver injury (DILI) is the leading cause of safety-related drug withdrawal. Conventional pre-clinical in vitro and animal tests fall short of predicting hepatotoxicity with confidence in humans, at least in part due to non-physiological culture conditions or species-specific differences, respectively. Compared to static 2D cultures, dynamic perfusion of 3D tissue culture models generated with human hepatic cells may improve the physiological relevance and predictiveness of in vitro DILI models. To address this need, we have combined a novel microfluidics-based bioprinting technology (RX1™ Bioprinter, Aspect Biosystems) with human iPSC-derived hepatocytes to create hollow, perfusable hydrogel fibres containing embedded hepatocytes with the goal of developing a scalable human cell-based model for DILI testing. Bioprinted human iPSC-derived hepatocytes demonstrated high viability when embedded in the shell of the hollow fibres printed using an alginate-collagen-1 bioink (AGC-10™ Matrix, Aspect Biosystems). The bioprinted hiPSC-hepatocytes were also shown to produce albumin in static culture over an extended (14 day) time period, a key indicator of hepatic functionality. We have also shown that human umbilical vein endothelial cells (HUVECs) are highly viable when embedded in a similar alginate-collagen bioink. In the future, we aim to combine both hiPSC-hepatocytes and human endothelial cells in these perfusable, hollow fibre tissue constructs, with the goal of creating a vascularized hepatic tissue model that could better mimic the metabolization of compounds within native liver sinusoids.

Keywords: 3D bioprinting, human iPSC-derived hepatocytes, drug-induced liver injury

MDD169

A MOLECULAR TRANSDIFFERENTIATION APPROACH TO STUDY CANCER

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Cancer is a complex genetic disease with more than 250 genes implicated in human tumors. Although several recurrently mutated driver genes have been identified for each tumor type, determining which specific mutations found in human patients lead to tumorigenesis has proven to be difficult. The goal of this project was to study the molecular determinants of human cancer in a systematic manner, and to identify the set of oncogenic drivers necessary and sufficient to transform normal human cells. We developed a defined molecular approach for cellular transformation by combining direct cell fate conversion and oncogene activation. Specifically, we used lineage-specific transcription factors FOXA3, HNF1A, and HNF4A to transdifferentiate human fibroblasts to induced hepatocytes (iHep), and exposed the iHeps to liver cancer-specific oncogenic drivers during cell fate conversion. Importantly, we identified a pool of oncogenes, i.e. constitutively active b-catenin (CTNNB1T41A), MYC and TERT that, with or without simultaneous TP53 inactivation, is able to transform iHeps to a highly proliferative phenotype. In contrast, the same oncogenes overexpressed in human fibroblasts or primary hepatocytes induce cellular senescence. The transformed iHeps are tumorigenic in nude mice, and bear gene expression signatures of liver cancer. Temporal analysis of the tumorigenic program using single-cell RNA-seq and RNA velocity analysis revealed that the cells progress along a common path to transformation, invariably acquiring liver progenitor cell identity prior to expressing markers characteristic of liver tumor cells. These

results, together with analysis of chromatin accessibility using ATAC-seq and NaNoMe-seq indicate that lineage-determining factors act by defining a cell identity and associated chromatin state that are permissive for transformation. In conclusion, we have developed a novel approach to transform normal human cells in a defined and controlled manner by combining cellular reprogramming to oncogene transduction. Our results highlight that tumorigenesis is triggered by a combination of three elements: the set of driver mutations, the cellular lineage, and the state of differentiation of the cells along the lineage.

Funding source: Academy of Finland

Keywords: transdifferentiation, oncogenic drivers, transformation

MDD171

COMBINING LIVE IMAGING AND SINGLE-CELL TRANSCRIPTOME ANALYSIS TO UNDERSTAND HUMAN PANCREATIC ENDOCRINE PROGENITOR DIFFERENTIATION

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To gain dynamic information on the process of pancreatic endocrine cell development in human, we constructed a heterozygote hESC line harbouring a double reporter enabling to monitor NEUROG3 protein via fusion of tagRFP-T to the open reading frame in the endogenous locus and a transcriptional EGFP reporter. Our reporter enables to monitor the emergence of endocrine progenitors, their proliferation and their differentiation into endocrine cells using live imaging over several days. We observe that some endocrine progenitors can proliferate, mostly at the onset of differentiation and with a lower frequency than pancreas progenitors. In addition, we observe heterogeneity in the peak intensity of NEUROG3 expression, and we quantified the half-life of the human NEUROG3 fusion protein. Live imaging also reveals changes in the motility of differentiating cells over time. In parallel, we performed indexed sorting of the cells in the course of differentiation and performed deep single-cell transcriptome analysis at the emergence point of endocrine progenitors. This enables to match NEUROG3 expression level of the cells to their transcriptome. Different steps of differentiation, associated markers and pathways were thereby identified. This includes surface markers that can potentially be used to isolate specific cell populations. Notably, the transcriptome does not reveal any sign of endocrine subtype allocation within 24 hours of NEUROG3 induction, such as differential gene expression between cells becoming alpha, beta or other hormonal cell types. Current attempts at correlating dynamic behaviours in movies (such as cell motility) to the transcriptome will be presented. In the future, our experiments can be extended to isolate endocrine progenitors at specific stages and promote their proliferation in vitro, possibly increasing the purity of endocrine cell production and its efficiency for the purpose of diabetes therapy.

Funding source: PhD Fellowship: NNF16CC0020994

Keywords: Pancreatic endocrine differentiation, Live imaging, Single-cell RNA sequencing

MDD186

HIGH-THROUGHPUT-COMPATIBLE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL LINES INTO KIDNEY ORGANOIDS FOR NEPHROTOXIC DRUG SCREENING

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Organoids are emerging to be an excellent tool for studying human development and disease. Furthermore, organoids hold great promise for drug discovery as they contain more physiological relevant cell types compared to commonly used transformed cell lines. To address the need for a miniaturized organoid culture model system for kidney research, we developed the STEMdiff™ Kidney Organoid Kit, a serum-free kit for the efficient differentiation of human embryonic and induced pluripotent stem cell (PSC) lines into kidney organoids. To do this, a panel of PSC lines (H1, H9, WLS-1C and STiPS-M001) were seeded into Corning® Matrigel®-coated 96-well plates in mTeSR™1. One day after seeding, the adherent cells were overlaid with an additional layer of Matrigel®, which promotes the formation of cavitated PSC spheroids over the next 48 hours. Differentiation of these spheroids was then initiated by switching media from mTeSR™1 to the STEMdiff™ Kidney Organoid Kit media. During the next 18 days of differentiation, cells were directed through stages of late primitive streak, posterior intermediate mesoderm, and metanephric mesoderm to give rise to convoluted kidney organoids that resemble segmented nephron-like structures and are composed of podocytes, proximal and distal tubules, with an associated endothelium and mesenchyme. To investigate the use of these organoids for drug screening, we exposed the cultures to gentamicin, an antibiotic with known nephrotoxic side effects. Exposure to gentamicin resulted in reduced cell viability in a dose-dependent manner, as measured by using a quantitative, luminescence-based viability assay. Next, organoids treated with either a sub-lethal dose (2.5 mg/mL) of gentamicin or a vehicle control were analyzed using immunocytochemistry and ELISA for the expression of kidney injury molecule 1 (KIM-1), a specific biomarker expressed in damaged tubules. KIM-1 was significantly upregulated with 1.5 + 0.2 (mean + SD; n = 5) fold higher expression in damaged tubules of drug-treated kidney organoids compared to controls. In summary, the STEMdiff™ Kidney Organoid Kit supports the highly efficient and reproducible differentiation of human PSCs into kidney organoids in high-throughput-compatible microwell plates, which can be used for nephrotoxic compound screening.

Keywords: Organoids, Drug screening, Direct differentiation

MDD497

INVESTIGATING THE ROLE OF MESENCHYMAL R-SPONDIN2 DURING HUMAN LUNG DEVELOPMENT

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Mammalian respiratory system development is regulated by complex reciprocal signaling events that take place between the epithelial cells lining the lung and the surrounding mesenchymal cells. Despite the lung mesenchyme being home to cell types that provide crucial signals during development, the identity and function of specific cell types in the lung mesenchyme are poorly defined, especially in the context of human development. To interrogate mesenchymal cell heterogeneity in the developing human lung, our lab has conducted single cell RNA sequencing on multiple human lung samples spanning 8-22 weeks of gestation. Using cell-clustering approaches, we identified a mesenchymal cell population highly enriched for expression of the WNT agonist R-Spondin2 (RSPO2). Strikingly, mutations in this gene cause severe lung aplasia in humans. Co-fluorescent in situ hybridization (FISH) and immunofluorescence (IF) data from human lung tissue sections show that RSPO2 is expressed adjacent to proliferating distal epithelial progenitor cells and that the RSPO2 receptor, LGR5, is expressed almost exclusively in the epithelial progenitor cells. These data suggest that RSPO2+ mesenchymal cells may have a critical role in regulating epithelial progenitor cells during human lung development. We are currently utilizing human in vitro organoid models and explant culture systems to determine the functional role of RSPO2+ mesenchymal cells in the context of epithelial progenitor cell regulation in the developing human lung.

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Keywords: human development, organoids, lung mesenchyme

EPITHELIAL

MDD199

A CRISPRI APPROACH TO INVESTIGATE THE ROLE OF FAM13A IN HUMAN IPSC-DERIVED LUNG EPITHELIAL CELLS

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Chronic obstructive pulmonary disease (COPD) is a debilitating disease affecting the lung epithelium. Recent studies show that low lung function in adulthood precedes COPD development and GWAS results demonstrate overlapping risk variants (e.g. HHIP, FAM13A and DSP) associated with both COPD and low lung function. However, how lung function variants contribute mechanistically to lung function and/or epithelial dysfunction in COPD is poorly understood. We aim to assess how lung function genes associated with COPD susceptibility affect epithelial cell function using human induced pluripotent stem cell (iPSC)-derived type 2 alveolar epithelial cells (iAEC2s). In this study, we focused initially on FAM13A as the function of this gene has been poorly described in the human alveolar epithelium. We used CRISPR to introduce frameshift mutations in FAM13A in iPSCs. We also employed a CRISPR interference (CRISPRi) system by targeting a tetracycline-inducible catalytically inactive Cas9 construct (dCas9) to the AAVS1 locus in iPSCs. Using established lung directed differentiation protocols, FAM13A sufficient or deficient iPSCs were specified to lung progenitors at which point NKX2-1+ cells were sorted and re-plated in media to differentiate iAEC2s. FAM13A deficient cells had drastically reduced NKX2-1 expression and failed to induce markers of iAEC2s. As FAM13A deficiency appeared to impair patterning of the cells to appropriate lung progenitors, we next decided to employ CRISPRi-targeted iPSCs to understand the cellular function of FAM13A in specified iAEC2s. FAM13A knock-down in lung progenitors impaired subsequent differentiation to iAEC2s. Interestingly, knock-down of FAM13A in established iAEC2s did not alter cell fate; however, loss of FAM13A perturbed maturation of these cells. In summary, we have found that FAM13A deficiency in iPSCs impairs directed differentiation to iAEC2s. Moreover, using CRISPRi we demonstrate that FAM13A is important in deriving iAEC2s at specific stages of the differentiation. In future studies we will employ CRISPRi to serially down-regulate

other genes of interest associated with lung function, following differentiation to lung epithelium to determine their contribution to cellular function.

Keywords: CRISPRi, type 2 alveolar epithelial cells, lung disease modelling

MDD209

THE EFFECTS OF BMAL1 ON MOUSE INTESTINAL TUMOURS

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The circadian rhythm is a highly conserved timekeeper which allows cells to coordinate activity in relation to internal and external cues over a 24 hour period. This coordination is governed by a core molecular feedback mechanism that requires the non-redundant clock gene BMAL1. Previous studies have implicated circadian clock disruption in cancer. It has been shown that mutations to core circadian clock genes are common in cancers, and that mutated clock genes in normally functioning tissue can lead to increased rates of tumour formation. However, it remains unclear how and to what extent circadian rhythms contribute to tumour initiation or progression. This study aims to identify if and to what extent loss of BMAL1 influences factors which affect the intestinal stem cell niche in mice, that might impact cancer biology. APC is a tumour suppressor of the Wnt signalling pathway, and its loss leads to aberrant growth in intestinal crypts that initiates tumour formation in the intestinal epithelium. When combined with a mutation to the APC gene, clock-deficient BMAL1-/- mice exhibit twice as many intestinal tumours as control BMAL1+/+ mice. Analysis of proliferation (Phosphohistone H3), apoptosis (Caspase 3), Wnt signalling (β-catenin), and Paneth cells (Lysozyme) reveal a significant decrease in the number of apoptotic cells in BMAL1-/- mice, although proliferation and Wnt signalling do not seem to be affected. Notably, there was also a significant increase in the number of Paneth cells in BMAL1-/- mice. Since Paneth cells are largely responsible for creating and maintaining the intestinal stem cell niche, these findings suggest there may be an expansion of the stem cell niche beyond its normal confines in BMAL1-/- mice. Such a scenario could lead to dysregulation of the stem cell niche, interfere with proper differentiation, and ultimately be a contributing factor in tumourigenesis. We posit the loss of stem cell regulation is a potential link between circadian rhythm disruption and tumour formation.

Keywords: Circadian, Intestinal, Niche

MDD212

CIRCADIAN TRANSCRIPTIONAL REGULATION IN INTESTINAL ORGANOIDS OF MICE

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The circadian clock assists in coordinating biological processes by regulating gene activity based on time of day. The clock comprises a 24-hour transcription-translation feedback loop that is synchronized by external factors such as photoperiod, hormones, and other physiological processes. When the circadian clock is disrupted genetically or though repeated re-entrainment, in the case of shiftwork, it will ceases to function as a time keeper. Several studies have linked loss of circadian clock function with increased risk for colorectal cancer. The majority of colorectal cases have mutations in the gene, Apc, which is modeled using the Apcmin mouse strain. Adenomas and healthy tissue can be isolated from these mice and cultured as organoids to observe the epithelium and its stem cells in an isolated context. The role of circadian gene regulation in healthy and adenoma organoids is unknown, and would help to understand the cellular programs that might become dysregulated during the transition from healthy tissue to neoplastic development. To elucidate this gap in knowledge, we derived organoids from the intestine of Apc+/+;Bmal1+/+, (clock-wildtype) Apc+/+;Bmal1-/- (clock-dead) Apcmin/-;Bmal1+/+(adenoma, clock-wildtype), and Apcmin/-;Bmal1-/- (adenoma, clock-dead) mice. We performed a high resolution 24h RNA sequencing experiment to reveal the differences in gene regulation of healthy and adenoma organoids, that either have or lack a functional circadian clock, due to the absence of the core circadian clock gene Bmal1. Our experiments reveal that the circadian clock regulates surprisingly few genes in the intestinal epithelium autonomously, but some of the genes it does regulate such as Nras, Ephb3, and Bmp3, are known to be important for intestinal stem cell function. We also found that adenoma organoids lacking a functioning clock have increased levels of genes including, Pcna, ki67, and Hras, suggesting that Apcmin/-; Bmal1-/- tumour organoids are more proliferative than those with a functioning circadian clock. Our work shows the importance of considering time of day in gene expression, and reveals processes regulated by the circadian clock gene Bmal1 in the intestinal epithelium during homeostasis and colorectal cancer.

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Keywords: Circadian Rhythms, Intestinal Organoids, RNA-Sequencing

MDD213

HUMAN BENIGN AND MALIGNANT ENDOMETRIAL ORGANOIDS RECAPITULATE PARENT TISSUE PHENOTYPE AND EPIGENETIC SIGNATURE

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3D organoid cultures are emerging as invaluable tools in the realm of developmental studies and disease modelling unveiling an infinite biomedical application spectrum, including drug screening, gene engineering and cell transplantation. The establishment and maintenance of benign and malignant endometrial organoids using a chemically defined medium and extracellular matrix scaffolding has only recently been described, yet many questions regarding the physiological relevance of the model remain unanswered. For a cell model to be considered credible, phenotypical and genetic/epigenetic resemblance to parent tissue is fundamental; otherwise, extrapolation of results is rendered unreliable. In this regard, we recruited 14 patients (12 patients with endometrial cancer of various histological types and grades, 2 patients with benign uterine conditions), generated endometrial organoids and investigated their histological, immunohistochemical, immunofluorescent molecular marker and DNA methylation (Infinium HumanMethylation450 BeadChip, Illumina) kinship to original endometrial tissue. Our findings aim to validate endometrial organoids as a credible cell-based platform for endometrial cancer research.

Keywords: endometrial cancer, organoids, epigenetics

MDD214

AIR-LIQUID INTERFACE CULTURE PROMOTES MATURATION OF HUMAN IPSC-DERIVED ALVEOLAR EPITHELIAL TYPE 2-LIKE CELLS

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Alveolar epithelial type 2 cells (AEC2s) are the facultative progenitors of the distal lung epithelium and play a central role in the pathogenesis of several poorly understood lung diseases. Primary human AEC2s are challenging to study due to their limited accessibility in vivo and poor proliferation in ex vivo cell culture. The directed differentiation protocol of human iPSC-derived AEC2s (iAEC2s) developed by our group has created a renewable source of AEC2-like cells that can be readily generated from patient samples. iAEC2s in 3D organoid culture show some features that differ from adult primary AEC2 controls, such as high cytoplasmic glycogen content, suggesting lack of full maturation. To promote further maturation of iAEC2s, we have developed an air-liquid interface (ALI) culture system for iAEC2s. When cultured at ALI, iAEC2s demonstrate retention of AEC2-like identity based on transcript and protein-level expression of the markers NKX2-1, the key lung epithelial transcription factor, and SFTPC, a surfactant protein and an AEC2-specific gene. iAEC2s also maintain their identity when profiled transcriptomically by single-cell RNA sequencing and compared to previously published transcriptomic datasets of adult human AEC2s. ALI culture likewise promotes maturation of iAEC2s relative to 3D culture. Our group has previously identified a gene set associated with primary human AEC2 maturation by RNA sequencing of second-trimester fetal lung samples and adult AEC2s sorted on a specific marker known as HTII-280. This gene set includes genes that encode proteins important in known AEC2 functions, including surfactant proteins SFTPA1 and SFTPA2, the secreted protease inhibitor SLPI, and the protease PGC. Based on single-cell RNA sequencing, iAEC2s significantly upregulate this maturation gene set when cultured at ALI versus 3D. iAEC2s at ALI also downregulate genes associated with earlier developmental stages of human fetal lung, including the lung epithelial progenitor transcription factor SOX9. In summary, we report that air-liquid interface culture of iAEC2s allows maintenance of AEC2-like identity, promotes maturation in vitro, and results in downregulation of genes associated with earlier fetal lung cells. This platform furthers our ability to study more mature AEC2-like cells.

Keywords: Alveolar epithelial type 2 cells (AEC2, AT2), Air-liquid interface culture, Maturation

MDD217

A COMPREHENSIVE INDUCED PLURIPOTENT STEM CELL-BASED MODEL OF CROHN'S DISEASE

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Crohn's Disease (CD) is an inflammatory disorder of the gastrointestinal tract increasing in both incidence and prevalence worldwide. It has a complex and multifactorial etiology comprising of significant genetic and environmental factors. Prior investigation has implicated a variety of both cell-intrinsic and extrinsic environmental stressors in CD pathogenesis; aberrant macrophage and T cell signaling have both been identified as critical to the CD pathologic inflammatory response. Because access to primary cells from patients is limited and animal models fail to recapitulate the true pathophysiology of CD, we decided to take advantage of patient-derived human induced Pluripotent Stem Cells (iPSCs), as they represent a bona-fide tool to study cell intrinsic macrophage defects and their potential impact on intestinal epithelial dysfunction. Here, we report the generation of a comprehensive, clinically relevant CD patient-derived iPSC library. CD patients present with a wide range of disease symptoms, which are broken up into four broad clinical

phenotypes: mild non-progressors, patients resistant to anti-TNF therapy, patients with significant perianal disease, and fulminant, rapid progressors who go on to require surgical intervention soon after diagnosis. We have generated a CD iPSC library from 16 patients representing all four groups, and have differentiated subsets of these patient cell lines into both macrophages (iMac) and intestinal epithelial organoids (HIOs) using a novel protocol that generates intestinal organoids devoid of mesenchyme. We have analyzed patient iMacs and assessed their cytokine secretion profile and response to canonical pro-inflammatory stimuli, and have also generated patient HIOs in order to determine the presence of an epithelial cell-intrinsic disease signature. In summary, we report the generation of a large, clinically relevant CD-iPSC library, and the successful directed differentiation of CD iPSCs to iMacs and mesenchyme-free HIOs. Using these tools, we have investigated CD macrophage function in the context of normal homeostasis and have established a robust, reductionist in vitro model of CD that can elucidate molecular and cellular defects that hinder macrophage and intestinal function, ultimately leading to the pathophysiology seen in CD patients.

Keywords: Patient iPSC-based Disease Modeling, Organoid Co-Cultures, Macrophages in Disease

MDD475

DIRECTED DIFFERENTIATION OF HUMAN IPSCS INTO AIRWAY BASAL STEM CELLS

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Airway basal cells serve as a resident multipotential stem cell population within the airway. These cells have been isolated and extensively utilized in in vitro studies, and are considered to be a population with significant interest for future transplantation. The derivation of tissue-specific stem cells from human induced pluripotent stem cells (iPSCs) would overcome the limitation of patient-derived resources and would enable applications for regenerative medicine using autologous cells. In this study, we focused on developing methods to derive airway basal cells from iPSCs, and assessed their resulting biological and functional properties. To facilitate the development of an efficient differentiation protocol, we introduced NKX2.1 and P63 fluorescent reporters within iPSCs using site-specific gene targeting. After careful molecular characterization of these double reporter iPSCs, we then applied a lung epithelium-directed differentiation protocol followed by a proximalization protocol. These published approches led to emergence of immature lung progenitors, evidenced by expression of the lung-specific NKX2.1GFP reporter, followed by the development of immature airway progenitors co-expressing NKX2.1GFP+ and P63tdTomato+, but not the adult basal cell surface marker NGFR. We show here that these immature airway progenitors upregulate NGFR expression in response to primary basal cell culture medium; importantly, this NGFR+ population exhibits the expected functional properties of airway basal stem cells, including the capacity to clonally self-renew or terminally differentiate into multilineage ciliated and secretory epithelial cells in air-liquid interface (ALI) cultures. Furthermore, the well differentiated airway epithelium generated in ALI cultures from iPSC-derived basal cells (iBCs) yields significant levels of CFTR-dependent chloride flux in Ussing chamber assays. Importantly, both cryopreserved and extended cultured iBCs retain these features. Thus, we demonstrate directed differentiation in vitro of human iPSCs into an airway basal stem cell-like population, which will facilitate disease modeling as well as offering the potential for future regenerative therapies.

Keywords: Directed differentiation, Airway basal cells, Induced pluripotent stem cells (iPSCs)

MDD498

DERIVATION OF MACAQUE LUNG PROGENITOR CELLS FROM PLURIPOTENT STEM CELLS AS THERAPEUTIC AGENT FOR LUNG INJURY

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One goal of our research is to optimize the engraftment of lung progenitor cells as therapeutic agents for lung injury. In addition to ongoing studies in mice, we are developing “preclinical” models of lung injury, repair and progenitor cell engraftment in rhesus macaques. We have isolated and characterized primary basal cells (BCs) from rhesus macaques (mmuBCs) (n=3) and compared mmuBCs to murine basal cells by RT-PCR, ICC and IHC. Despite structural differences between the airways of mice and macaques, the basal cells of the two species displayed molecular similarities. We sequenced 320 primary mmuBCs (from 2D culture at passage 1) at the single cell level using the 10X

Genomics platform. From these data, we conclude that mmuBCs are a relatively homogenous population that expresses high levels of Cytokeratins 5 (Krt5) and 14 (Krt14). Moreover, our dissociation and enrichment protocol does not show contamination by basal cell progeny such as ciliated or secretory cells. We further have established protocols to maintain, manipulate and differentiate mmuBCs. We are currently optimizing directed differentiation protocols to generate lung epithelial lineages, including BCs, from wild type and NKX2-1-GFP rhesus macaque iPSCs. We have successfully induced ckit+, CXCR4+ endoderm from mmuIPSC that can be pushed towards a lung fate using dual Smad inhibition followed by Wnt and BMP activation. We have dissociated and replated these cultures as spheres that can be further directed toward a proximal lung epithelial fate (9.0 ±1.7 % Nkx2.1+ and 40.9±0.6% spheres with a partial or full Krt5+ identity) after 14 days in differentiation. We will further optimize these protocols and utilize proximal and distal lung progenitors for proof-of-concept engraftment studies in rhesus macaques after viral, bleomycin-induced and mechanical injury.

Keywords: macaque basal cells, lung-directed differentiation, cell-based therapy

EYE AND RETINA

MDD220

DEREGULATION OF NEURO-DEVELOPMENTAL GENES AND PRIMARY CILIUM CYTOSKELETON ANOMALIES IN IPSC RETINAL SHEETS FROM HUMAN SYNDROMIC CILIOPATHIES

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Ciliopathies are heterogeneous genetic diseases affecting primary cilium structure and function. Meckel-Gruber (MKS) and Bardet-Biedl (BBS) syndromes are severe ciliopathies characterized by skeletal and neuro-development anomalies, including polydactyly, cognitive impairment, and retinal degeneration. We describe the generation and molecular characterization of human iPSC-derived retinal-sheets (RSs) from controls, MKS (TMEM67) and BBS (BBS10) cases. MKS and BBS RSs displayed significant common alterations in the expression of hundreds of developmental genes and members of the WNT and BMP pathways. Induction of crystallin molecular chaperones was prominent in MKS and BBS RSs suggesting a stress response to misfolded proteins. Unique to MKS photoreceptors was the presence of supernumerary centrioles and cilia, and aggregation of ciliary proteins. Unique to BBS photoreceptors was the accumulation of DNA damage and activation of the mitotic spindle checkpoint. This study reveals how combining cell reprogramming, organogenesis and next-generation sequencing enables the elucidation of mechanisms involved in human ciliopathies.

Funding source: Foundation Fighting Blindness (FFB) Canada, National Science and Engineering Research Council of Canada , and Maisonneuve-Rosemont Hospital Foundation, Université de Montréal, National Institutes of Health (NIH)

Keywords: cilia; ciliopathy; iPSC; photoreceptor;, retinal degeneration; Bardet-Biedl Syndrome, Meckel-Gruber syndrome; MKS; BBS

MDD223

GENE-CORRECTED INDUCED PLURIPOTENT STEM CELL LINES AS A NEW PATIENT-SPECIFIC MODEL TO STUDY LCA5-ASSOCIATED RETINAL DISEASE

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Patient-specific iPSC-derived organoids offer new opportunities for disease modelling and drug discovery. In the field of inherited retinal diseases (IRDs), a genetically heterogeneous group of progressive vision disorders, retinal organoids hold great promise to mimic the pathophysiological mechanisms. Leber congenital amaurosis (LCA) is a subtype of IRD that can be caused by mutations in different genes, including LCA5 that encodes the protein lebercilin. This protein is regulating the transport of phototransduction proteins to the photoreceptor outer segments. We corrected both alleles of a homozygous nonsense mutation in exon 5 of the LCA5 gene (c.835C>T; p.Q279*), in patient-derived iPSCs via homology-directed repair CRISPR-Cas9 gene editing using the ribonucleoprotein delivery system. We validated the rescue of LCA5 expression via RT-PCR, and are currently performing sequence analysis of predicted off-target sites to exclude the introduction of unwanted genetic variants. Currently, we are differentiating control and patient cell lines into retinal organoids following an extensive (>180 days) protocol. We will validate the emergence of the photoreceptor layer with rudimentary outer segments using rod and cone photoreceptor markers (OPN1SW, OPN1MLW, and RHO). Expression and localization of lebercilin will be accessed with qPCR and immunohistochemistry, respectively. We will use this patient-specific cellular model to better understand the molecular mechanisms underlying LCA5-associated IRD, as well as for testing genetic or other treatments to slow down or prevent the degeneration of photoreceptors in these blinding disorders.

Funding source: This work was supported by TOP-subsidie from NWO and by Proefdiervrij Foundation.

Keywords: CRISPR, retinal organoids, retinal disease

MDD230

A COMMUNITY RESOURCE OF PATIENT CELL LINES FOR THE STUDY OF AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is the leading cause of vision loss in people over 60. While the loss of retinal pigmented epithelial cells (RPEs) has been implicated in the disease, the mechanisms driving degeneration remain elusive and vary across patients, reflecting the complex underlying genetics. While many AMD patient iPSC lines are available, large cohorts are necessary to capture the complexity of this disease. Cohorts can be assembled by combining cell lines made across multiple labs, but these suffer from a large amount of technical variation due to differences from lab to lab in cell culture processes and reagents. To mitigate this technical variation, NYSCF developed the NYSCF Global Stem Cell Array®, a fully automated pipeline for the generation of iPSCs and derivative cell types, capable of producing hundreds of lines per month. Here we present a large community resource for AMD research, including iPSC and RPE progenitor lines generated from hundreds of blood samples collected through the NEI's Age-Related Eye Disease Study 2 (AREDS2; https://www.nei.nih.gov/research/clinical-trials/age-related-eye-disease-study-2-areds2). This genetically defined cohort was selected based on input received from a vision community working group that prioritized genetic mutations related to AMD pathogenesis. The cell lines have been fully validated, meeting NYSCF's rigorous quality control standards as well as standard hallmarks of these cell types. This collection of cell lines is accompanied by comprehensive phenotypic metadata, including imaging studies, serving as a large-scale resource for the community to advance AMD research. Initial lines are now available for distribution from the NYSCF repository (nyscf.org/repository) and the corresponding patient data through the NIH Biomedical Research Informatics Computing System (BRICS) (https://brics.nei.nih.gov/). NEI expects these lines to be used by the research community for drug screening and disease modeling and leveraged in the future to advance our understanding of AMD and identify new effective diagnostics, treatments or cures.

Keywords: AMD, iPSC, RPE

MDD238

AAV-DELIVERED GENE THERAPY FOR DOMINANT CRX-LEBER CONGENITAL AMAUROSIS USING PATIENT-DERIVED RETINAL ORGANOIDS

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CRX is a homeodomain transcription factor essential for the differentiation and maturation of retinal photoreceptors. Mutations in the CRX gene lead to distinct retinopathy phenotypes, including autosomal dominant Leber congenital amaurosis (LCA). No effective treatment is currently available for CRX-retinopathies. In this study, we used retinal organoids derived from iPSCs of CRX-LCA patients to examine disease mechanism(s) and develop potential therapies. Immunohistochemistry revealed impaired upregulation of rod and cone opsin visual pigments and diminished formation of outer segment-like structures in patient organoids. Bulk and single cell transcriptomic analyses further demonstrate

that patient organoids fail to upregulate key genes related to visual signal detection, suggesting impaired maturation as the underlying molecular pathology. Overexpression of the normal CRX allele using an AAV vector partially restores expression of the visual pigment Rhodopsin, which is severely reduced in photoreceptors of patient retinal organoids. Our studies provide a proof-of-concept for future development of gene therapy for CRX-LCA and other CRX-retinopathies.

Funding source: National Institutes of Health Intramural Program

Keywords: Gene therapy, Disease modeling, Retinal organoids

HEMATOPOIETIC SYSTEM

MDD244

DEPLETION OF SCFA-FERMENTING GUT BACTERIA ALTERS THE EPIGENOME OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Gut dysbiosis alters the development and severity of atopic disease. We previously demonstrated that nursing dams and newborn mice treated with low-dose vancomycin alters gut microbial diversity with a marked loss of bacteria that produce short-chain fatty acids (including butyrate). Vancomycin-induced gut dysbiosis enhances the TH2 response to lung allergens due to altered dendritic cell trafficking and activation in addition to modifying the behavior of other mature leukocyte lineages. Butyrate supplementation reverses the vancomycin-induced TH2 pro-inflammatory phenotype. Butyrate is known to exert some of its effects on target cells by inhibiting histone deacetylases with consequent effects on gene expression. Consistent with a role for epigenetic skewing of the hematopoietic compartment, we found that engraftment of total bone marrow from dysbiotic mice transferred enhanced TH2 proclivity in normobiotic recipients. Strikingly, we found unique regulatory states (H3K27ac marks) in purified hematopoietic stem and progenitor cells (HSPC) of TH2-skewed recipient mice. Single-cell RNA sequence analyses identified a distinct transcriptomic signature in HSPC of dysbiotic mice that were reversed by butyrate supplementation. Together, these data suggest that the gut microbiome alters gene expression in blood progenitor cells with long term consequences on the immune response to peripheral allergens.

Keywords: Allergy and asthma, Microbiome, Epigenetics

MDD250

TRANSCRIPTOME MAPPING OF HEMATOPOIETIC PROGENITORS IN SYSTEMIC LUPUS ERYTHEMATOSUS REVEALS MYELOID SKEWING AND ALTERNATIVE GRANULOPOIESIS

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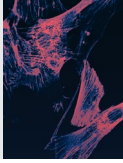
Hematopoietic stem and progenitor cells (HSPCs) are multipotent cells giving rise to both myeloid and lymphoid lineages. HSPCs actively respond to inflammatory stimuli by myeloid skewing which may result in exhaustion, decreased function, increased risk for inflammation, decreased adaptive immunity and increased cardiovascular mortality. Since most cells participating in the pathogenesis of systemic lupus erythematosus (SLE) originate from bone marrow (BM)-HSPCs,



we reasoned that the aberrancies of immune cells could be traced back to HSPCs. To this end, global gene expression map of BM-HSPCs was completed by RNA-seq followed by pathway and enrichment analysis. Cell cycle and apoptosis status of SLE HSPCs were monitored by flow cytometry while DNA damage via immunofluorescence. Transcriptomic analysis of Lin-Sca-1+cKit+ (LSK) hematopoietic progenitors from diseased lupus mice demonstrated a strong myeloid signature with expanded frequencies of common myeloid progenitors (CMPs) -but not of lymphoid progenitors (CLPs)- reminiscent of a "trained immunity" signature. CMP profiling revealed an intense transcriptome reprogramming with suppression of granulocytic regulators. This is indicative of a differentiation arrest with downregulation of major regulators such as Cebpe, Cebpα and Spi1, and disturbed myelopoiesis. Despite the differentiation arrest, frequencies of BM neutrophils were markedly increased in diseased mice suggesting an alternative granulopoiesis pathway. In SLE patients with severe disease, hematopoietic progenitor cells (CD34+) demonstrated enhanced proliferation, cell differentiation and transcriptional activation of cytokines and chemokines that drive differentiation towards myelopoiesis thus mirroring the murine data. Comparative transcriptomic analysis between human and mouse reveals intense granulopoiesis signature in active disease. Moreover, comparison with published whole blood transcriptomic data suggests that BM-HSPCs signature can be traced back to the periphery of patients. Priming of HSPCs and aberrant regulation of myelopoiesis may contribute to inflammation and risk for flare. Re-establishment of the homeostatic myeloid versus lymphoid balance may improve transplantability of HSPCs and restore immune function.

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Keywords: Systemic Lupus Erythematosus, Hematopoietic stem cells, Transcriptomics

IMMUNE SYSTEM

MDD258

THE UNEXPECTED INTERPLAY BETWEEN THE STEM CELL FACTOR LIN28 AND INFLAMMATION RESPONSE

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Lin28 is a stem cell factor which is mainly expressed in stem and progenitor cells and undergoes a significant downregulation during cell differentiation. Ectopic expression of Lin28 is associated with abnormal development, cell transformation and tumor formation. We have previously found that specific Lin28 expression in the nephrons results in the formation of cystic kidneys. To explore the mechanism by which Lin28 expression leads to kidney damage we performed global gene expression analysis by RNA seq. Strikingly, this analysis revealed a very strong inflammation response in the transgenic kidneys. We validated this finding by blood tests, FACS analysis, ELISA and H&E staining. This inflammation response did not appear in any other organ that we analyzed. Moreover, Lin28 overexpression in the stromal cells of the kidney did not lead to inflammation. Therefore, we concluded that the inflammation is a local response that occurs upon Lin28 expression in specific cell types. Finally, our preliminary results suggest that the inflammation is the cause of the cystic kidney phenotype and not its result. To conclude, while Lin28 has been studied so far mainly in the context of embryogenesis, stem cells and tumor development, this study demonstrates, for the first time, that ectopic expression of Lin28 plays a role also in inflammation development. Therefore, we show that Lin28 has a broader effect on cell fate regulation than has been suggested so far.

Keywords: Lin28, Kidney, Inflammation

MDD262

A NOVEL TARGETED APPROACH TO ACHIEVE IMMUNE SYSTEM RESET: A SINGLE DOSE OF CD45-TARGETED ANTIBODY DRUG CONJUGATE ENABLES AUTOLOGOUS HSCT AND HALTS DISEASE PROGRESSION IN A MURINE ARTHRITIS MODEL

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Resetting the immune system through autologous hematopoietic stem cell transplant (autoHSCT) is a highly effective treatment in selected patients with autoimmune diseases. AutoHSCT can induce long-term remission with 80% progression free survival

in multiple sclerosis patients (Muraro 2017, Burt 2019). Use of autoHSCT in scleroderma patients has achieved superior outcomes in two randomized studies compared to standard of care (Tyndall 2014, Sullivan 2018). These impressive results are achieved by a combination of the eradication of autoreactive immune effector cells and re-establishment of self-tolerance, i.e., immune system reset. However, only a small fraction of eligible patients undergo autoHSCT, largely due to toxicity associated with current conditioning regimens. To explore a safer alternative to enable immune reset, we generated a novel ADC targeting murine CD45 and evaluated its ability to enable congenic HSCT and eliminate pathogenic host-reactive cells in the context of proteoglycan-induced arthritis (PGIA), a model that recapitulates key features of rheumatoid arthritis with contributions from both B and T effector lymphocytes. Inflammatory arthritis was generated by immunizing Balb/c mice (CD45.2+) with the recombinant human G1 core aggrecan in the adjuvant DDA 3 times over 42 days. Ten days after the third immunization, when all animals had active disease and the mean clinical score was half-maximal, mice were randomized and treated. Animals were transplanted with 20x106 congenic (CD45.1+) bone marrow cells 48 hours after ADC administration. A single dose of 2 mg/kg of CD45-ADC was well tolerated, enabled full donor chimerism and halted disease progression. Disease control after CD45-ADC conditioning and HSCT matched disease control obtained by multiple infusion of anti-TNF mAbs. No chimerism or disease modification was observed in animals conditioned with 2 mg/kg isotyope ADC and HSCT. These results suggest that targeted immune depletion with a single treatment of CD45-ADC can result in effective auto-HSCT and halts disease progression. Targeted CD45-ADCs may represent a safer and better tolerated approach for conditioning patients prior to immune reset through autoHSCT and significantly reduce the side effects associated with current conditioning.

Keywords: Autoimmune Disease, Arthritis, Antibody Drug Conjugate

MUSCULOSKELETAL

MDD268

3D MICROFLUIDIC MODEL OF HETEROCHRONIC PARABIOSIS TO STUDY SYSTEMIC REGULATION OF SKELETAL MUSCLE AGING

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Muscle satellite cells (MuSCs) are skeletal muscle-specific stem cells and play a key role in skeletal muscle regeneration after injury. However, aging alters their regenerative capacity. Interestingly, studies using parabiosis which makes two animals sharing their blood circulations with a surgical technique, demonstrated that circulating factors from young animals can rejuvenate the regenerative capacity of aged animals. However, due to the complexity of in vivo parabiosis, identifying putative youthful factors has yet to be elucidated. To overcome this challenge, we employed microengineering technologies and developed a 3D vascularized muscle-on-a-chip (VMoC) circuit with biophysical and biochemical properties similar to in vivo MuSC niche, where FACS purified quiescent MuSCs are cultured in between myofibers and 3D hydrogel (ECM mimetic) underneath vascular endothelial cells. With the VMoC, we further integrated it to model parabiosis by co-culturing cells/sera from young and aged animals. By adding the serum from young, aged, and Sod1-/- (oxidative stress model) mice to VMoCs, we demonstrate distinct myogenic activities of young MuSCs; VMoCs with aged and Sod1-/- sera showed significantly reduced myogenesis as measured by fusion index. Next, we replicated heterochronic parabiosis using our integrated chip-to-chip platform to assess the rejuvenating effects of young serum/cells. Interestingly, similar to in vivo parabiosis, when aged VMoCs were continuously exposed to the young systemic environment, the myogenic activity of aged MuSCs were significantly boosted. In addition, genes regulating different steps of myogenesis (Desmin, MyoD, and Myogenin) were upregulated in the aged VMoC exposed to the young systemic environment. Furthermore, this rejuvenation effect in myogenesis was correlated with increased VEGF, which has been shown to play a crucial role in MuSC function in parabiosis-VMoC. Collectively, our parabiosis VMoC will provide a pre-clinical testing tool that will facilitate our understanding of the dynamic regulation of circulating humoral factors and potential drug discovery. More importantly, our approach can be translated into human clinical studies by replacing the murine cells with the corresponding human cells/serum.

Funding source: NIH R21AR072287 (YJ), NIH R03AG062976 (YJ), and NIH DP2HL142050 (YK)

Keywords: Rejuvenation, Skeletal muscle, Parabiosis

NEURAL

MDD277

AN HIPSC-BASED GENETIC SCREENING SYSTEM TO MODEL AGGREGATION OF TAU PROTEIN ALLOWS THE IDENTIFICATION AND VALIDATION OF POSSIBLE TARGETS FOR ALZHEIMER'S DISEASE THERAPIES

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One of the hallmarks of Alzheimer's Disease (AD), as with some other neurodegenerative diseases, is the misfolding and aggregation of proteins, such as amyloid-beta and tau. Tau pathology is also believed to propagate trans-synaptically from neuron to neuron. Either prevention of propagation, or the removal of aggregated tau, is a potential approach for AD modification. Deubiquitinating enzymes (DUBs) maintain ubiquitin homeostasis by removing ubiquitin modifications from target proteins, thereby altering protein function, stability, and signaling. We hypothesize that the modulation of the ubiquitin-proteasome system with DUB inhibitors will lead to decreased tau aggregation either directly (by inhibiting the removal of ubiquitin from tau thereby increasing its degradation) or indirectly (via modulation of other relevant pathways, like autophagy). To select relevant DUBs, we developed a phenotypic assay to assess the effect of knocking down ~100 DUBs on the clearance of tau aggregates in hiPSC – derived neurons, assayed using high content image analysis. We show that addition of recombinant tau seeds to hiPSC – derived cortical neurons expressing a fluorescent tau reporter construct, leads to formation of tau aggregates, which we can robustly and reproducibly reduce by knocking down specific DUBs. The DUB enzymes revealed in our phenotypic screen have been validated in confirmatory studies and have the potential to become novel targets for the treatment of AD. In collaboration with Mission Therapeutics we will develop selective, potent DUB inhibitors for preclinical target validation.

Funding source: The design, study conduct, and financial support for the study were provided by Abbvie. Abbvie participated in the interpretation of data, review, and approval of the publication.

Keywords: hiPSC - based disease model, Alzheimer's Disease, Proteostasis of tau protein

MDD282

THE ROLE OF CD47/SIRP-ALPHA INTERACTION IN HUMAN SYNAPTOSOME AND MYELIN UPTAKE

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system mediated by immune-cell induced demyelination and axonal injury. In addition to loss of neurites, synaptic loss is reported in MS patients in both lesion and normal-appearing areas. The process of phagocytosis is tightly regulated via a number of positive ("eat me") and negative ("don't eat me") signals that lead to proper uptake of damaged cells or foreign molecules while preserving healthy cells. One of these "don't eat me" signals is mediated by the interaction between CD47 on target cells and its receptor SIRPA on phagocytes, which has been shown to be involved in preventing excess synaptic pruning by microglia in developmental stages. Although a decrease in CD47 is observed in MS lesions, it is not clear how CD47 is reduced and if this reduction contributes to phagocyte-mediated synaptic loss and myelin stripping in MS. Thus, we first aimed to identify conditions that could mediate CD47 reduction. To determine the effect of pro-inflammatory conditions on neuronal CD47 expression, primary human neurons (HN), and neurons differentiated from human induced pluripotent stem cells (iPS-Nn) were incubated with activated human lymphocyte supernatant (via CD3 and CD28 costimulation) or specific inflammatory cytokines, IFNγ and TNFα. We detected an increase in CD47 levels, with a 2.66-fold increase (n=3) in activated PBMC supernatant treated iPS-Nns (n=3), a 1.95-fold increase (n=2) in activated PBMC supernatant treated HNs, and a 4.36-fold increase in IFNγ treated iPS-Nns (n=1) in CD47 mRNA levels. TNFα did not affect CD47 expression at mRNA levels. Next, we tested whether CD47-SIRPA interaction is important in myelin debris and synaptosome uptake. Human myelin fractions and synaptosomes isolated from iPS-Nn were labelled with a pH sensitive dye, pHrodo, and phagocytosis was measured by flow cytometry. CD47 blockade significantly increased both myelin and synaptosome uptake by phagocytes. Overall, unexpectedly, we have observed that under inflammatory conditions, neurons upregulate CD47 expression, which might have protective functions. Also, CD47 interaction with SIRPA is involved in myelin debris uptake as its inhibition increases myelin uptake. The role of CD47 expression in myelin stripping needs further investigation.

Keywords: Neuroimmunology, phagocytosis, Inflammatory conditions

MDD283

A MODEL OF HUMAN IPSC DERIVED MICROGLIA TO STUDY ALZHEIMERS DISEASE

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Research on the biology of aging strives to understand the causes of increased disease vulnerability with age. Senescent cells are abnormal cells that accumulate with age. They promote inflammation as part of their senescence-associated secretory phenotype (SASP). We have shown that inflammation associated with SASP depends on a mitochondria-to-nucleus retrograde signaling pathway. Senescence of glial cells in the brain has been associated with inflammation in Alzheimer's Disease

(AD). However, the mechanism and cell type(s) involved are incompletely understood. Here we show progress on models of microglial senescence, including assays for markers of senescence and inflammation using human induced pluripotent stem cell (iPSC) approaches in vitro and animal models in vivo. We are developing these cell lines as a novel model to screen for drugs that block senescence-associated inflammation in microglia, including drugs which are already clinically available. This work can identify novel therapeutic targets for treatment of AD and novel mechanisms of senescence that underlie age-associated disease.

Funding source: California Institute for Regenerative Medicine, National Institute on Aging

Keywords: biology of aging, Alzheimer's disease, Microglia

MDD285

ANALYSIS OF DISTINCT CELL POPULATION FATE IN HUMAN CEREBRAL ORGANOID MODEL OF MULTIPLE SCLEROSIS.

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Multiple sclerosis (MS) is an autoimmune neurological disorder characterized by inflammation, demyelination and neural degeneration. MS also affects neural progenitor cell proliferation and differentiation capacity in the subventricular-zone as well as glial cell number, among other things. However, the origin and evolution of the disease are still poorly understood due to relative inaccessibility of human brain tissues and inadequate animal models to study MS. Recent advances in 3D cerebral organoid cultures derived from induced pluripotent stem cells (iPSC) provide new avenues to implement reproducible models to study cell type- and stage-specific effects of MS. Cerebral organoids contain ventricle-like structures aligned by neural stem cells, progenitor cells in various stages of differentiation and migration, and cortical neurons in a stereotypical inside-out stratified layout. Moreover, it has been previously shown that neurons present in c-organoids were able to get myelinated. We propose here to develop an innovative model of MS using human iPS derived cerebral organoids. We derived cerebral organoids from iPS cells of patients suffering of primary progressive MS and relapsing remitting MS. Stem cell proliferation, migration and differentiation in neuronal and glial lineage, as well as neuronal differentiation was assessed in the different type of MS organoids and compared to control organoids. Size of cortical structure in MS c-organoids was significantly smaller compared to control c-organoids. Immunofluorescence for stem cell marker SOX2 revealed that the stem cell pool localized in the VZ/SVZ, was reduced in MS organoids compared to control. Further investigations are needed to understand the mechanisms involved. In parallel analysis of apoptosis marker CC3 showed an increase of CC3+ cell numbers in MS organoids, particularly in the neuroblast/neurons population. This study will give new insight on the origin and evolution of the disease and will help to identify potential target for therapeutic strategies designed to promote myelin repair in the different types of MS.

Funding source: This work was supported by Tisch MS Research Center of New York

Keywords: Multiple sclerosis, cerebral organoids, IPS cells

MDD287

16P13.11 DELETION LEADS TO INCREASED NEURONAL NETWORK ACTIVITY IN HIPSC-DERIVED EXCITATORY NEURONS

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16p13.11 copy number variants (CNVs) are associated with numerous neurodevelopmental and neuropsychiatric disorders, including autism, intellectual disability, epilepsy schizophrenia and psychosis. The majority of 16p13.11 deletions or duplications occur within three well-defined intervals. Despite precise mutations within intervals I - III, the molecular mechanisms that underlie commonly observed clinical phenotypes remain largely unknown. Patient-derived induced pluripotent stem cells (iPSCs) provide a platform for investigating the morphological, electrophysiological and gene-expression changes that result from 16p13.11 CNVs in human-derived neurons. We have generated iPSCs from three probands with varying sizes of 16p13.11 deletions. Proband 1 comprises a micro-deletion within interval 1 while probands 2 and 3 encompass larger deletions spanning both intervals I and II. In addition, we have generated and characterized iPSCs from familial healthy controls. Here, we show that 16p13.11 deletions result in increased neurite branching of glutamatergic neurons. Additionally, immunocytochemistry analysis shows an increase in overall synapse number in 16p13.11 deletion neurons when compared to healthy controls. To determine functional consequences of changes in synapse number, we conducted multi-electrode array (MEA) recordings on neurons generated from deletion and control neurons. Preliminary data demonstrates a significant increase in the firing rate of glutamatergic neurons derived from probands,

compared to controls. Similarly, there were significant increases in the number and frequency of network bursts. Intriguingly, there was a decrease in overall network synchrony of neurons derived from probands. Overall, these results indicate that there are morphological and functional alterations as a result of 16p13.11 deletion in glutamatergic neurons in culture. In addition to confirming these phenotypes in additional probands with 16p13.11 CNVs, comparing the common phenotypes between neurons derived from probands with various 16p13.11 deletions sizes will further assist in ascertaining common pathways and targets that could be utilized for screening drug candidates. These studies aim to improve identification of novel treatment options for 16p13.11 deletion patients.

Funding source: Tommy Fuss Center, Boston Children's Hospital Neurodevelopment Institutional National Research Service Award (NRSA), T32 MH 112510

Keywords: 16p13.11, Neuropsychiatric, Neurodevelopmental

MDD288

ABERRANT SIGNALING IN COPY NUMBER VARIATION ASSOCIATED AUTISM

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Copy number variation (CNV) caused by deletion or duplication in 16p11.2 chromosomal locus is the most common genetic cause of autism. While both 16p11.2 deletion and duplication patients exhibit common developmental, behavioral and social impairment, they are associated with opposing structural changes in brain size. While 16p11.2 deletion patients exhibit macrocephaly, 16p11.2 duplication patients are associated with smaller brain size or microcephaly. Mechanisms through which 16p11.2 CNVs lead to reciprocal changes in brain size during early human development are unknown, and the contribution of 29 genes present in the 16p11.2 locus to brain size changes has yet to be investigated. Using neuronal progenitor cells differentiated from induced pluripotent stem cells (iPSC) reprogrammed from 16p11.2 CNV patient fibroblasts, we employed quantitative phosphoproteomics to detect global differences in phosphorylated proteins in patient derived NPCs. Pathway analysis of differentially affected proteins might contribute to our understanding of the neuropathology of autism associated with CNVs.

Keywords: iPSC, Autism, Aberrant signaling

MDD293

FABRICATION OF NEURAL TISSUE-LIKE BY 3D BIOPRINTING MURINE NEURAL STEM CELLS DERIVED FROM REACTIVE ASTROCYTES

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Diseases that affect the central nervous system have limited treatment alternatives due to the complexity of the tissue. Tridimensional (3D) bioprinting has emerged as a promising technology for tissue engineering due to its ability to construct in vitro models using layer-by-layer deposition of cells and biomaterials. In this work, we constructed a biomimmic neural tissue by 3D bioprinting neurospheres from reactive astrocytes-derived neural stem cells (NSC) and evaluated viability and neuronal differentiation capacity. Briefly, newborn mice astrocytes were isolated, cultured as a monolayer, and activated by scratching a patch of cells off. After three days, reactive astrocytes were detached by trypsinization and were plated on non-adherent plates to induce the formation of neurospheres. Dedifferentiated astrocytes-derived NSC formed floating neurospheres and reached an average diameter of ~100 µm after three days. Neurospheres were mixed with a bioink composed of gelatin-alginate blend, already tested for printability, rheological, mechanical, and physical properties. Neurospheres behavior in the 3D bioprinted tissue was evaluated for viability, cell migration, and neuronal and astrocytes differentiation. Results showed that the optimal gelatin-alginate bioink composition is 5% gelatin-2.5% alginate, which presented shear-thinning behavior, 50% of network porosity, and Young's modulus of 6.9 ± 0.2 kPa, within the range of in vivo neural tissue. 3D bioprinted neurospheres in gelatin-alginate bioink showed a higher percentage of living cells when compared to dead cells after 7 days of culture of the bioprinted tissue. We observed cells migrating out from the neurospheres, suggesting that the biomaterial is a suitable substrate. Gene expression of cells at day 14 in vitro was evaluated by qPCR, and results showed expression of GFAP and MAP2, markers for astrocytes and neurons, respectively, indicating the capacity of the NSC to differentiate into neuronal lineages in the bioprinted structures. In conclusion, our results show that it is possible to 3D bioprint reactive astrocytes-derived neurospheres using gelatin-alginate bioink. This suggests that we can fabricate neural tissue-like models for in vitro studies on neural development, degeneration and regeneration using 3D bioprinting.

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Keywords: 3D bioprinting, neural stem cells, astrocytes

MDD300

4 STEPS TO MAKE EVERY NEURON IN THE HUMAN SPINAL CORD

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The spinal cord contains billions of neurons, with a huge diversity of subtypes enabling sensory, proprioceptive, and motor function. However, current human stem cell-based in vitro models and prospective cell transplantation therapies fail to reflect the significant regional specificity of spinal cells. Here we describe methods to recapitulate the full diversity of spinal cell types along both the rostrocaudal (R/C) and dorsoventral (D/V) axes with chemically defined, scalable protocols using human pluripotent stem cells (hPSCs). We first induce R/C patterning to generate neuromesodermal cells from a defined anatomical level, then instruct these cells to become early spinal progenitors. By providing appropriate D/V signaling, spinal progenitors can be subspecified to generate tunable ratios of motor neurons (MNs) and locomotor interneurons (INs) from the ventral spinal cord, or TGF-β-dependent proprioceptive INs and TGF-β-independent sensory INs from the dorsal spinal cord. Cultures with over 95% neuronal yield can be generated in as little as 19 days, and these protocols can be used modularly to generate phenotypes from different anatomical levels. Single-cell RNA-sequencing reveals regionally-specified neurons with discrete Hox gene profiles spanning the hindbrain through lumbar spinal cord, representation of all major motor and somatosensory spinal cell types, and the presence of human-specific cell populations. Altogether, this dataset enables characterization of the diversity of human spinal neurons for the first time. We anticipate that access to these cells will advance a mechanistic understanding spinal development, expand the potential and accuracy of in vitro models, provide insight into novel region-specific markers and therapeutic targets, and represent clinically relevant populations for cell transplantation.

Keywords: Spinal cord, direct differentiation, Single Cell RNA-sequencing

MDD324

MODELING AMYOTROPHIC LATERAL SCLEROSIS WITH HUMAN IPSCS REVEALS AN ABERRANT INTERPLAY BETWEEN THE RNA-BINDING PROTEINS FUS AND HUD/ELAVL4

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We use human induced Pluripotent Stem Cells (iPSCs) to model the motor neuron disease Amyotrophic Lateral Sclerosis (ALS). Several RNA-binding proteins (RBPs) have been genetically linked to ALS and we propose that an aberrant interplay between the neural RBP HuD (also known as ELAVL4) and ALS-linked RBPs, such as FUS and TDP-43, exists in ALS patients' motor neurons. By analyzing the RNA interactome of wild-type and mutant FUS (P525L) in human iPSC-derived motor neurons (MNs), we have identified a number of novel RNA targets. In particular, many transcripts are preferentially bound by mutant FUS in the 3'UTR and neural ELAV-like RBPs, including HuD, are among those targets. HuD is a RBP playing a role in neuron-specific RNA processing, regulating the expression level of genes involved in cell growth and differentiation. Mutant FUS binding results in increased number of sites of translation, leading to aberrant increase of HuD protein levels. These findings have been confirmed in primary MNs from a FUS-ALS mouse model. Moreover, HuD and mutant FUS interact at the protein-protein level and co-localize in cytoplasmic speckles with peculiar biomechanical properties. Interestingly, upon oxidative stress both HuD and mutant FUS are engaged in stress granules. Notably, in the spinal cord of FUS ALS patients, HuD represents a neural-specific component of FUS-positive cytoplasmic aggregates, whereas in sporadic patients it co-localizes with phosphorylated TDP-43-positive inclusions. Finally, we observed peculiar axon branching and regeneration phenotypes in both human iPSC-derived MNs and mouse primary MNs. These effects could be mediated by up-regulation of two known HuD targets, NEURITIN and GAP43. In line with this, mass spectrometry analysis revealed alteration of proteins involved in the motor axon biology, including axogenesis, in FUS mutant MNs. Collectively, these results suggest a possible novel pathogenetic mechanism in ALS, triggered by FUS mutations and involving the RBP HuD.

Funding source: Fondazione Italiana di Ricerca per la Sclerosi Laterale Amiotrofica (AriSLA); Istituto Pasteur Italia - Fondazione Cenci Bolognetti

Keywords: Amyotrophic Lateral Sclerosis, FUS, Motor neuron

MDD331

ALTERED GENE EXPRESSION AND DNA METHYLATION IN IPSC-DERIVED NEURAL PROGENITOR CELLS (NPCS) FROM PATIENTS WITH SMITH-MAGENIS SYNDROME (SMS) CAUSED BY MUTATIONS IN RAI1

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SMS is a rare neurodevelopmental disorder with a global incidence of ~1/15,000, characterized by variable presentations including behavioral disturbances, circadian rhythm dysfunction, distinctive facial features, and intellectual disability. In ~90% of cases, SMS is caused by an interstitial deletion of chromosome 17p11.2, while mutations in retinoic acid induced 1 (RAI1), a gene encoded by this region, underlie the basis for the rarer non-deletion cases. Prior studies suggest that RAI1 acts as a transcription factor, but its function in human brain remains to be fully defined. To investigate the role of RAI1 in the neurobiology of SMS, we are conducting iPSC-based modeling studies. iPSCs carry the replica of the donor's genome and can be differentiated into neural derivatives, permitting studies in disease-relevant, renewable cells. In our preliminary work, iPSCs were generated by reprogramming adult somatic cells of four unrelated SMS cases, each carrying its unique, deleterious RAI1 mutation. iPSCs from six gender-matched, unaffected controls were also analyzed in this study. Differentiation into NPCs was checked by immunostaining with neural stem cell markers, nestin and PAX6. Transcriptome and methylation profiles in SMS- and control-derived NPCs were analyzed through RNA sequencing and Illumina EPIC methylation arrays, respectively. RNA-seq detected significant differential expression of 35 genes unveiling enrichment for biological processes that include cell adhesion, extracellular matrix organization, cell motility, and signal peptide secretion. Methylation profiles revealed 17 differentially methylated CpG sites. Two genes showed an inverse relationship between DNA methylation and gene expression in SMS-derived NPCs. PHACTR3, shown in GTEX as most highly expressed in various brain regions, was both under-expressed and hyper-methylated. TNFAIP2, disclosed in prior studies to be a target of retinoic acid, was both hypo-methylated and overexpressed. Similar studies on CRISPR-derived isogenic lines would permit analysis in greater depth, genotype-phenotype relationships in SMS. Overall, these investigations may illuminate disrupted neurobiological mechanisms that could reveal targets toward the development of novel therapeutics.

Funding source: National Institute of Mental Health Intramural Research Program

Keywords: Smith-Magenis Syndrome, iPSC-derived neural progenitor cells, gene expression and DNA methylation

MDD334

A PHENOTYPIC SCREEN USING PATIENT-DERIVED MOTOR NEURONS IDENTIFIES PIKFYVE AND SYF2 AS NOVEL THERAPEUTIC TARGETS FOR DIVERSE FORMS OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease characterized by the massive degeneration of motor neurons. Given that >30 genes contribute to the disease pathogenesis and 80% of ALS cases have unknown mutations, we hypothesized that therapeutics that are effective in one form of ALS may not be effective in other forms and that screening patient-derived motor neurons may enable the identification of therapeutics that target points of convergence in multiple forms of ALS. To test this notion, we performed an unbiased phenotypic screen of 2,000 FDA approved drugs and 1800 tool compounds to identify compounds that rescue the survival of induced motor neurons (iMNs) generated from iPSCs derived from multiple ALS patients with known or unknown causal mutations. We found that targets whose perturbation is broadly efficacious across iMNs from many ALS patients are rare, providing experimental confirmation that patient-based target identification tools are critical. One broadly-efficacious target, PIKFYVE kinase, normally promotes autophagy. Surprisingly, inhibition of PIKFYVE rescues neurodegeneration mechanistically by blocking autophagosome-lysosome fusion, which induces secretory autophagy to robustly clear misfolded proteins that normally accumulate in the cytoplasm and drive neurodegeneration. Moreover, antisense-mediated suppression of PIKFYVE rescued motor deficits in a mouse model of ALS, confirming the utility of this target in vivo. A second broadly-efficacious target was SYF2, an RNA-binding protein. Antisense-mediated knockdown of SYF2 abrogated the pathological mislocalization of another RNA-binding protein, TDP-43, from the nucleus to the cytoplasm, which occurs in most cases of ALS. Our results demonstrate that convergent therapeutic targets in ALS are relatively rare, but two ways to broadly rescue ALS motor neuron degeneration include activating secretory autophagy and modulating RNA-binding protein dynamics.

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Keywords: Therapeutics, Amyotropic lateral sclerosis (ALS), Autophagy and RNA binding protein

MDD346

IPSC-DERIVED CEREBRAL ORGANOIDS TO ANALYZE THE MOLECULAR TRIGGERS AND CELLULAR CONSEQUENCES OF G4C2 REPEAT INSTABILITY IN C9ORF72-ALS/FTLD

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Instable expanded G4C2 hexanucleotide repeats in the C9orf72 (C9) gene represent the most frequent genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Patients with C9-ALS/FTD typically carry between hundreds to thousands of G4C2 repeats compared to fewer than 23 repeats in healthy subjects. The molecular triggers and cellular consequences of G4C2 repeat instability remain largely unknown, chiefly for two reasons: 1. Current G4C2 repeat sizing assays are hampered by low sensitivity, lack of accuracy, and high error rate. 2. Tractable C9 experimental models such as patient iPSC-derived neuronal cultures or transgenic BAC mice display limited or no repeat instability. To begin to understand the molecular triggers of G4C2 repeat instability, we generated iPSC-derived cerebral organoids from five members of a British-Canadian ALS/FTD family with pronounced repeat instability in germline and somatic tissues. The father was unaffected by ALS or FTD and carried a 70-repeat C9 allele; the mother showed no C9 expansion. Four of the five children carried ~1750 repeat expansions in blood and one daughter showed normal repeat size. To explore the cellular consequences of expanded G4C2 repeats, we analyzed fresh-frozen and formalin-fixed CNS and peripheral tissues from family members autopsied at age 90 years (father) and 59 or 66 years (affected children). Mosaic expansions of C9 repeats, RNA foci, and DPRs were found across different CNS tissues. Single nucleus RNA-Seq from frontal cortex uncovered alterations in cell type composition between the two affected family members and the two unaffected controls. Furthermore, several pathways related to DNA damage and heat shock response were found to be dysregulated, in line with the reported cytoplasmic mislocalization and aggregation of TDP43 in C9-ALS/FTD. In conclusion, cerebral organoids represent a new promising model to better understand the molecular triggers and cellular consequences of repeat instability in C9-ALS/FTD path.

Funding source: This project was supported by a grant from eRare3 (Repetomics). PMM is supported by the ALS Association Milton Safenowitz Postdoctoral Fellowship.

Keywords: amyotrophic lateral sclerosis, DNA repeat instability, cerebral organoids

MDD353

AN IN-DEPTH ANALYSIS OF DISRUPTED MOLECULAR PATHWAYS IN PITT-HOPKINS SYNDROME PATIENT IPSC DERIVED OLIGODENDROCYTES

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Pitt-Hopkins Syndrome (PTHS) is a rare and relatively understudied autism spectrum disorder that is caused by an autosomal dominant mutation or deletion in the gene transcription factor 4 (TCF4). TCF4 is a basic helix-loop-helix (bHLH) transcription factor that plays a critical role in neuronal development through known interactions with other proneural bHLH proteins. Through these bHLH protein interactions, human TCF4 expression peaks during corticogenesis and patients with TCF4 mutations have profound developmental delays and autistic behaviors. We have previously reported significant reduction in oligodendrocyte numbers and myelin in our PTHS mouse models. To study the role of TCF4 in human oligodendrocyte development, we have developed a platform to differentiate PTHS patient and control induced pluripotent stem cells into mature human oligodendrocytes (OLs) using a 2D and 3D culture system. We have reprogrammed iPSCs and differentiated OLs from 6 control and 6 PTHS patients (4 with a point mutation in the bHLH domain of TCF4 and 2 with large truncations of TCF4). Over the course of 90 days (2D system) and 160 days (3D system), we have identified key differences between PTHS and controls. We are using qPCR to monitor OL development and to identify differences in the expression of maturation markers between patient and control lines, based on the phenotypes observed in our mouse models. Moreover, we are validating our expression profiles using immunofluorescence

to quantify the proportions of oligodendrocyte precursor cells and mature oligodendrocytes produced by PTHS patient and control lines. Lastly, we have optimized a magnetic-activated cell sorting (MACs) protocol to improve our ability to make accurate comparisons between PTHS patient and control lines. With this stem cell-based platform, we aim to better understand the role of TCF4 in oligodendrocyte development in a human context, and to identify molecular mechanisms that underlie oligodendrocyte pathophysiology in PTHS.

Keywords: Stem Cells, Oligodendrocytes, Pitt-Hopkins Syndrome

MDD361

INVESTIGATING THE ROLE OF SQSTM1 (P62) IN MITOCHONDRIAL FUNCTION AND CLEARANCE IN HUMAN CORTICAL NEURONS

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Mitochondrial dysfunction has been linked to numerous neurodegenerative diseases. For instance, several disease-associated mutations are harboured in genes involved in mitophagy – the selective clearance of damaged mitochondria by autophagy. This includes PINK1 and PRKN mutations in Parkinson's disease patients. To induce mitophagy, PINK1 and parkin interact with various autophagic adaptor proteins including SQSTM1 (p62; gene mutations have been identified in frontotemporal dementia patients). The specific role of SQSTM1 in mitochondrial quality control is currently unclear. We aimed to elucidate the significance of its role in mitochondrial function and clearance in human cortical neurons. To this end, we generated human cortical neurons from induced pluripotent stem cell (iPSC) lines with unedited or mutated SQSTM1 producing premature termination of translation. We then assessed mitochondrial function in neurons with or without SQSTM1 using high-content screening systems (e.g., Opera Phenix and Seahorse XF Analyzer). We also established treatment regimens to induce mitochondrial depolarisation and biochemical methods to detect mitophagy. Cortical neurons generated from the SQSTM1-knockout (KO) iPSCs were morphologically identical to those derived from the unedited iPSCs, as judged by immunohistochemistry. SQSTM1 KO neurons displayed impaired autophagic flux. However, mitochondrial membrane potential, distribution, respiration and ATP production in iPSC-derived cortical neurons appeared to be unaffected. Furthermore, mitochondrial PINK1 recruitment and loss of mitochondrial proteins in iPSC-derived cortical neurons occurred in the absence of SQSTM1 following mitochondrial depolarization. In conclusion, SQSTM1 does not appear to be essential for mitochondrial respiration, energy production and mitophagy in our current human iPSC neuronal systems.

Keywords: SQSTM1, mitochondria, mitophagy

MDD372

MODELING LATE-ONSET SPORADIC ALZHEIMER'S DISEASE THROUGH BMI1 DEFICIENCY

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Late-onset sporadic Alzheimer's disease (AD) is the most prevalent form of dementia, but its origin remains poorly understood. The Bmi1/Ring1 protein complex maintains transcriptional repression of developmental genes through histone H2A mono-ubiquitination, and Bmi1 deficiency in mice results in growth retardation, progeria, and neurodegeneration. Here, we demonstrate that BMI1 is silenced in AD brains, but not in those with early-onset familial AD, frontotemporal dementia, or Lewy body dementia. BMI1 expression was also reduced in cortical neurons from AD patient-derived induced pluripotent stem cells but not in neurons overexpressing mutant APP and PSEN1. BMI1 knockout in human post-mitotic neurons resulted in amyloid beta peptide secretion and deposition, p-Tau accumulation, and neurodegeneration. Mechanistically, BMI1 was required to repress microtubule associated protein tau (MAPT) transcription and prevent GSK3beta and p53 stabilization, which otherwise resulted in neurodegeneration. Restoration of BMI1 activity through genetic or pharmaceutical approaches could represent a therapeutic strategy against AD.

Funding source: Canadian Institutes of Health Research, National Science and Engineering Research Council of Canada, Molecular Biology Program of Université de Montréal.

Keywords: Alzheimer's disease, dementia, sporadic, BMI1, polycomb, amyloid, Tau, p53, GSK3b, MAPT

MDD373

DOLUTEGRAVIR DISRUPTS NEURAL ROSETTE ORGANIZATION IN HUMAN IPSC-DERIVED FOREBRAIN ORGANOIDS

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Prevention of mother-to-child-transmission of HIV remains a prominent global health priority. Antiretroviral drugs have drastically reduced the incidence of vertical transmission but evaluating the safety of specific drugs on fetal development remains a challenge. Based on interim observations from a birth-outcomes surveillance study in Botswana, the US Food and Drug Administration and the European Medicines Agency released warnings in 2018 that the antiretroviral drug dolutegravir may increase the risk for severe neural tube deficits in newborns if it is taken by the mother at the time of the conception or early in pregnancy. An updated report in 2019 suggests a slightly higher prevalence of neural tube defects with exposure to dolutegravir at conception. Given these clinical observations, this project aims to investigate the effect of dolutegravir on neural development using 3D cortical forebrain organoids. Forebrain organoids are derived from human induced pluripotent stem cells and recapitulate the structural organization and cell type diversity of early stages of cortical neurogenesis in a human-specific context. These forebrain organoids are composed of numerous neural rosettes in which neural progenitor cells are organized in a distinctive radial orientation. To simulate prenatal exposure to dolutegravir, we treated these organoids daily and observed a robust phenotype after two weeks exposure to the drug in media. Forebrain organoids exhibited a dose-dependent response to dolutegravir resulting in a disruption of the neural rosette structures, suggesting deficits in the function and organization of early neural progenitor cells. Drug-exposed organoids also appear smaller in overall size, with fewer and thinner rosettes as compared to either vehicle-treated control organoids, or organoids exposed to raltegravir, another antiretroviral integrase inhibitor. Future studies will focus on identifying molecular and cellular mechanisms of this phenotype by characterizing specific pathways that are altered upon dolutegravir exposure. Moreover, these findings add to a growing body of work demonstrating the utility of forebrain organoid models as a platform for toxicity screening and studying the neurodevelopmental effects of prenatal drug exposure.

Funding source: NIH Grant R21MH118037

Keywords: Organoids, iPSCs, Integrase inhibitors

MDD376

ASTROCYTE CONTRIBUTIONS TO RETINAL GANGLION CELL NEURODEGENERATION USING GLAUCOMA PATIENT PLURIPOTENT STEM CELLS

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Retinal ganglion cells (RGCs) serve as the essential connection between the eye and the brain, with this connection disrupted in blinding disorders such as glaucoma. Human pluripotent stem cells (hPSCs) can serve as powerful tools for the in vitro analysis of human neurodegenerative diseases including glaucoma, as well as a platform for pharmacological screening of these diseased cells. Thus, the ability to derive RGCs from hPSCs was applied to an in vitro model of glaucoma using cells derived from a patient with an OPTN(E50K) mutation, as well as the use of Crispr/Cas9 gene editing to generate appropriate isogenic controls as well as new disease models. Using this system, hPSCs were differentiated to generate three dimensional retinal organoids, from which RGCs could be analyzed and isolated. Upon differentiation, OPTN(E50K) RGCs exhibited morphological changes including significant deficits in neurite complexity as well as the demonstration of more highly excitable properties by patch clamp analysis, suggesting a potential role for excitotoxicity in the deficits observed in glaucomatous RGCS. Subsequently, non-cell autonomous contributions to RGC neurodegeneration were identified through co-cultures with hPSC-derived astrocytes. Astrocytes derived from glaucoma hPSCs exhibited dysfunction in the autophagy pathway and altered mitochondrial dynamics compared to isogenic controls. Furthermore, upon co-culture with hPSC-derived RGCs, glaucomatous astrocytes exerted disease-related phenotypes upon healthy RGCs, while healthy astrocytes were capable of rescuing disease phenotypes in glaucomatous RGCs. The results of the current study are the first of its kind to identify neurodegenerative phenotypes in hPSC-derived RGCs from a glaucomatous source, as well as identify a role for astrocytes in the neurodegenerative process through non-cell autonomous mechanisms.

Funding source: NIH Grants R01EY024984 and R21EY031120, Indiana State Department of Health Grants 15779 and 26343

Keywords: astrocyte, neurodegeneration, stem cell

MDD379

APOE4 PERTURBS LIPID HOMEOSTASIS AND INCREASES CELLULAR REQUIREMENT FOR PHOSPHOLIPID SYNTHESIS

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The E4 allele of apolipoprotein E (APOE) increases risk for many diseases including Alzheimer's disease (AD), yet its mechanism of action remains poorly understood. Treatments addressing and ameliorating the risks associated with APOE4 have not yet been developed. APOE is best known to function as a lipid transport protein. Dysregulation of lipids has emerged as a key feature of several neurodegenerative diseases including AD, however it is unclear if and how APOE4 perturbs the intracellular lipid state. We have found that APOE4 disrupts the cellular lipidome in human iPSC-derived astrocytes, resulting in increased unsaturation of fatty acids and accumulation of lipid droplets. Genetic and chemical perturbations of this phenotype resulted in improved function, and suggest that it may be possible to environmentally modify this phenotype. In particular, we examined whether supplementation with a soluble phospholipid precursor is sufficient to restore cellular lipidome and rescue defects in an iPS and mouse model of APOE4. We find that phospholipid precursor supplementation shows robust effects in APOE4 iPS-derived astrocytes, reducing the accumulation of lipid droplets and restoring lipid homeostasis and lipid buffering capacity. We are currently examining dietary supplementation in a mouse model of APOE4 in a 5xFAD background (E4FAD). Our work suggests the simple application of a lipotropic molecule that can restore lipid homeostasis in cells harboring the APOE4 allele.

Funding source: The Neurodegeneration Consortium, the Robert A. and Renee E. Belfer Foundation, the Howard Hughes Medical Institute, and NIH grants RF1 AG048029 and RF1 AG062377 (to L-H T.).

Keywords: APOE, lipid homeostasis, Alzheimer's Disease

MDD466

PLURIPOTENT STEM CELL DERIVED MODELS OF NEUROLOGICAL DISEASES REVEAL EARLY TRANSCRIPTIONAL HETEROGENEITY

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Many neurodegenerative diseases (NDs) develop only later in life, when cells in the nervous system lose their structure or function. In genetic forms of NDs, this late onset phenomenon remains largely unexplained. Analyzing single cell RNA sequencing (scRNA-seq) from Alzheimer's disease (AD) patients, we find increased transcriptional heterogeneity in AD excitatory neurons. We hypothesized that transcriptional heterogeneity precedes ND pathologies. To test this idea experimentally, we used juvenile forms (72Q; 180Q) of Huntington's disease (HD) iPSCs, differentiated them into committed neuronal progenitors, and obtained single cell expression profiles. We show a global increase in gene expression variability in HD. Autophagy genes become more stable, while energy and actin-related genes become more variable in the mutant cells. Knocking-down several differentially-variable genes resulted in increased aggregate formation, a pathology associated with HD. We further validated the increased transcriptional heterogeneity in CHD8+/- cells, a model for autism spectrum disorder. Overall, our results suggest that although NDs develop over time, transcriptional regulation imbalance is present already at very early developmental stages. Therefore, an intervention aimed at this early phenotype may be of high diagnostic value.

Keywords: Huntington's disease, transcriptional heterogeneity, stem cell model

MDD467

TECHNIQUES FOR LABELING AND CHARACTERIZING CALCIUM DYNAMICS IN HUMAN NEURAL PRECURSOR CELLS

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In neuronal circuit development, intracellular calcium plays an essential role in processes like proliferation, differentiation, migration, and synapse formation. While a great deal is known about calcium's role in development from work in model systems, it remains unclear if the same roles for calcium function are preserved in human neuronal development. For instance, in human cortical neurons there are calcium-mediated dendritic action potentials, which have not been observed in homologous rodent neuronal types. These differences arise during development, as both dendritic and spine maturation follow different trajectories as circuits are being formed. Consequently, an open question is what role intracellular calcium plays in the development of human neural precursors (NPCs) and neurons. To investigate this question, the development of methods and tools for studying calcium dynamics in human cells is needed. To address this, we used an in vitro platform for monitoring both structural changes and calcium activity in human neural precursors derived from induced pluripotent stem cells (iPSCs). First, we determined the multiplicity of infection (5 vs 20) and infection time (24 vs 48 hrs) necessary for a Lenti-CMV-GCaMP6f viral vector to stably label Passage-6 (P6) NPCs with a calcium indicator (N= 4 wells). Following this, we recorded spontaneous calcium transients in NPCs (N=7 movies) and found that the calcium transients were spatially and temporally heterogeneous. The temporal patterns of calcium activity in NPCs were highly variable and included brief transients as well as longer periods of sustained calcium activity. Furthermore, there was no spatial structure to the calcium activity as measured by correlations in calcium activity between cells (N=221 cells). Finally, we were able to monitor examples of NPCs where cell structure and calcium activity varied over our recording period, opening up the possibility that this approach will allow us to relate structural changes observed in human NPCs with changes in calcium activity that occur over development. Taken together, our methods will allow for a better understanding of the role of calcium in human NPCs and could provide insights into human neural development.

Funding source: This work is supported by NIH grant MH101634, NSF Career Award 1749772, and the Cystinosis Research Foundation.

Keywords: human neural precursor cells, calcium imaging, in vitro

MDD468

MTOR SIGNALING REGULATES THE MORPHOLOGY AND MIGRATION OF OUTER RADIAL GLIAL CELLS IN THE DEVELOPING HUMAN CORTEX

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Outer radial glial (oRG) cells are a population of neural stem cells prevalent in the developing human cortex that contribute to its cellular diversity and expansion. The mammalian Target of Rapamycin (mTOR) signaling pathway is active in human oRG cells and mTOR mutations are linked to a variety of neurodevelopmental disorders, including autism and Fragile X Syndrome, and cortical malformations such as Focal Cortical Dysplasia and Tuberous Sclerosis. In our studies of mTOR signaling in the developing human cortex we observed specific activation and function in oRG cells, but not in other neural progenitor types, during peak neurogenesis. Using multiple human models of cortical development, including primary human tissue samples and pluripotent stem cell-derived cortical organoids, we identified unique roles for the mTOR signaling pathway in oRG morphology and function. First, dysregulation of mTOR signaling resulted in significant truncation of the oRG primary process and disruption to the glial scaffold. Using dynamic imaging of primary oRGs we additionally observed that changes to morphology had consequences on cell behavior. oRG cells have a characteristic division behavior called mitotic somal translation (MST) where cells 'jump' prior to division. After manipulation of mTOR signaling the MST 'jump' distance significantly decreased and oRG cells migrated significantly smaller distances than paired controls. To better understand the changes to oRG morphology we evaluated differences in cytoskeletal regulation and observed a significant decrease of F-actin levels in oRG cells with dysregulated mTOR. We also identified changes to the actin regulator and Rho GTPase, CDC42, and its targets, Cofilin and Arp2/3, after manipulation of the mTOR signaling pathway in primary human oRG cells. Last, we rescued the truncation of the oRG basal process after mTOR manipulation by using an activator of CDC42, confirming the relationship between mTOR signaling and CDC42-mediated actin regulation. Together, our studies conclude that mTOR signaling regulates oRG cellular morphology and migration behavior during normal human cortex development and may provide insights into how dysregulation contributes to neurodevelopmental disease.

Keywords: Human Cortex, Radial Glia, Organoid

MDD469

USING STEM CELL-DERIVED MICROGLIA AND BRAIN ORGANOIDS TO MODEL THE ROLE OF MICROGLIA IN HUMAN BRAIN DEVELOPMENT AND AUTISM SPECTRUM DISORDER.

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Substantial evidence indicates microglial dysfunction plays a role in autism spectrum disorder (ASD). Microglia are the resident immune cells of the brain derived from the embryonic yolk sac that play a role in immune responses, brain homeostasis, neuronal differentiation, and synapse maintenance. ASD has been associated with increased microglial activation, deficits in synaptic connections, and a reduction in functional connectivity. However, the mechanistic contribution of microglial dysfunction in ASD pathology remains unclear. The histone demethylase KDM6B has been associated with ASD and shown to play a role

in the switch of microglial activation states and is predominantly expressed in microglia. As mice fail to recapitulate hallmark features of human brain development, there is focus on human pluripotent stem cells (hPSCs) to model human specific features. hPSCs can be directed to form 3D structures termed brain organoids that recapitulate transcriptional, structural, and functional properties of the human fetal brain (Watanabe et al. 2017). However, current brain organoid models lack cell types such as microglia. This study aimed to optimize the production of hPSC-derived microglia, demonstrate hPSC-derived microglia are functional, and then integrate these cells into the organoid system to then probe the function of KDM6B in brain development. hPSCs were differentiated to microglia using a modified version of a published protocol (McQuade et al. 2018) and KDM6B activity was modified using the highly specific inhibitor GSK-J4. Expression of microglia marker genes was confirmed by FACS and qPCR analysis. hPSC derived microglia responded to inflammatory stimuli and phagocytosed pHrodo-e. coli bioparticles and showed altered activity with addition of GSK-J4. Lastly, hPSC-derived microglia integrated into brain organoids and survived up to six weeks in culture. Future studies aim to develop assays to measure microglial activity inside the organoids, and create disease specific mutations to better understand disease mechanisms and screen for novel therapeutics.

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Keywords: Autism Spectrum Disorder, Brain Organoid, Microglia

MDD477

SCALED EXPANSION OF HUMAN PLURIPOTENT STEM CELLS (HPSC) IN SUSPENSION CULTURE FOLLOWED BY HIGH-YIELD FACTOR-DRIVEN GENERATION OF INDUCED NEURONS FROM 3D PSC SPHEROIDS

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Culture systems for PSC expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While two-dimensional (2D) feeder-free expansion of PSC is well established, the scale at which PSCs and subsequent PSC-derived cell types can be efficiently manufactured using traditional methods is limited without a significant increase in hands-on time, as well as a potential risk of 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 describe a new system for highly scalable expansion of human hPSC as three-dimensional (3D) spheroids in suspension, followed by rapid conversion of hPSC spheroids to functional neurons by forced expression of a single transcription factor NGN2 (hPSC-iN). Generation of hPSC-iNs is traditionally done in 2D and can produce highly pure neurons from PSC in <10 days. While expansion potential is an important parameter for assessing a fit for purpose medium system (i.e., 2D vs. 3D), another important consideration is compatibility with downstream differentiation protocols. In recent years, 3D aggregate cell culture has been gaining traction as an enhanced culture technique which provides more physiologically relevant cell-cell interactions over the traditional 2D culture protocols. When determining whether to move from 2D to 3D culture environments, a number of considerations need to be made; including the quantity of desired cell type(s) required for downstream applications, compatibility of reagents and experimental endpoints designed for 2D, and importantly, how cells derived using 2D and 3D methodologies compare and contrast to each other. Here, we demonstrate the feasibility of generating hPSC-iN's from expanded hPSC 3D spheroids. Key parameters for both hPSC expansion and hPSC-iN generation are presented and discussed, which include scalability, neuronal yield, and differentiation efficiency. Notably, conversion in 3D resulted in significantly higher yield of iN's compared to standard 2D method. Finally, the impact of 2D vs 3D induction on iN maturation will be presented.

Keywords: 3D, Large-scale, Neural

MDD485

DEVELOPMENT OF IPSC-DERIVED HUMAN NEUROPROGENITOR CELLS (NPCS), NEURONS, AND ASTROCYTES FROM LYMPHOBLASTOID CELL LINES (LCLS) OF GUAM PARKINSON-DEMENTIA COMPLEX (PDC) PATIENTS

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The Parkinsonism-Dementia Complex (PDC) is one phenotype of a disappearing neurodegenerative disease (Guam ALS-PDC) that shows clinical and neuropathological relationships

with amyotrophic lateral sclerosis (ALS), atypical parkinsonism and Alzheimer's disease. ALS-PDC has been linked with both genetic and environmental factors (i.e., certain metal ions, cycad plant neurotoxins), but evidence from human and animal studies is inconclusive. Patient-specific induced pluripotent stem cells (iPSCs) that generate neural progenitor cells (hNPCs), neurons, and astrocytes provide a powerful in vitro system to explore the underlying cause of PDC. We derived iPSC lines from lymphoblastoid cell lines (LCLS) of a PDC-affected Guam Chamorro female patient and an age- and gender-matched healthy Chamorro from Saipan using the protocol developed by Barrett et al. (2014). iPSCs were cultured for a minimum of 10 passages and characterized with established pluripotency markers (Oct4, SSEA-4, TRA-1-60, Sox2) prior to the generation of hNPCs. Protocols for development of both Chamorro iPSC-derived hNPCs and neurons matched those for the NIH-established iPSC line ND50031 (healthy control). An embryoid body protocol (STEMCELL™ Technologies) was used to derive hNPCs from the iPSC lines and the BrainPhys™ hNPC Neuron Kit (STEMCELL™ Technologies) was used to differentiate the hNPCs into neurons. hNPCs from both iPSC lines were immunoprobed with established neuroprogenitor markers (nestin, Sox2), and neurons immunoprobed for markers of maturing neurons (β-tubulin, Map2, doublecortin, synaptophysin). hNPCs and neurons from the ND50031 and Chamorro lines were comparable based on the morphology and expression of neural markers. hNPCs from both Chamorro-derived iPSC lines were also differentiated into astrocytes that expressed markers (GFAP, S-100β, vimentin, GLAST, AQP4) similar to those observed in mature astrocytes from a commercial source (Axol Biosciences). Studies are underway to differentiate the Chamorro-derived hNPCs into neurons and astrocytes to determine their response to metal ions and cycad toxins, the environmental chemicals implicated in the etiology of Guam PDC. These studies should help clarify the role of environmental factor(s) in ALS-PDC and possibly other related neurodegenerative diseases.

Funding source: Funding from NIH 1R41ES026225-01 and an Intramural Grant from Western University of Health Sciences

Keywords: neurodegeneration, Lymphoblastoid cell lines (LCLs), Neural stem cells

MDD494

OPTOGENETIC INSTRUCTION OF SHH INDUCES SYMMETRY BREAKING EVENTS THAT POLARIZED NEURAL STRUCTURES IN-VITRO, A TOOL TO SYSTEMATICALLY STUDY EARLY EVENTS DURING DORSAL-VENTRAL BRAIN PATTERNING

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Embryonic development requires both spatial and time orchestration of fate acquisition and morphogenesis. These events are strictly related to correct positioning along the body axes and their relative distance from morphogens sources. In the forebrain, structures like the neural ridge, cortical hem, and pre-cordal plate secrete in a polarized fashion, morphogens, such as BMPs, WNTs, FGFs and SHH, that are responsible for neural tube development and patterning. We are taking advantage of a light induced gene expression system to control SHH expression in a spatiotemporal regulated fashion. We paired this optogenetic set up with hPSCs in order to systematically obtain Dorsal-Ventral like polarized neural structures in vitro. We are taking advantage of this system to describe and dissect in-vitro the networks involved in the earliest stages of ventral forebrain development.

Keywords: Neural development, Optogenetics, SHH

MDD503

EFFECT OF GENETIC BACKGROUND ON OUTCOME IN A STROKE MODEL USING MOUSE EMBRYONIC STEM CELL-DERIVED CORTICAL NEURONS

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Stroke is the sudden loss of neuronal function, local or generalized, associated with infarction or hemorrhage in the brain, spinal cord, or retina. Stroke ranks second as a cause of mortality and third in disability-adjusted life-years. Human studies to address the genetic cause of clinical variation in stroke outcomes require high statistical power. Limited reports in animal models have demonstrated a strong influence of genetic background on stroke morbidity, though such studies typically only examine a few strains. The Collaborative Cross was designed to generate mice emulating human diversity on a large scale, but it is difficult for many labs to apply this tool because of the number of animals required. However, mouse embryonic stem cells have been derived from genetically diverse strains, and can be used as a faster initial screen to study stroke in ESC-derived neurons. Nonetheless, no protocols were available to differentiate mESC into neurons other than 129 strain. Therefore, we developed a novel protocol to differentiate the eight mESC strains representing the founding strains of the Collaborative Cross. into neurons. We also developed a stroke model in vitro that emulates a mild and a severe stroke, to enable us to identify the most susceptible and resilient strains. Mature neurons were subjected to the stroke model, and phenotypical differences were analyzed. Cell, survival, apoptosis, cytotoxicity, oxidative stress, mitochondrial dysfunction, caspase activation, glial reactivity, and electrical activity were measured. As expected, we found neuronal cells from certain strains were extremely susceptible, and others quite resilient, while the rest of them lay somewhere in between. We are conducting single-cell RNAseq to evaluate what gene expression patterns are associated with these two phenotypes. This in vitro neurogenetics platform will help us understand how genetic diversity impacts the outcome of a stroke and might lead to discovery of new therapeutic approaches.

Funding source: JAX scholar grant

Keywords: stroke, genetic diversity, brain injury

NEW TECHNOLOGIES

MDD391

APPLICATION OF HIGH EFFICIENCY GENOME ENGINEERING PLATFORMS FOR GENERATION OF CONDITIONAL HUMAN IPSC MODELS OF PEDIATRIC SARCOMA

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Pediatric sarcomas are composed of highly heterogeneous subtypes and the two most prevalent pediatric sarcomas are Osteosarcoma (OS) and Ewing sarcoma (ES) which, despite being rare are in desperate need of new therapeutic options because survival rates have remained steady for the last 40 years. OS is thought to originate from osteoblasts and driven primarily by mass genomic instability from loss of tumor suppressor genes such as P53 or overexpression of oncogenes such as c-MYC. Whereas ES does not have a known cell-of-origin and is largely driven by a single translocation between EWSR1 and FLI1 (EWS/FLI1). Despite their contrasting genetics, both OS and ES are dependent on developmental stage and timing of the oncogenic events, something human tumor cell lines are unable to recapitulate. To overcome this, we are taking a new approach to sarcoma modeling through the use of human induced pluripotent stem cells (iPSC) and CRISPR/Cas9-based genome engineering. Although developmentally regulated conditional gene expression systems are well described in mice, installing similarly complex systems in human iPSC remains prohibitively inefficient. To overcome this limitation we have implemented two new approaches for targeted gene insertion in human iPSC; one based upon short-homology directed integration (GeneWeld) that excels at large cargo integration (>4kb), and one based on the use of recombinant adeno-associated virus (rAAV) that is limited by cargo size but affords high efficiency (>80%). We first demonstrate the utility of GeneWeld over traditional HR for the creation of conditional reporter systems by selection-free targeting of a T2A-GFP-P2A-ERT2-CRE-ERT2 construct into three sarcoma related genes, namely OSX, PDGFRα, and PTHLH. In a separate approach we leverage the high efficiency of rAAV-based targeting to install a conditional gene trap cassette within a CRISPR/Cas9-induced EWS/FLI1 translocation, thus allowing developmental control over expression of the fusion transcript. The activity of these systems during iPSC differentiation into mesenchymal stromal cells (MSC) and osteoblasts (OB) is currently being assessed. Future efforts will focus on timing of Cre induced genetic manipulation as an effort to precisely model the initiation and early development of OS and ES.

Keywords: iPSC based models of pediatric sarcomas, CRISPR/Cas9, Site specific homologous recombination

MDD414

A NOVEL, MASSIVELY PARALLEL SYSTEM TO DISSECT DE NOVO NEOPLASTIC TRANSFORMATION USING HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION TO TERATOMAS

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Understanding the molecular mechanisms behind neoplastic transformation and cancer progression are longstanding goals in oncology. Accurate models can help unravel the biology of cancer progression, inform the development of early detection methods, guide clinical practice and choose therapeutic targets. Human models of transformation have historically drawn on knowledge of aberrant genes from clinical samples but have typically been investigated in low throughput and rely on the availability of healthy cells. To overcome these challenges, we present here a novel approach that leverages the teratoma as a multi-lineage source of primary human cells to enable massively parallel in vivo multi-lineage screens. Towards this, first, we introduce a barcoded open reading frame lentiviral overexpression library of 51 key cancer drivers into human pluripotent stem cells which are then injected into immunodeficient mice to form teratomas. Next, we assay the effects of these cancer drivers via single cell RNA-seq, profiling >40,000 teratoma-derived cells spanning >20 cell types across all three germ layers. Via this we multiplex analyze cell type populations, lineage-specific driver enrichment and transcriptomic shifts driven by these oncogenic perturbations. Finally, to enrich tissue-specific drivers and validate tumorigenicity, we further proliferate the tumors by serial dissociation and re-injection for two additional rounds in immunodeficient mice, leading to robust hits for neoplastic transformation demonstrating the hallmarks of malignancy. Given the embryonic nature of teratoma derived cells, we then assess transformation potential in a pediatric context. For instance, we find that c-MYC alone or in tandem with myristoylated AKT1 is a potent driver of proliferation in neural lineages, especially progenitors, and leads to development of an embryonal tumor phenotype similar to medulloblastomas. Taken together, our approach provides a powerful new platform to dissect the cascading genetic and epigenetic changes driving cellular transformation, enabling systematic study of cancer formation and evolution, and we anticipate thus derived new in vivo cancer models will enable the rational design of therapies and the discovery of new therapeutic targets.

Keywords: De novo transformation, Single cell screening, Cancer systems biology

MDD415

A STRESS-FREE STRATEGY TO REPAIR A POINT MUTATION IN PATIENT IPS CELLS

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When people use the patient specific iPS cells as a disease model, the ideal control would be the isogenic line with a repaired point mutation rather than iPS cells from siblings or other healthy subjects. CRISPR-Cas9 technology is widely used for its convenience and high efficiency for such tasks. However, repairing the point mutation in iPS cells is not easily accomplished even with CRISPR-Cas9. Finding a suitable Cas9 cutting site as close as possible to the point mutation to increase efficiency, introducing silent mutations to generate a restriction enzyme site for screening, sequencing the genomic DNA of a large number of clones to ensure no additional changes exist around the cutting sites, are all possible obstacles. Here we report a strategy that will make the Cas9 “knock-in” method both hassle-free and error-free. Instead of choosing the Cas9 recognition site close to the point mutation, we used sites located in the nearest intron. We constructed a donor template with the fragment containing the corrected point mutation as one of the homologous recombination arms flanking a PGK-PuroR cassette. After selection with puromycin, positive clones were identified and further transfected with a CRE vector to eliminate the PGK-PuroR cassette. We successfully repaired the point mutation G2019S of LRRK2 gene in a PD patient iPS line, and will use the line for future neuroprotection studies.

Funding source: RO1-NS0758390-06A1

Keywords: CRISPR-Cas9, isogenic line, iPSC

MDD418

NUCLEOLAR PHENOTYPES DETECTED BY FACS ENABLE A CRISPRI POOLED SCREENING IN HUMAN IPS CELLS

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The Allen Institute for Cell Science is creating a dynamic visual model of human induced pluripotent stem cell (hiPSC) organization. We use CRISPR/Cas9 to fluorescently tag genomic loci whose products localize to specific organelles, enabling the study of stem cell states. hiPSC lines, plasmids, images, and other tools are available at allencell.org. Among the structures tagged, we included the nucleolus, a non-membranous nuclear organelle central for ribosome biogenesis. We double-tagged a hiPSC line for nucleophosmin (NPM1-mTagRFP-T) and fibrillarin (FBL-mEGFP), illuminating the granular and the dense fibrillar nucleolar components, respectively. To systematically investigate genomic perturbations to the nucleolus, we inserted a constitutively expressed CAG-dCas9-KRAB-TagBFP construct into the CLYBL safe harbor of the double-tagged line, creating a platform for CRISPRi screens. Since large-scale arrayed genomic screens using microscopy are expensive, we employed a flow cytometry-based phenotypic sorting strategy to enrich for changes in fluorescence distribution within single cells. The strategy is based on the signal pulse width of NPM1-mTagRFP-T and FBL-mEGFP fluorescent tags. The diffuse nucleolar phenotype displays a wide signal pulse, while the condensed nucleolar phenotype displays a narrow signal pulse. We performed a pooled CRISPRi screen of a nuclear-focused sgRNA library targeting ~2,300 genes. We sorted hiPSCs into diffuse or condensed nucleolar phenotypes based on NPM1-mTagRFP-T signal pulse width and used next-generation sequencing to determine sgRNA enrichment. We detected distinct patterns of perturbed genes in the different phenotypic bins. ENRICHR analysis revealed that genes related to histone biogenesis were enriched in the diffuse phenotype bin, while positive regulators of transcription were enriched in the condensed phenotype bin. We plan to perform a secondary hit validation for nucleolar disruption for a subset of individual genes by flow cytometry and microscopy. In summary, a flow cytometry binning strategy successfully captured phenotypic differences associated with genomic perturbations to the nucleolus, demonstrating that fluorescently-tagged proteins in combination with flow cytometry can shed light into hiPSC nucleolar states.

Keywords: CRIPSRi, Nucleolus, hiPSCs

MDD424

A RETROSPECTIVE ANALYSIS OF CYTOGENETIC CHANGES SEEN IN HUMAN PLURIPOTENT STEM CELL CULTURES

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Over a 10-year span (Jan 2009- Jan 2019), we performed routine G-banded karyotype analysis on 2,665 human embryonic stem cell (hESC) cultures and 12,479 human induced pluripotent stem cell (hiPSC) cultures. Our data reveal several new recurrent abnormalities (including presence of isochromosome 7p, gain of chromosome 14, gain of chromosome Y) as well as confirming earlier findings that the overall rate of acquiring a cytogenetic abnormality was similar between both hESC (23%) and hiPSC (21%) cell types. However, there are clear differences in the rate of particular cytogenetic abnormalities between cell types, such as gains on chromosomes 4, 10, 15, and 16, which are seen in hiPSCs and not hESCs. hESCs displayed fewer types of cytogenetic abnormalities while hiPSCs displayed a wider range of cytogenetic events, possibly due to fewer hESC lines in existence and therefore less genetic diversity in that starting material. In addition, we did not find any statistically significant correlation between increasing passage number and the presence of cytogenetic abnormalities in our data set.

Keywords: pluripotent stem cells, recurrent abnormalities, G-banded karyotype

MDD489

MULTIPLEXED AUTOMATED ASSAYS FOR NEUROTOXICITY EVALUATION USING INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL 3D CELL MODELS

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Cell-based phenotypic assays have become an increasingly attractive alternative to traditional in vitro and in vivo testing in pharmaceutical drug development and toxicological safety assessment. The effectiveness of automated imaging assays combined with the organotypic nature of human induced pluripotent stem cell (iPSC)-derived cells opens new opportunities to employ physiologically relevant in vitro model systems to improve screening for new drugs or potential chemical toxicities. In our studies, we used human iPSC-derived neural cultures to test functional and morphological end points for toxicity evaluation in a multi-parametric assay format.

For neurotoxicity assessment we employed iPSC-based 3D neural platform composed of mature cortical neurons and astrocytes. Neural spheroids generated spontaneous synchronized calcium oscillations. We used fast fluorescence kinetic imaging to measure the patterns and frequencies of the Ca2+ oscillations. Characterization of oscillation profiles was recorded by performed through multi-parametric analysis presenting more than 15 read-outs including frequency, amplitude, characterized primary and secondary peaks, peak width, and waveform irregularities. In addition, cellular and mitochondrial toxicities were assessed by high-content imaging. The assay was optimized for high throughput screening in 384-well plates and displayed a highly consistent performance. We evaluated neuroactive and neurotoxic profiles of more than 120 compounds, including set of neuromodulators with known mechanisms of action, set of various pharmaceutical drugs, sample library of cannabinoids, and a library of neurotoxic chemicals including flame retardants, pesticides, and poly-aromatic hydrocarbons.

Our results show that the iPSC-derived 3D neurospheroid assay platform is a promising biologically-relevant tool to assess the neuroactive and neurotoxic potential of pharmaceutical drugs and environmental toxicants.

Funding source: none

Keywords: compound screening, high content imaging, toxicity assessment

Theme: Tissue Stem Cells and Regeneration

ADIPOSE AND CONNECTIVE TISSUE

TSC104

HUMAN DERMAL AND ADIPOSE TISSUE MESENCHYMAL STROMAL CELLS VERSUS THEIR CORRESPONDING CONDITIONED MEDIA: A COMPARATIVE STUDY OF SKIN WOUND HEALIN

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Extensive wounds can lead to death if not quickly stabilized. Besides, the healing process restores tissue homeostasis but not its functionality. Thus, new strategies in tissue engineering are needed to accelerate wound closure and improve the quality of healing. Previously, we demonstrated that mesenchymal stromal cells (MSCs) derived from the dermis and adipose tissue (DSCs and ASCs, respectively) isolated from human abdominoplasty discards remained stable over time in culture with a low frequency of nuclear alterations and were overall equivalent in promoting in vivo cutaneous wound healing in mice. Considering the paracrine effects of MSCs, here, we comparatively investigated DSCs or ASCs with their corresponding conditioned media (CM; DSC-CM and ASC-CM, respectively) in the healing of skin wounds in vitro and in vivo. Although DSC-CM was more efficient than ASC-CM in promoting wound closure in dermal fibroblast and keratinocyte monolayers by scratch assay and vessel formation using HUVEC cell line, both were superior than the untreated condition. Proteomic analysis of DSC-CM and ASC-CM revealed 95% of similarity including the expression of TSG-6 and VEGF, involved in tissue repair. However, DSC-CM presented higher levels of angiopoietin-1 and decreased expression of TGF-beta, MMP-9, and TIMP-1 in comparison with the ASC-CM, which could explain dissimilar results. MSCs and their CMs were associated with IntegraTM dermal template as a scaffold and used in the treatment of cutaneous wounds in C57BL/6 mice. Clinical and

histopathological evaluations of the healing process (3 to 21 days after surgery) revealed that DSC and ASC treatments were more efficient in promoting skin wound closure than empty Integra (control), reducing inflammation, promoting angiogenesis (vessel number) and remodeling the extracellular matrix (collagen I/III and elastin), thus improving scar quality. DSC-CM and ASC-CM were equivalent although less efficient than DSCs and ASCs concerning the maturation and general quality of healing. Taken together, our findings suggest that MSCs act by additional mechanisms besides paracrine signaling and/or improvements in the current protocols for CM obtaining and/or administration need to be developed to increase the efficiency and applicability of DSC-CM and ASC-CM in cutaneous repair.

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Keywords: Cutaneous repair, Secretome, wound closure

TSC109

UVC RADIATION DECREASES HUMAN MESENCHYMAL STEM CELL-DERIVED SMALL EXTRACELLULAR VESICLES REGENERATIVE PROPERTIES

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Small Extracellular Vesicles (sEV) range from 30 nm to 150 nm that originate from the endosomal pathway. They contain proteins, lipids, and nucleic acids, suggesting a possible role in cellular communication. Perturbing the cellular homeostasis provides a way to study how this transmitted message can change. In our study, we sought to elucidate the effect genomic damage provoked by UV-C may have on the exosomal secretion of human mesenchymal stem cells derived from induced pluripotent stem cells (hiPS-MSC). Here, hiPS-MSC were irradiated with three different UV-C intensities (0,001 J/cm2, 0,01 J/cm2, and 0,1 J/cm2) and the expression of genes involved in the sEV biogenesis pathway was evaluated by RT-qPCR. TSAP6, ALIX, Syntenin, and Rab27a did not show a significant difference in the three conditions. Given these results, sEV secreted by irradiated and not irradiated hiPS-MSC were isolated from conditioned media using a Size Exclusion Chromatography column and the concentration of extracellular vesicles was measured by Tunable Resistive Pulse Sensing. No significant differences were shown between conditions. To assess if there is a change in intercellular communication due to a distinct exosome content, functional assays were performed. A wound-healing assay showed that cells incubated with not irradiated hiPS-MSC derived exosomes had significantly more wound closure than those incubated without exosomes, and the effect is lost when cells were incubated with irradiated hiPS-MSC derived exosomes. To examine a likely change in exosome content, proteomic analysis has been performed. Preliminary results indicate enrichment in proteins involved in cell migration in non-irradiated hiPS-MSC, which is lost under the effect of genomic stress. Taken together, our results suggest that, while genes of the sEV biogenesis pathway and the number of total sEV secreted by irradiated and non-irradiated hiPS-MSC do not show differences, sEV composition could be altered due to genomic damage caused by UV-C.

Keywords: Mesenchymal Stem Cells, Extracellular Vesicles, DNA Damage

TSC415

SIMULTANEOUS 2D AND 3D CULTURE OF SINGLE DONOR PRIMARY ADIPOSE-DERIVED STROMAL CELLS

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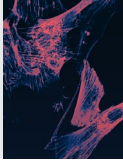
Complex soft tissue regeneration requires simultaneous regeneration of cells within both 2-dimensions (2D) and 3-dimensions (3D). For example, breast regeneration will require simultaneous regeneration of epithelial ductal lining (2D) and mesenchymal adipose (3D). As adipose-derived stromal cells (ASCs) differentiate across germ layer pathways, they may satisfy these simultaneous 2D and 3D structural requirements. Herein we present a technique for the simultaneous culturing of single donor ASCs in both 2D and 3D. This is an observational descriptive study. Human adipose-derived stromal vascular fraction (SVF) was isolated from lipoaspirate using a standardized Kit (Reviticell, Inc.). 300,000 SVF cells were added to 6 mls of ASC-defining media and 2 mls of the media / SVF suspension were added to each of three attachment substrate-treated wells of a 6 well plate and then cultured in humidified 5% CO2. On culture day 5, 1 ml of the supernatant was transferred to the three non-attachment substrate-treated wells to develop spheroids (supernatant spheroids). 1ml of fresh media was added to all of the wells. All cells were then cultured for the next five days and morphologic features were then assessed. Spheroids were compared to ASC spheroids simultaneously developed from SVF with a hanging drop method (300,000 SVF cells: 18 drops, 16,500 SVF cells per drop). ASCs exhibited typical 2D branching patterns and became confluent by day 8, with adherent spheroids developing by day 10. The supernatant developed spheroids by culture day 5, as did the spheroids developed by the hanging drop method. 2D cultured spheroids measured 900 µm, supernatant spheroids 807 µm, and hanging drop spheroids 810 µm. From the initial 300,000 suspended SVF cells, the 2D method yielded 1.5M adherent ASCs and 4.5 spheroids and the respective supernatant yielded 7.5 spheroids, for a total of 12 spheroids. The hanging drop yielded 27 spheroids. This study reveals that single donor ASCs may be cultured simultaneously



in 2D and 3D. Further research will explore the reason that supernatant spheroids only developed once the suspended ASCs were passaged. We suspect cross talk between adhering ASCs and suspended ASCs was occurring. Such crosstalk may be necessary for self-organization of complex soft tissues using ASCs.

Keywords: adipose derived stromal cells, spheroids, tissue regeneration

CARDIAC

TSC113

AMPK-MEDIATED SIRTUIN ACTIVATION REGULATES HUMAN IPSC-DERIVED CARDIOMYOCYTE DIFFERENTIATION

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The application of stem cell-derived cardiomyocytes (CMs) for cardiac disease modeling, drug screening, and potential clinical therapies is highly dependent on production of mature cardiomyocytes. Based on recent studies, metabolic maturation may be the key for improving overall maturation of iPSC-derived CMs; however, the mechanism underlying metabolic maturation is not well described. AMP-activated protein kinase (AMPK) is a master regulator of metabolic activities, and we investigated whether AMPK regulation participates in iPSC-CM maturation. We observed that AMPK phosphorylation at Thr172 increased at day 9 of differentiation but then decreased after day 11 of differentiation. Chemical inhibition of AMPK with Compound C (also known as dorsomorphin) resulted in reduced mean fluorescence intensity of TNNT2 by flow cytometry ($p<0.005$) and significantly reduced mRNA ($p<0.001$) and protein expression of TNNI3 and TNNT2 by qPCR and western analysis, respectively ($p<0.005$). Moreover, sustained activation of AMPK using AICAR from day 9-14 increased fluorescence intensity of TNNT2 by flow cytometry ($p<0.005$) as well as TNNI3 and TNNT2 by both mRNA and protein levels. We characterized the effect of AMPK activation on sarcomere length using TNNT2 and a-actinin antibodies and AICAR treated cells and showed sarcomere length increased from 1.59 (±0.16) to 2.31 (±0.17) µm ($p<0.001$). AMPK can interact with the SIRTUIN family, NAD+ class 3 histone deacetylases that can act as metabolic sensors. We observed that AMPK activation enhances SIRTUIN activation by increasing cellular NAD+ levels post differentiation in day 9-16 significantly ($p<0.002$). We also found that AICAR decreased acetylation of Histone3 at lysine 9 and 56 and histone 4 at lysine 16 (which are the known target sites for nuclear localized SIRTUIN (SIRT1 and SIRT6)), suggesting that AMPK activation enhances SIRTUIN activity. We used DAPI intensity as hallmark for heterochromatin status and found that DAPI intensity was significantly higher in AICAR-treated versus control cells ($P<0.001$) by imaging and flow cytometry. Thus, AMPK-induced SIRTUIN-mediated deacetylation of histone proteins may regulate chromatin accessibility and condensation and consequently enhance differentiation.

Keywords: Cardiomyocytes, AMPK, SIRTUIN

TSC126

DIFFUSION TENSOR MRI REVEALS MYOCARDIAL ARCHITECTURE AFTER STEM CELL-DERIVED CARDIOMYOCYTE TRANSPLANTATION IN GUINEA PIG MODEL OF INFARCTION

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Heart regeneration using human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have presented as a novel mechanism for cardiac repair after myocardial infarction (MI). In vivo response assessment, therapy efficacy and the associated mechanisms of repair are challenging to probe and warrant the development of advanced imaging methods. The objective of our study was to develop a comprehensive high-resolution Magnetic Resonance Imaging (MRI) protocol to localize and characterize structural changes after hPSC-CMs transplantation in an ex vivo guinea pig model of MI. Guinea pig hearts were cryoablated to induce infarction and scar regions were injected either with hPSC-CMs (n = 4) or vehicle (n=2) 10 days later. Hearts were harvested 28 days post-transplantation and perfusion fixed. Ex vivo MRI was performed on a 7T Bruker MRI system. We utilized 3D Diffusion Tensor Imaging (DTI, resolution 0.3x0.3x0.3 mm) to quantify myocardial structure parameters such as fractional anisotropy (FA, directionality) and mean diffusivity (MD, water mobility). Contrast enhancement (CE, resolution = 0.2x0.2x1 mm) was used to distinguish infarcted from healthy tissue via accumulation of the gadolinium agent. A double dose of gadolinium was injected pre-sacrifice to ensure contrast accumulation during ex vivo imaging. Cell therapy subjects demonstrated a higher level of tissue anisotropy FA within the engraftment (0.23 +/- 0.02) compared to the infarction (0.12 +/- 0.07, $p < 0.0001$), and approached that of remote tissue (0.31 +/- 0.09, $p < 0.0001$). Similarly, MD was not significantly different in graft versus remote tissue (1143 µm2s-1 +/- 230 vs. 1140 µm2s-1 +/- 180, respectively) and was improved compared to infarct (1580 µm2s-1 +/- 240, $p<0.0002$). hPSC-CM engraftment was in agreement between CE-MRI and histology (8.6% vs.10.1% relative to infarct). Overall, the DTI measures within graft tissue were indicative of greater structural organization within the infarct zone (close to remote tissue) due to the presence of hPSC-CMs. Our findings show that MRI serves to be a valuable non-invasive method for graft localization and myocardial architecture characterization and thus, evaluate therapy efficacy after MI. Future studies can incorporate in vivo MRI sequences to perform serial response to therapy.

Keywords: Magnetic Resonance Imaging, Pluripotent stem cells, Cellular therapy

TSC410

PPAR DELTA SIGNALING INDUCES METABOLIC MATURATION IN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES THROUGH ENHANCED FATTY ACID OXIDATION

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Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) provide a powerful approach for regenerative therapies, drug discovery and disease modeling. One caveat, however, is that PSC-CMs remain functionally and structurally immature, resembling first trimester fetal heart cells. In vivo heart development involves a postnatal metabolic switch from anaerobic glycolysis in the fetal heart to fatty acid oxidation (FAO) in the adult heart, and this switch coincides with an increase in PPAR signaling activity. Here we show that inducing PPAR signaling in vitro in PSC-CMs induces the metabolic switch observed in vivo, resulting in enhanced PSC-CM maturation. PPAR delta (PPARd) signaling activation resulted in PSC-CM structural maturation through improved myofibril organization, reduced circularity standard deviation and increased binucleation. However, no difference in contractility was observed in either 2D or 3D culture formats. Interestingly, PPARd activation upregulated genes involved in long chain fatty acid (LCFA) metabolism and inhibition of glycolysis-dependent oxidative phosphorylation. Accordingly, PPARd-activated PSC-CMs showed increased expression of LCFA membrane transporters (CD36) and FAO enzymes (ACADVL), resulting in increased LCFA uptake and processing. PPARd induction also increased mitochondrial content, mitochondrial size, maximal respiration rates and FAO flux rates, confirming FAO induction in PSC-CMs. PPARd-mediated metabolic maturation can be induced in various culture protocols, including 3D Engineered Heart Tissues or after metabolic selection, indicating broad reliability for this mechanism. In summary, we identify a role for PPARd signaling activation in enhancing PSC-CM metabolic maturation by inducing a metabolic switch to FAO, with no effect on contractility, thus uncoupling a metabolic prerequisite from contractile maturation during early heart development.

Funding source: NYSTEM-C32561GG

Keywords: PPARdelta signaling, Metabolic maturation, Enhanced Fatty Acid Oxidation

EARLY EMBRYO

TSC134

A NEW APPROACH TO AN OLD PROBLEM: ILLUMINATING EARLY HUMAN MORPHOGENESIS USING OPTOGENETICS

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In comparison to fully differentiated somatic cells, stem cells have an enormous range of cell fates which they can potentially access. This enormous decision space underlies both the promise and difficulty of utilizing stem cells in a therapeutic context. Thus, understanding and ultimately controlling, how stem cells undergo cell fate transitions promises to herald the therapeutic potential of Regenerative Medicine. Here we employed photo-switchable proteins from plants to control the signaling flux of critical developmental pathways, including the Wnt and BMP signaling pathways, to dissect the early cell fate decision underlying human gastrulation. By virtue of our ability to precisely pattern light in both space and time, we are using these novel tools to both dissect how signaling dynamics are decoded into cell fates and how spatial patterns of signaling activity organize the collective behavior of stem cells during gastrulation. In addition, we have developed a user-friendly set of live-cell, fluorescent reporters of cell state to observe these decisions in real-time. In the future, we hope to connect these cell state outputs with our optogenetic inputs to develop an all-optical feedback-controlled platform for guiding stem cell fate choices.

Keywords: Morphogenesis, Optogenetics, Human development

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

TSC137

WNT SIGNALING IN HUMAN INDUCED PLURIPOTENT STEM CELLS AND DIFFERENTIATED HEPATOCYTE-LIKE CELLS

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Wnts are secreted glycoproteins that activate an intracellular signaling cascade with intracellular free calcium (Ca^{2+}(i)) and β-catenin as major transducers. The Wnt pathway is important for stem cell self-renewal and differentiation. We have developed a Wnt signaling readout using a ratiometric assay (with Ca^{2+} dyes; Fluo4 and FuraRed) and confocal microscopy to measure Wnt-induced Ca^{2+}(i) release in real time. By using this assay, we reported Wnt-induced Ca^{2+}(i) release by multiple Wnt ligands in various mammalian cells and proposed a convergent Wnt model, indicating synergy between Ca^{2+}(i) release and β-catenin stabilisation. We asked the question whether Wnt-induced Ca^{2+}(i) release occurs in human induced pluripotent stem cells (hiPSCs) and whether levels of Wnt signaling alter as hiPSCs differentiate? To address this question, we used a human iPS cell line (CGT-RCiB-10) derived from peripheral blood mononuclear cells and hiPSC-derived hepatocytes (hiHeps) at different stages of hepatic differentiation and measured Ca^{2+}(i) release in response to Wnt9B. We observed that peripheral and medial cells of the hiPSC colonies differ in their basal Ca^{2+}(i) levels. Addition of Wnt9B (400 ng/mL) activated Ca^{2+}(i) release in hiPSC colonies; the time kinetics of the Ca^{2+}(i) waveform was segmented into rise, dwell and fall time (19.29 ± 1.27 s, 20.09 ± 1.23 s and 36.03 ± 1.91 s respectively, n=130). Interestingly, Wnt-induced Ca^{2+}(i) release in hiPSCs caused a change in the surface area of these cells that appeared to be inversely proportional to Ca^{2+}(i) release. Initially, the surface area of the cells reduced from 210.04 ± 13.82 µm to 197.83 ± 13.94 µm with about 2-fold rise in the amplitude of Ca^{2+}(i) waveform (n=20). We also observed a difference in Wnt-induced Ca^{2+}(i) release as hiPSCs progress through differentiation with significantly ($P < 0.0001$, Mann-Whitney U test) reduced dwell time for the Ca^{2+}(i) in hiHeps (13.20 ± 1.62 s, n=56) when compared to hiPSCs (20.09 ± 1.23 s, n=130). To our knowledge, this is the first study to report Wnt-induced Ca^{2+}(i) release in hiPSCs and differentiated hiHeps.

Funding source: This work is sponsored by Punjab Educational Endowment Fund (PEEF), Government of Pakistan via Chief Minister Merit Scholarship 2016 awarded to B.Kanwal.

Keywords: Induced pluripotent stem cells, iPSC-derived hepatocytes, Wnt/Ca^{2+} signaling

TSC139

STEM CELL DERIVED FUNCTIONAL PANCREATIC ISLETS FOR TREATMENT OF TYPE 1 DIABETES

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Type 1 Diabetes is a rapidly expanding endocrine disease affecting millions of people worldwide. Type 1 diabetes can be treated with transplantation of cadaveric pancreatic islets, but this approach is limited by shortage of donor islets and low viability of islets after transplantation. Transplantation of human pluripotent stem cell derived pancreatic islets (SC-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. Here in this study, we generated islets-like aggregates from hESCs and then incorporated them into 3D porous scaffolds made of recombinant spider silk protein. The aggregates containing scaffolds were injected into ACE of a large-eyed pre-clinical animal model. Our results show that the injected aggregates were integrated onto the iris tissue and became vascularized one month after transplantation. The aggregates closely resemble human islets in both size and morphology, and contain mainly mono-hormonal endocrine cells expressing either insulin, glucagon or somatostatin. Because successful stem cell therapy for type 1 diabetes relies on production of clinical-grade functional islets, we optimized protocol for in vitro differentiation into functional SC-islets. The SC-islets were able to increase insulin secretion by 10-folds in response to high glucose stimulation in vitro. The functionality of the SC-islets was also evaluated by transplantation into ACE of diabetic animal model. Together these results indicate that transplantation of functional SC-islets into ACE could be the ideal solution for treatment of type 1 diabetes in the future.

Funding source: This work is supported by Sweden's innovation agency Vinnova.

Keywords: Stem cell derived functional beta cells, Transplantation therapy for Type 1 diabetes, Anterior chamber of eye

TSC148

ENHANCED IN VITRO HIPSC-DERIVED HEPATOCYTE-LIKE CELLS MATURATION

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The liver is a crucial organ regarding metabolic, immune and homeostatic regulation. Chronic and acute liver diseases, genetically determined or acquired, account for approximately 2 million deaths per year. The only therapeutic options to severe liver diseases are partial or total liver transplantation. Hepatic tissue engineering, combined with human induced pluripotent stem cells (hiPSCs) technology, offers an alternative to traditional therapeutic procedures. Hepatocytes differentiation protocols, nevertheless, result in hepatocytes with fetal phenotype, hampering the comprehension about adult liver cells mechanisms of regeneration, potential tissue engineering approaches, in vitro disease modeling and drug development applications. Here we show that the application of a formulation composed of cell death inducer molecules in hiPSC-derived hepatocytes (HLCs) for 24 h contributed for induction of hepatic maturation, which was confirmed through gene (RT-qPCR) and protein (immunofluorescence and flow cytometry) expression analyses. Mature hepatocyte differentiation markers, such as ALB, G6PC and TDO2, were overexpressed in the treated group, which also secreted significantly more albumin in culture. Finally, KRT7 and KRT19, hepatic biliary duct cells (i.e. cholangiocytes) related genes showed reduced expression. This new formulation may enhance current in vitro liver development assays, increase the accuracy of liver diseases modeling and of hepatotoxicity assays and improve current stem cell-based bio-artificial liver engineering.

Funding source: This study is supported by grants from FAPESP (2019/19380-4)

Keywords: hiPSC, Hepatocyte, Maturation

TSC153

DECELLULARIZED URETERAL SCAFFOLD (PIG URETER) LOADED WITH ADIPOSE MESENCHYMAL STEM CELLS (SHEEP ADIPOSE TISSUE) PROMOTES URETER REGENERATION IN A XENOTRANSPLANT MODEL (SHEEP URETER)

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Ureteral injuries account for about 3% of urogenital traumas. Decellularized tissues have emerged as an alternative to ureteral repair, but the available protocols have failed in functional host integration. The aim of this study was to develop and validate in vivo a ureteral graft from a porcine ureteric scaffold, seeded with adipose mesenchymal stem cells (aMSCs). Ureteral samples from healthy pigs were used. Tissues were decellularized using Triton X-100 1% and SDS 0.1% under continuous intraluminal perfusion in a bioreactor designed by our group. Decellularization and structural integrity were characterized by histological analysis, β-actin western blot, residual DNA content, and scanning electron microscopy. Extracellular matrix (EMC) proteins and VEGF were studied by immunohistochemistry. Furthermore, 41 growth factors were analyzed by protein array. Recellularization was performed with aMSCs extracted from sheep adipose tissue, and it was evaluated histologically. Ureteral grafts were implanted into seven host sheep, and the functionality was analyzed by ureterography. At ten weeks, the implant was extracted, and integration was evaluated by histologically. Decellularized grafts showed high structural integrity and low DNA contamination and β-actin levels. EMC proteins and VEGF were observed. After cellularization with aMSC, the grafts showed the presence of groups of cells, and 32 growth factors were differentially detected. Sheep implants showed peristaltic movements and the regeneration of all ureteral tissue components. These results indicate that the protocol used is successful in achieving a decellularized ureter with an intact native architecture and recellularization with aMSCs. Also, the porcine ureteral scaffold seeded with aMSCs showed a high functional integration with the host tissue. Therefore, this type of graft may be a suitable alternative to ureteral regeneration.

Funding source: Fundación Científica Felipe Fiorellino, Ciudad Autónoma de Buenos Aires, Grant number: intramural funding.

Keywords: Ureter, Decellularized scaffold, Xenotransplant

EPITHELIAL

TSC160

CIRCADIAN REGULATION OF REGENERATION IN THE INTESTINE OF DROSOPHILA MELANOGASTER

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The circadian clock is a 24-hour molecular transcription-translation feedback loop that drives rhythms in behaviours and physiological processes. Following injury, the circadian clock can regulate the timing of mitosis in the intestine of Drosophila melanogaster. The intestine of Drosophila melanogaster is a pseudostratified epithelium consisting of intestinal stem cells that divide into progenitors which differentiate into absorptive and secretory cells. The wide variety of genetic tools, and its striking similarity to the mammalian gut make the Drosophila intestine a useful model for biomedical research. We have previously shown that the circadian clock is active in the intestinal stem cells, progenitors and absorptive cells and that it is active during regeneration. However, the mechanism for circadian regulation of stem cell activity remains unknown. We hypothesize that, in stem cells, the circadian clock may regulate differentiation and proliferation thereby gating regeneration to specific times of day. Jak/STAT signaling, one of the key pathways involved in intestinal stem cell differentiation and proliferation in the Drosophila intestine, is conserved in mammals and it is involved in a number of diseases including inflammatory bowel disease and cancer. We sought to uncover if Jak/STAT signaling shows a circadian rhythm and if there is a time-of-day difference in regenerative response. Using a reporter of Jak/STAT activity we show that clock mutants have altered Jak/STAT signaling and that there is a time-of-day difference in Jak/STAT activity when irradiated. We tested a candidate Jak/STAT regulator, IPK2 that we have previously shown to be regulated by the circadian clock, and found that it increases Jak/STAT activity. Furthermore, clonal loss of IPK2 in stem cells results in a failure of maintenance. Together these results suggest that the circadian clock may play a role in regulating a circadian rhythm in regenerative response of the intestine through IPK2.

Funding source: NSERC, CFI, Government of Ontario, University of Windsor

Keywords: Intestine, Circadian Clock, Jak/STAT

TSC161

THE CLOCK GENE BMAL1 AFFECTS REGENERATION OF INTESTINAL EPITHELIAL CELLS IN MICE WITH INFLAMMATORY BOWEL DISEASE

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The circadian clock is a highly conserved molecular system that drives the oscillation of biological rhythms with a 24 hour period. These rhythms are achieved through a core clock mechanism involving CLOCK and BMAL1 transcription factors that positively regulate the expression of CRY1/2 and PER1/2/3, their negative regulators. Disruption of the circadian clock has been shown to cause an increased risk of Inflammatory Bowel Disease (IBD). Patients with IBD experience chronic inflammation along with impaired regeneration of intestinal epithelial cells. Inflammation and regeneration are closely linked. Recently, inflammatory factors have been shown to activate regenerative pathways (ie. YAP, Notch) using IBD models in mice. We tested the role of the clock in IBD with an emphasis on the epithelial regenerative response using BMAL1+/+ (wild type) and BMAL1-/- (null mutant) mice. Dextran Sulfate Sodium (DSS) was applied to induce acute colitis in mice, acting as an effective model for ulcerative colitis: one of the two categories of IBD. We observe a drastic decrease in the survival of mice lacking functional BMAL1 that were treated with 4% DSS over 7 days. Disease activity and cytokine analyses reveal time-dependent severity in inflammatory response that is worse in BMAL1 mutants. To test the circadian rhythm of IBD, we performed a 24 hour analysis comparing epithelial cell proliferation and cell death, and inflammation in colon tissue. Our results indicate a significant rhythmic expression of mitosis throughout the day in BMAL1+/+ mice while mitosis in BMAL1-/- mice is arrhythmic and at lower levels. Based on these results, poor regeneration during IBD is in part attributed to decreased and arrhythmic regeneration. To determine the relative contribution of inflammation and epithelial regeneration in IBD severity, a conditional intestinal knockout of BMAL1 in these tissues will be examined. These data will provide insight into how the core clock affects the inflammatory and regenerative abilities of intestinal epithelial cells as well as surrounding blood cells.

Funding source: University of Windsor, Government of Ontario, CIHR, Crohn's and Colitis Canada, NSERC

Keywords: Intestine, Circadian Clock, Regeneration

TSC171

EXTRACELLULAR VESICLES DERIVED FROM AMNIOTIC FLUID STEM CELLS ATTENUATE INTESTINAL EPITHELIAL INJURY OF EXPERIMENTAL NECROTIZING ENTEROCOLITIS

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Necrotizing enterocolitis (NEC) is one of the most devastating intestinal diseases in preterm neonates, with a high incidence and mortality rate. We have shown that the Wnt/β-catenin pathway and intestinal regeneration were impaired in NEC. Amniotic fluid stem cells (AFSC) can improve the intestinal injury in experimental NEC but their administration is potentially problematic and consequently we focused on their extracellular vesicles (EV). We hypothesize that EV derived from AFSC attenuate the intestinal injury of NEC by activating the Wnt pathway. Experimental NEC was induced from post-natal day (P) 5-9 by gavage feeding of hyperosmolar formula four times daily, hypoxia for 10 minutes prior to feeds, and lipopolysaccharide administration. AFSC administration rescued intestinal stem cell (ISC) impairment and restored epithelial regeneration in experimental NEC, however, Wnt-deficient AFSC were unable to. Organoids derived from NEC intestine were smaller in size and had more budding due to lack of Wnt signaling. Supplementing the culture media with Wnt reversed these changes. Administration of EV derived from AFSC induced similar morphological changes in organoids to those exposed to Wnt. Intestinal proliferation and intestinal stem cell were rescued by EV administration either before or after experimental NEC induction. However, the beneficial effects of EV administration were only achieved if EV were administered during the progression of NEC injury. EV derived from AFSC exert significant beneficial effects in NEC by stimulating the Wnt pathway. Most importantly for translational clinical application, the intestinal injury observed in NEC is attenuated by EV administration as a rescue strategy but not for prevention of the disease.

Funding source: This project was supported by CIHR Foundation Grant 353857 and the Robert M. Filler Chair from The Hospital for Sick Children.

Keywords: intestinal epithelial regeneration, necrotizing enterocolitis, extracellular vesicles

TSC186

USING A THREE-DIMENSIONAL GELATIN MATRIX TO SUPPORT TYPE II ALVEOLAR EPITHELIAL CELL DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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The type II alveolar epithelial cell (AECII) has been recognized as the alveolar stem cell, capable of proliferating and transdifferentiating into terminal type I alveolar epithelial cells (AECIs) that function in gas-exchange. This renders AECIIs as an attractive candidate for use in exogenous cell therapy for lung injury. However, the isolation of primary AECIIs for alveolar regeneration is precluded by technical challenges, including slow turnover and the inability to maintain the AECII phenotype ex vivo. Induced pluripotent stem cells (iPSCs) can be differentiated into lung progenitor cells and subsequently into AECIIs in vitro with the step-wise addition of developmentally-relevant signaling factors in culture. We differentiated iPSCs into lung bud organoids (LBOs) that were then inoculated onto a three-dimensional gelatin matrix to facilitate further AECII differentiation. We observed that the inoculated cells remained viable and proliferated on the matrix. Additionally, cells acquired expression of both epithelial cell adhesion molecule and the AECII-specific marker, pro-surfactant protein C, indicating that the gelatin matrix supports the differentiation of lung progenitor cells into AECIIs. Furthermore, when cells grown on the matrix were sub-cultured onto two-dimensional tissue culture plates, they exhibited a squamous AECI-like morphology. This phenotype was not observed in cells that were differentiated in the absence of the gelatin matrix, which remained cuboidal in morphology after transitioning to 2D culture. In depth characterization of these putative AECIs is currently underway. Taken together, our results point to the feasibility of supporting AECII differentiation with this gelatin matrix.

Funding source: Canadian Institutes of Health Research (CIHR) grants awarded to Haibo Zhang

Keywords: Induced pluripotent stem cells, Type II alveolar epithelial cells, cell therapy

EYE AND RETINA

TSC194

3D-PRINTED HUMAN ABCB5-POSITIVE STEM CELLS FOR THE TREATMENT OF CORNEAL BLINDNESS

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Limbal stem cells (LSC) residing at the edge of the cornea continually repopulate the corneal epithelium. Limbal stem cell deficiency (LSCD) occurs when these LSC are damaged (due to infection, burns, or other trauma) or missing (due to genetic conditions). Patients with LSCD are unable to regenerate the corneal epithelium, resulting in blindness due to invasion of the conjunctiva and concomitant neovascularization. For patients with unilateral LSCD, transplantation of autologous limbal tissue or ex vivo expanded limbal cells from the unaffected eye can be successfully used to treat LSCD. However, patients with total, bilateral LSCD have no source of autologous LSC and must rely on allograft transplantation, which is associated with poor outcomes. Such patients would greatly benefit from an alternative autologous source of stem cells for ocular surface reconstruction. We previously demonstrated that human ABCB5+ LSC were capable of restoration of the corneal epithelium in an immunodeficient mouse model of LSCD. We found that ABCB5 is also expressed by dermal stem cells (DSC)and hypothesized that these ABCB5+ DSC could provide an alternative source of stem cells for corneal epithelial regeneration. We tested this by subjecting ABCB5+ DSC to corneal differentiation conditions in vitro and saw significant induction of the corneal markers PAX6 and KRT12. Transplantation of ABCB5+ DSC to immunodeficient recipient mice with mechanically induced LSCD resulted in clear corneas. We are currently working on a method to 3D print ABCB5+ DSC in fibrin gel using our custom-designed droplet-based bioprinter to increase the efficiency of turning dermal stem cells into limbal stem cells. Our results reveal the potential of unmodified ABCB5+ DSC for corneal regeneration in the setting of experimental LSCD and set the stage for the use of ABCB5+ DSC as an alternative autologous source of stem cells to regenerate the corneal epithelium in patients with bilateral LSCD.

Funding source: Grant support: HSCI, NIH/NEI, NIH/NCI, NIH/NINDS, NIH/NIBIB, Department of Veteran Affairs, Organ Design and Engineering (ODET) T32 EB016652-05

Keywords: Limbal stem cells, 3D printing, ABCB5

TSC202

INVESTIGATING THE ROLE OF PTEN IN REGULATING MURINE MULLER GLIA REGENERATION IN THE RETINA

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While recent advances have been made in stem cell transplantation for vision repair in humans, their effectiveness is limited, and these technologies have not yet been translated to the clinic. An attractive alternative strategy is to activate endogenous stem cells for self-repair. Müller glia are endogenous glial cells in the retina that act as stem cells in teleost fish and amphibians; wherein they can differentiate and recover any lost retinal cell types in response to injury or degeneration, essentially self-healing a damaged eye. In contrast, mammalian Müller glia have lost this ability to self-heal and instead activate a negative cytotoxic process known as reactive gliosis, that serves to limit the spread of injury. In teleost fish and amphibians, where Müller glia have a proliferative response, the first step in Müller glia activation is delamination of glial cell bodies into the outer nuclear layer, where photoreceptors reside. This initiating event for Müller glia regeneration is poorly understood at the molecular level. Phosphatase and tensin homolog (Pten) deletion or knockdown has emerged as a powerful strategy to promote axonal regeneration in the central nervous system, including in the retina, but a potential role in cellular regeneration has yet to be reported. Using a tamoxifen inducible Müller glia specific Cre driver, Sox9CreERT2, we deleted exons 4 and 5 in Pten fl/fl; Rosa-zsGreen mice and analyzed adult retinas at 1, 3, 7- and 21-days post tamoxifen induction. We traced the lineage of Müller glia using the zsGreen reporter and showed that Müller glia cell bodies delaminate into the outer nuclear layer where photoreceptors reside, which is the initiating event of regeneration in teleost fish and amphibians. We are in the process of conducting bulk RNA-seq analysis of the transcriptomes of Pten conditional knock-out retinas to identify differentially expressed genes that may provide molecular insights into how Pten regulates this first step of Muller glia regeneration. By identifying these latent regulators, we will be better positioned to design gene therapies that could be used to activate Müller glia for regenerative purposes in the clinic

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Keywords: Retinal Stem Cells, Müller glia, Pten

TSC203

MAGNETIC RESOLUTION OF MOUSE RETINAL STEM CELLS REVEALS QUIESCENT VERSUS PRIMED TO PROLIFERATE POPULATIONS

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The adult mammalian eye contains quiescent stem cells located within the pigmented ciliary epithelium (1:500 cells) with the potential to make all the cell types in the retina, however they do not regenerate damaged adult tissue in vivo. The lack of validated markers or strategies to prospectively identify these rare cells within the retina in order to target them for potential therapeutic purposes has remained a significant challenge. We used a microfluidic platform that uses magnetic resolution to facilitate efficient sorting to enrich and capture retinal stem cells (RSCs) using cell surface biomarkers to enrich the stem cells to approximately 1:4. We combined the microfluidic platform with single-cell RNA sequencing and identified 5 clusters of cells in a tSNE plot of which 2 contain RSCs. Through this analysis we discovered 4 novel cell surface markers that when combined in the microfluidic platform yielded an even higher 1:2 enrichment of RSCs. We also analyzed the transcription factors that are differentially expressed in the 2 RSC clusters which based on gene expression may indicate that Cluster 2 RSCs may be in a super quiescent state, while Cluster 4 RSCs may be in a more primed to proliferate state. In support of this hypothesis, sorted Cluster 4 cells had a 3 times greater probability of forming clonal RSC spheres in vitro than Cluster 2 cells. The Creb1 gene is highly expressed in the two RSC clusters. Indeed, we found that although homozygous Creb1 hypomorphic mice have normal size eyes, they have 50% reduction in the absolute number of adult RSCs as assayed by clonal sphere formation in vitro. Knock-down of Creb1 in vitro using siRNAs in wildtype primary ciliary epithelial cells was able to recapitulate this 50% decrease in RSC number with a significant decrease in their ability to self-renew. Bioinformatic re-analysis of only the two RSCs clusters revealed the appearance of a third significant separate sub-cluster of RSCs, which may be a transitional population between the more quiescent and the poised to proliferate RSCs. This may indicate that all three RSC clusters may be a single population of RSCs, but in three different states – a super quiescent state, a second transitional state, and a primed to proliferate state.

Keywords: enrichment, ciliary epithelium, retinal stem cell

HEMATOPOIETIC SYSTEM

TSC209

A CD45-TARGETED ANTIBODY DRUG CONJUGATE ENABLES ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT) AS A SINGLE AGENT IN MICE

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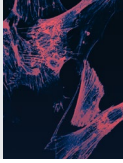
Allo-HSCT is a potentially curative treatment for malignant and non-malignant blood disorders. However, current conditioning regimens are associated with significant conditioning-related toxicities. As a result, many patients are not able to undergo this potentially curative therapy. To pursue a safer alternative conditioning strategy, we developed a novel antibody drug conjugate (ADC) targeting mouse CD45, engineered for rapid clearance, to provide a translatable approach that may be fully myeloablative as a single agent. We used a tool ADC to evaluate the feasibility of conditioning mice for autologous and



allogeneic HSCT. In a congenic model of autologous transplant, we established that a single dose of CD45-ADC (3 mg/kg) was fully myeloablative and enabled complete donor chimerism. We further evaluated this tool CD45-ADC in two murine models of allo-HSCT. In a minor histocompatibility antigen mismatch model of allo-HSCT, a single dose of CD45-ADC (3 mg/kg) enabled full multilineage donor chimerism (>95% at 16 weeks) in DBA/2 (CD45.2+, H-2d) mice) following transplant with 2x107 bone marrow (BM) cells from Balb/c congenic mice (H-2d, CD45.1+). The donor chimerism was comparable to myeloablative TBI (9 Gy). A matched dose of isotype-ADC (Iso-ADC) had no effect. In a fully mismatched model, a single dose of CD45-ADC (3 mg/kg) enabled partial, transient chimerism (~27%) at 4 weeks in C57BL/6 mice (H-2b, CD45.2+) transplanted with 2 x107 BM cells from Balb/c CD45.1 mice (H-2d, CD45.1+). In the same model, CD45-ADC (3 mg/kg) supplemented with TBI (2 Gy) enabled durable, multilineage donor chimerism >95% over 16 weeks, comparable to 9 Gy TBI. A matched dose of Iso-ADC had no effect. These data demonstrate that single agent CD45-ADC enables transplant in a mouse model of matched unrelated donor transplant and CD45-ADC supplemented with low dose TBI enables full allo-transplant. This targeted and translatable approach for safer conditioning could improve the risk-benefit profile for allogenic and haploidentical HSCT and may allow the curative potential of HSCT to more patient.

Keywords: HSCT, CD45, ADC

TSC217

TAILORING FOR DEVELOPMENT: A TRANSIENT WAVE OF HSC-DERIVED INNATE LYMPHOID PROGENITORS COLONIZES AND SHAPES THE MOUSE EMBRYO THYMIC ARCHITECTURE.

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Successive waves of hematopoietic progenitors from fetus to adults generate different immune cell subtypes with distinct function and tissue homing capacity. In line with this view, the perinatal thymus is colonized by two distinct waves of thymic settling progenitors (TSP) where they contribute to thymic organogenesis and homeostasis. However, it has remained unclear what is the developmental program by which embryonic TSP waves are produced and what is the relative contribution of each wave to shaping the embryonic thymic architecture. We found that the first wave TSP are generated through a unique developmental program initiated in hematopoietic stem cells (HSCs) derived lympho-myeloid primed progenitors that transiently exist in the fetal liver between embryonic day (E) 11.5 and E15.5. These progenitors lose myeloid and B cell potential and further migrate to the thymus to give rise to embryonic T cell receptor-invariant T cells like Vγ5 γδ T cells beside differentiating into innate lymphoid cells (ILC) with bias towards the lymphoid tissue inducer (LTi) lineage. Moreover, transcripts that define the molecular signature of ILC, and more precisely the LTi lineage, are upregulated exclusively in the first wave TSP on the single-cell level. At later developmental stages, adaptive T lymphocytes are derived from lympho-myeloid progenitors that colonize the thymus and retain B-cell, ILC and myeloid potential. Temporally controlled TSP removal reveals the tissue-inducing potency of the first wave TSP progeny represented in their indispensable role in the differentiation of autoimmune regulator (Aire)-expressing medullary thymic epithelial cells (mTEC) during embryogenesis and shortly after birth. The crosstalk between the first wave TSP progeny and Aire+ mTEC prior to αβ T-cell positive selection, ensures that they are in place to induce tolerance in the nascent αβ T cell repertoire. Collectively, our work unravels the distinctions of early versus late thymopoiesis and the dominance of innate development gene programs during embryonic thymopoiesis. This sequence of events highlights the impact of developmental timing on the emergence of different lymphoid subsets tailored to the distinct developmental age of the animal.

Keywords: Thymus, Hematopoietic Stem Cells, Lymphopoiesis

TSC219

ADULT HEMATOPOIETIC STEM CELL (HSC) CLONALITY IS DETERMINED BY EMBRYONIC MACROPHAGE SENSING OF CALRETICULIN ON HSCS

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In development, local signals induce hematopoietic stem cell (HSC) emergence, trafficking, and division, and thereby dictate the number of HSC clones that ultimately support long-term hematopoiesis. Using a brainbow color barcoding system, we have previously demonstrated that zebrafish produce 20-30 HSCs in the developing aorta. Using spinning disk confocal microscopy, we imaged runx1+23:mCherry+ HSPCs in the fetal niche and found surprising interactions with mpeg1:GFP+ primitive macrophages. From 56-96 hours post fertilization (hpf), macrophages contacted newly lodged HSPCs and scanned the cell surface for 30-45 minutes, whereupon they either took up a portion of membrane and cytoplasm or engulfed the entire cell. To evaluate the effect this may have on HSC clonality, we depleted embryonic macrophages in our brainbow labeling system and raised fish to 2-3 months. Embryonic macrophage depletion via the irf8 morpholino or clodronate liposome injection

significantly reduced the number of HSC clones present in adulthood with an average of 14 clones vs 24.6 in sibling controls (p = 0.0002). To better characterize the interaction between niche macrophages and HSPCs we performed single-cell RNA sequencing and low-cell input proteomics. Macrophages that had recently engaged HSPCs were FACS purified via mCherry protein taken up from runx1+23:mCherry+ HSPCs. Proteomic analysis identified 416 differentially enriched peptides present in recently-engaged macrophages, which included three isoforms of calreticulin. Normally ER bound, previous work in cancer biology has shown that calreticulin may become surface bound under stress conditions, and that surface bound calreticulin can act as a 'come-eat-me' signal for macrophage mediated clearance of tumor cells. Antibody staining confirms surface calreticulin on HSPCs and scRNA-seq identifies the canonical receptor for calreticulin in recently-engaged macrophages. Morpholino knockdown of calreticulin reduces the prevalence of macrophage-HSPC interaction from 29% to 13% (p = 0.0058). Together, our data support a model in which the environmental stimuli that induce HSCs also result in variable surface display of calreticulin, leading to either macrophage grooming or phagocytosis, which ultimately regulates HSC clonality.

Keywords: Hematopoiesis, Clonality, Macrophage

TSC230

IDENTIFICATION OF NOVEL SURFACE MARKERS OF EXPANDED HUMAN HEMATOPOIETIC STEM CELLS

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With the advent of genetically engineered hematopoietic stem cells (HSCs) being used as part of therapeutic strategies for the treatment of multiple hematological diseases, the ex vivo expansion of HSCs represents a thriving avenue of interest. Our laboratory has identified the molecule UM171 that promotes the expansion of HSCs in vitro and we recently reported the clinical benefits of transplantation of cord blood grafts expanded with this molecule for the treatment of hematological malignancies. The expanded HSC immunophenotype remains to be precisely defined, as known HSC markers become unreliable when HSCs are introduced in culture, hampering the study of these cells. Our group previously identified 2 culture compatible HSC surface markers: the endothelial protein C receptor (EPCR) and integrin α3 (ITGA3). In order to further define expanded HSC immunophenotype, we performed RNA-sequencing of EPCR+ and ITGA3+ cell populations isolated from UM171-expanded CB cells combined with surface proteomic analysis of EPCR+ to identify novel culture compatible HSC markers. Comparison of cell populations expressing EPCR and ITGA3 with their negative counterparts led to the identification of Carcinoembryonic Antigen Related Cell Adhesion Molecule 1 (CEACAM1) and Glycoprotein A33 (GPA33) as a new markers of long-term HSCs. CEACAM1+ cells show multi-lineage differentiation potential in vivo and serial reconstitution ability in immunocompromised mice. Elevated GPA33 expression also associates with long-term reconstitution ability in vivo and GPA33+ cells show multi-lineage potential. Further characterization of these markers is currently ongoing. Defining the expanded HSC immunophenotype is the first step to better characterize these cells. The identification of CEACAM1 and GPA33 contributes to this endeavor to ultimately facilitate the design of improved strategies to manipulate HSCs for therapeutic purposes.

Funding source: This research is funded by a CIHR grant.

Keywords: Hematopoietic stem cells (HSCs), Surface markers, Umbilical cord blood cells

TSC232

CLONAL TRACKING UNCOVERS BARRIERS AND VALIDATES NEW STRATEGIES TO ENHANCE GENE EDITING IN HUMAN HEMATOPOIETIC STEM CELLS

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Gene editing (GE) by programmable nucleases holds great therapeutic promise by allowing in situ gene correction and function restoration with physiological expression. However, low efficiency of homology-directed repair (HDR) in hematopoietic stem cells (HSCs) has constrained its perspective clinical applications in several diseases. We recently showed that delivery of DNA double-strand break by nucleases and transduction by Adeno-Associated Vector (AAV) carrying the HDR-template in human HSCs trigger a p53-dependent DNA damage response delaying proliferation and decreasing hematopoietic reconstitution after xenotransplantation. Suppression of this response by transient expression of a dominant negative p53 released cell cycle block

and rescued hematopoietic reconstitution. Yet, the underlying biology remained unknown as well as the impact of GE on clonal dynamics of HDR-edited HSCs upon transplantation. Here, we devised a novel technology (BAR-seq) which enables clonal tracking of individual HDR-edited HSCs by introducing a unique heritable barcode (BAR) in the HDR-template. Deep sequencing of integrated BARs in human hematochimeric mice showed that only few (5-10) dominant HDR-edited HSCs robustly contributed to the hematopoietic graft long-term after transplant. Transient p53 inhibition during GE substantially increased polyclonal graft composition without altering individual HSC output, thus explaining the improved engraftment and highlighting the p53-mediated response as culprit of an otherwise oligoclonal hematopoiesis. Importantly, BAR-seq provided the first direct evidence that human HDR-edited HSCs maintain multilineage potential and undergo multiple rounds of symmetric and asymmetric divisions in primary and secondary xenogeneic hosts. We then overcame HSC constraints to HDR by forcing cell cycle progression and concomitantly upregulating the HDR machinery through transient expression of the Adenovirus5-E4orf6/7 protein. Combined E4orf6/7 expression and p53 inhibition enhanced >50% HDR efficiency within human graft and preserved all essential stem cell properties. Altogether, we expect that the substantial gains obtained in HDR efficiency and polyclonal repopulation by our improved protocol will now enable safe and effective clinical translation of HSC GE.

Keywords: Hematopoietic stem cell, Gene editing, Clonal tracking

TSC234

S100A6 IS A CRITICAL REGULATOR OF HEMATOPOIETIC STEM CELLS

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The fate options of hematopoietic stem cells (HSCs) include self-renewal, differentiation, migration and apoptosis, but how precisely intracellular Ca2+ (Cai2+) signals are transduced to regulate fate options in normal hematopoiesis is unclear. HSCs self-renewal divisions in stem cells are required for rapid regeneration during tissue damage and stress. S100A6 is members of the EF-hand family of calcium-binding proteins and is found increased when quiescent cells are stimulated to proliferate. S100A6 expression is known to be upregulated in mixed-lineage leukemia (MLL)/AF4 positive leukemia cells with poor prognosis. However, as therapeutic toward S100A6 is being developed to target leukemia, it is important to understand how loss of S100A6 affects normal blood development and the role of S100A6 in HSC maintenance. Therefore it is important to explore how S100A6 regulates HSCs fate options under normal physiological condition. Our murine model of S100A6 knockout (KO) HSCs have reduced total cell numbers in HSC compartment, a decreased of myeloid output and accompanied by an increased necrotic HSCs number in steady state. Importantly, S100A6KO HSCs have impaired self-renewal and regenerative capacity, fail to respond to stressor 5FU. Intriguingly, S100A6KO HSCs show a decreased of Akt and Hsp90 levels, with an impairment of mitochondrial oxidative phosphorylation and a reduction of mitochondrial Ca2+ levels. We unravel the mechanistic insights into how S100A6 regulates intracellular and mitochondria Ca2+ buffering of HSC. We demonstrate that Akt activator SC79 reverts the levels of intracellular and mitochondrial Ca2+ in HSC. Reduced colonies forming capacity and the decreased Hsp90 activity of S100A6KO are rescued through Akt pathway. Our transcriptomic and proteomic profiling suggest that S100A6 is a critical HSC regulator and Akt is the prime downstream mechanism of S100A6 in the regulation of HSC self-renewal with specifically governing mitochondrial metabolic function and Hsp90 protein quality. Our work describes the impact of the loss of S100A6 in the blood system and indicates the importance of this pathway in normal hematopoietic development. We anticipate investigating the true therapeutic window to target S100A6 in MLL/AF4 simultaneously to preserve the normal HSCs.

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Keywords: S100A6, Self-Renewal, Hematopoietic

IMMUNE SYSTEM

TSC238

A HUMAN IN VITRO MODEL FOR TYPE-1 DIABETES REVEALS GENE EDITING TARGETS FOR IMMUNE PROTECTION OF STEM CELL DERIVED BETA CELLS

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Type 1 diabetes (T1D) is an autoimmune disorder leading to the destruction of insulin-producing β-cells in the pancreas. Despite recent scientific advances, questions remain regarding the initial trigger and the downstream mechanisms of disease progression. Human induced pluripotent stem cells (hiPSCs) provide new opportunities for cell replacement therapy of T1D. Therapeutic quantities of human stem cell-derived β-cells (SC-β) can be attained in vitro following a stepwise differentiation protocol. Yet, preventing immune rejection of grafted cells, without the use of life-long immunosuppressants, remains a major challenge. Using T1D patients' hiPSC derived β-cells (iPSC-β), we developed a human in vitro platform in an autologous setting that recapitulates aspects of the effector/target interactions in an autoimmune response. A donor-matched β-cell-specific response was achieved by co-cultures with perihelial blood mononuclear cells (PBMCs) derived from the same donors' blood. We performed a droplet based single-cell RNA sequencing (scRNA-seq) of T1D iPSC-β co-cultured with their autologous PBMCs. scRNA-seq data analysis of co-cultured cell populations identified upregulated genes that contribute to the inflammatory microenvironment of a T1D pancreatic islet. Subsequent co-culture experiments have shown that CRISPR-depletion of such genes in SC-β, can reduce activation of T-cells and increase β-cell survival. These results provide insights into the nature of immune destruction of β-cells during T1D and suggest a path to prevent it in cell replacement approaches.

Funding source: Juvenile Diabetes Research Foundation
Sigma Millipore

Keywords: Autoimmunity of type-1 diabetes, Human induced pluripotent stem cells, Beta cells

MUSCULOSKELETAL

TSC256

GLUCOSE PROMOTES MESENCHYMAL STEM CELL PARACRINE FUNCTIONS PERTINENT TO ANGIOGENESIS BY REGULATING UNFOLDED PROTEIN RESPONSE

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Mesenchymal stem cells, also referred to as multipotent stromal cells (MSCs), are a promising source of cells for a wide range of tissue engineering and regenerative medicine applications because they possess the capacities to differentiate into various tissues of mesodermal origins as well as to secrete paracrine growth factors including angiogenesis. The low MSC retention rate within the injured tissue upon transplantation in vivo, caused by disruption of nutrient and oxygen supplies, however, has been a major roadblock for MSC use in therapeutic applications. Our previous studies established that the lack of glucose (but not of oxygen) is fatal to human MSCs (hMSCs) because it acts as a pro-survival molecule for hMSCs upon transplantation. The aim of this study is to provide further insights about the effects of glucose on hMSCs paracrine function pertinent to angiogenesis in vitro and in vivo. We found that conditioned media (CM) collected from hMSCs cultured with 1 or 5 g/L glucose for 3 days in severe hypoxia promoted significant ($p < 0.05$) increased HUVECs migration when compared to the ones collected from hMSCs cultured without glucose. In fact, hMSCs cultured in the presence of glucose released significantly ($p < 0.05$) higher amounts of Angiogenin, VEGF-A, a VEGF-C, Angiopoietin-1, Endostatin, and CCL2 when compared to hMSCs cultured without glucose. Most importantly, implanted hMSC-containing hydrogels loaded with either 5, 10, and 20 g/L glucose exhibited a 2.4-, 2.8-, and 2.4-fold increase ($p < 0.05$) in the volume of newly-formed blood vessels when compared to hMSC-containing hydrogels without glucose, respectively(n=8). The volume of newly-formed blood vessels in cell-containing hydrogels without glucose and in cell-free hydrogels loaded with glucose were similar and minimal. Mechanistically, inhibition of protein synthesis, mediated by the unfolded protein response via PERK, due to failure to provide glucose to hMSCs exposed to near anoxia in vitro may be responsible for the limited paracrine function pertinent to angiogenesis observed. These data demonstrate that glucose promotes hMSC paracrine functions pertinent to angiogenesis via regulation of unfolded protein response.

Keywords: Mesenchymal stem cell, Tissue regeneration, Glucose

TSC257

OPTIMIZING IN VITRO OSTEOGENESIS IN CANINE AUTOLOGOUS AND IPS-DERIVED MESENCHYMAL STROMAL CELLS WITH DEXAMETHASONE AND BONE MORPHOGENIC PROTEIN (BMP)-2

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The dog is an excellent translational model for cell-based orthopedic treatments. However, ideal sources of canine mesenchymal stromal cells (cMSCs) (i.e. autologous vs iPS-derived) and optimal osteogenic culture conditions remain

undetermined. The objective of this study was to evaluate early- and late-stage in vitro osteogenesis of autologous and iPS-derived cMSCs cultured with varying concentrations of BMP-2 and dexamethasone. Autologous cMSCs were isolated from bone marrow aspirates of skeletally mature dogs, while iPS-derived cMSCs were generated from adult canine fibroblasts. Osteogenic cultures were evaluated for early-stage osteogenesis at day 7 using alkaline phosphatase (ALP) activity and real-time quantitative PCR assays. Cells were evaluated for late-stage osteogenesis at day 21 using the alizarin red (ALZ) staining assay. Data were reported as mean +/- SD and analyzed using two-way ANOVA with Tukey's post hoc test. Significance was established at $p<0.05$. BMP-2 was required to induce robust ALP activity, whereas inclusion of dexamethasone decreased ALP activity in the presence of BMP-2. Inclusion of dexamethasone reduced Runx2 and osterix expression in autologous cMSCs. However, inclusion of BMP-2 increased expression of osteocalcin and osterix in autologous cMSCs. In contrast to early-stage results, inclusion of dexamethasone and BMP-2 in late-stage osteogenic assays produced the greatest monolayer mineralization for autologous cMSCs. However, dexamethasone was inhibitory to mineralization of iPS-derived cMSCs. This is the first study to extensively evaluate osteogenesis of iPS-derived cMSCs. While autologous and iPS-derived cMSCs respond similarly to early-stage osteogenic media, they exhibit unique responses to dexamethasone and BMP-2 in late-stage assays. Results of this study indicate that the in vitro osteogenesis of cMSCs can be modulated by varying the timing and concentration of osteogenic stimuli. However, conditions that drive robust late-stage monolayer mineralization are inhibitory to early-stage osteogenic differentiation, suggesting that optimal conditions for early- and late-stage in vitro differentiation are not identical. The results of this study will prove useful for investigators considering cMSCs for translational bone regeneration studies.

Funding source: Funded by the AKC-CHF, the Bone & Joint Fund, and NIH 5T32OD011083-09 (SG).

Keywords: canine mesenchymal stromal cells, induced pluripotent cells, osteogenic differentiation

TSC423

CYCLIC MECHANICAL LOAD REGULATES SINGLE CELL REGENERATIVE DIFFERENTIATION STAGE IN MOUSE OSTEOPROGENITORS VIA CDKN1A

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Forces generated by gravity loading are thought to stimulate tissue stem cell-based regenerative processes. Conversely, with profound tissue mechanical unloading in microgravity mesenchymal and hematopoietic precursors have decrease regeneration with increased stemness and differentiation markers as well as higher p21/CDKN1a levels. Interestingly, when p21/CDKN1a is knocked out, it results in a mouse with a regenerative phenotype. These findings suggest that mechanical loading may regulate tissue stem cell regenerative progression decisions via p21/CDKN1a. In this work we tested the hypothesis that cyclic mechanical loading differentially regulates primary mouse osteoprogenitor proliferation and differentiation progression at specific cell cycle stages via p21/CDKN1a. Mouse bone marrow cells from Cdkn1a-/- (null) or wildtype mice were cultured unloaded under osteogenic conditions for 7-days, then subjected to 48-hours of cyclic stretch or static control. After 48-hours loading, we used a 10X Genomics controller to generate bar-coded single cell Illumina libraries and performed single cell RNAseq for 6,000 static control and 6,000 cyclic stretch cells of each genotype. Using osteogenesis markers and single cell expressome analyses, we identified osteoprogenitor, early osteoblast, and late mineralizing osteoblasts populations. We found that Cdkn1a-/- osteogenic cultures without loading contain more differentiated late stage osteoblasts with fewer progenitors, and that cyclic stretch further promotes the conversion of progenitors to osteoblasts in both wildtype and Cdkn1a-/- backgrounds. The proportion of cells identified as late mineralizing osteoblasts doubled in Cdkn1a-/- cultures relative to wildtype but is not affected by load. Finally, single cell expression of Cdkn1a in wildtype cells, is most suppressed by cyclic stretch both in early and late osteoblasts, and minimally expressed or suppressed in progenitors. Collectively, our results support the hypothesis that Cdkn1a suppresses tissue stem cell regeneration in the absence of mechanical loading during late differentiation, and that loading reverses this effect by suppressing p21/CDKN1a expression. These studies suggest a novel molecular target to counter tissue degeneration caused by disuse on earth and in space.

Funding source: NASA Space Biology Grant to E. Almeida

Keywords: Cyclic loading, Regeneration, CDKN1A

NEURAL

TSC307

A NOVEL SELF-ASSEMBLING BIOMATERIAL TO ENHANCE HUMAN NEURAL STEM CELL-BASED REGENERATION AFTER SPINAL CORD INJURY

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Human iPSC-derived neural stem cells (hiPS-NSCs) are an exciting therapeutic approach for regeneration after traumatic spinal cord injury (SCI). Unfortunately, most patients are in the chronic phase of injury where ex vacuo microcystic cavitations form a formidable barrier to regenerative cell migration and neurite outgrowth. QL6 (K2(QL)6K2) is a novel, biodegradable, peptide biomaterial which self-assembles into an extracellular matrix (ECM)-like lattice in vivo. It has previously been shown to reduce inflammation and support endogenous and exogenous mouse cell survival. However, its ability to support translationally-relevant hiPS-NSCs continues to be a critical knowledge gap. Nonvirally-derived hiPS-NSCs were cultured on QL6 biomaterial versus a Geltrex ECM control. The mechanism of adhesion was assessed by EDTA assay and qPCR. hiPS-NSC survival, proliferation, and neurosphere formation were extensively characterized in vitro through immunohistochemistry and

scanning electron microscopy techniques. T-cell deficient RNU rats (N=60) capable of supporting a human graft were given a clinically-relevant C6-7 clip-contusion injury or sham surgery. In the chronic injury phase, animals were randomized to: (1) vehicle, (2) hiPS-NSCs, (3) QL6, or (4) QL6+hiPS-NSCs. All rats received delayed daily treadmill rehabilitation. A subset of animal cords underwent high throughput single-cell RNA sequencing (scRNAseq). hiPS-NSCs proliferated robustly on self-assembled QL6 vs control as demonstrated by Ki67+/DAPI+ immunocytochemistry (29%vs6%; p<0.01). EDTA adhesion assay demonstrated that human NSC binding to QL6 is largely driven by calcium-independent mechanisms. Importantly for NSCs, QL6 enhanced the formation of adherent neurospheres, the native conformation of NSCs. SEM imaging demonstrated an interwoven human NSC-biomaterial interaction in vitro. Blinded sensorimotor assessments of transplanted rats are ongoing with a 22-week post-injury endpoint. Early scRNAseq differential gene expression analyses demonstrate enhanced mature oligodendrocyte marker expression (MBP, MAG) by the graft when co-transplanted with QL6. This work provides key proof-of-concept data that QL6 self-assembling peptide can support translationally-relevant human iPS-NSCs for use in traumatic SCI.

Funding source: We thank the Canadian Institutes of Health Research (CIHR) for providing operating funds and a 5-year postdoctoral research fellowship to CSA. We also thank the Krembil Foundation, Phillip and Peggy DeZwirek and Wings for Life,

Keywords: Spinal cord injury, Biomaterial, Neural stem cells

TSC409

UNIQUE NEUROGENIC AGGREGATES IN THE HUMAN MEDIAL GANGLIONIC EMINENCE GENERATE LARGE NUMBER OF GABAERGIC INTERNEURONS

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Disruptions in GABAergic interneurons have been implicated in several neuropsychiatric disorders, yet their development in the human brain is poorly understood. We show that the human medial ganglionic eminence (hMGE) is organized into unique DCX+ cell-enriched aggregates (DEAs), which are not observed in the mouse MGE. DEAs form as early as 14 gestational weeks (GW) and persist as late as 39 GW in post-mortem samples. These cell-dense aggregates also express SOX2, DLX2, and the proliferation marker Ki-67, suggesting that this microstructure provides a long-lasting neurogenic niche for young GABAergic interneurons. In support of DEAs as persistent neurogenic regions, transplantation of hMGE cells into the neonatal mouse cortex re-established the highly proliferative DCX+ aggregates. At later stages, transplanted hMGE cells further differentiated into functionally mature GABAergic interneurons. Our study identifies a novel hMGE-specific organization and diversity of neural progenitors. This persistent gestational structure could underlie the expanded production and diversity of interneurons needed to populate the human brain.

Keywords: GABAergic Interneurons, Ganglionic Eminence, Neurodevelopment

NEW TECHNOLOGIES

TSC321

RAPID NON-INVASIVE TEST FOR ROUTINE DETECTION OF RECCURENT GENETIC ABNORMALITIES IN HUMAN PLURIPOTENT STEM CELLS

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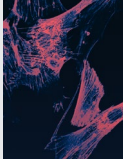
Preserving the genetic integrity of human pluripotent stem cells (hPSCs) is essential to guarantee the accuracy of disease models and the safety of downstream clinical applications. Because genetic abnormalities can accumulate at critical stages of the hPSC workflow such as hPSC generation, amplification or during genome editing, the genome of hPSCs should be frequently monitored. However, the current methods to assess hPSC genomic integrity are not optimized for regular screenings



as these approaches are often limited in term of resolution, flexibility, cost or processing time. Our aim is to improve and facilitate the procedure of hPSC genome integrity quality control. Based on a large meta-analysis compiling hPSC genetic abnormalities reported in more than 100 publications, we were able to exhaustively identify recurrent genetic abnormalities occurring in hPSCs. We then developed a rapid test (iCS-digital) that can detect more than 90% of hPSC-specific recurrent genetic abnormalities. This technology, based on digital PCR, is able to process DNA extracted directly from culture supernatant samples.This test was successfully used to detect copy number variations that arose during prolonged culture or after critical hPSCs stages such as gene editing. By enabling frequent monitoring of hPSC genomic integrity, the iCS-digital test contributes to secure many phases of the hPSC workflow, while minimizing time and financial losses associated with the use of abnormal hPSC lines.

Keywords: Human pluripotent stem cells, Genetic abnormalities, Quality control

TSC333

STORAGE AND SHIPMENT OF HUMAN IPSC-DERIVED CORTICAL NEURONS AT ROOM TEMPERATURE USING WELLREADY

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Human iPSC-derived neural progenitor cells are matured for at least 2 weeks before dispatch to customers at 37°C. This necessitates the use of sophisticated storage and shipping mechanisms to maintain the cells in a viable condition during transport to the customer. Atelerix has developed a hydrogel-based technology (WellReady™) capable of preserving biologics at room temperature and also protecting them from the adverse effects of mechanical forces that occur during shipment. This allows the convenient transportation of cells, whilst avoiding the known problems associated with cryopreservation and subsequent thawing, as well as those arising from shipping in liquid media. iPSC derived cortical neurons were stored for 24 hours at 15°C before being shipped a distance of 230 miles by courier in Controlled Room Temperature (CRT) packaging, stored overnight and returned. The total time for storage and transit was 3 days. Neuronal cultures that had no WellReady™ protection were greatly damaged during transit with considerable shearing of cells from the culture substrate and a total viable cell recovery approximately 40% of the non-stored control. Cells protected with WellReady™, on the other hand, exhibited no decrease in viable cell recovery compared to the non-stored control. The protection exhibited by WellReady™ represented a 2.6±0.7-fold increase over those cultures that were not protected. Even after 7 days of storage at 15°C, cell viability was maintained. Cells exhibited a morphology similar to that of the non-stored control with axonal connections also being preserved. WellReady™ offers a quick, convenient method to protect, store and ship fragile high value cell cultures in 384-, 96-, 48-, and 24-well plates.

Keywords: Cortical Neurons, Room Temperature Storage, Shipment

TSC340

ASSESSING QUALITY OF PLURIPOTENT STEM CELLS (PSCS) EXPANDED IN 3D SUSPENSION CULTURE

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Human pluripotent stem cells (PSCs) due to their capacity to self-renew and ability to differentiate into multiple cell types, are an invaluable source for cell therapy and regenerative medicine. PSC-based therapeutic products require large numbers of cells and large-scale expansion. In addition, it is important to maintain quality and purity of cells during expansion. Conventionally, stem cells are grown as colonies in 2-dimensional (2D) culture. These methods are convenient, easy to maintain and homogeneous. However, 2D methods have limited scale and are not ideal for mass production. Further, 2D systems do not mimic cell growth or function in vivo. Three-dimensional (3D) suspension culture systems offer a great advantage of enhanced surface to volume ratio making scale-up of PSCs more feasible. 3D cultures provide cell-cell interactions in all directions, perhaps closely mimicking the in vivo situation. PSCs can be grown in suspension as multicellular aggregates or on extracellular matrix-coated microcarriers either in low adherent cell culture vessels or in dynamic suspension in spinner flasks or bioreactors. Although other studies showed adequate expansion of PSCs using different 3D systems, not much is known about the quality of the cells. Culturing cells in 3D could result in morphological, metabolic and epigenetic changes that could be different from 2D cultured cells. These changes could ultimately affect cellular function and lead to safety issues, such as genomic instability. Our plan is to test different culture parameters and expansion methods that promote adequate growth of PSCs in spinner flasks, vertical bioreactors and in perfusion-enabled bioreactor systems. We will use these culture systems to gain understanding of the effect of 3D suspension culture conditions on different cell quality parameters, including but not limited to, stem cell and pluripotency markers, replication rate, mitochondrial dynamics, and genetic and epigenetic changes.

Funding source: Intramural FDA Advanced Manufacturing Grant

Keywords: pluripotent stem cells, suspension culture, bioreactors

TSC348

A SINGLE IV DOSE OF PLINABULIN INDUCES SUSTAINED MOBILIZATION OF CD34+ CELLS IN HUMANS THROUGH A MECHANISM OF ACTION (MOA) INDEPENDENT FROM G-CSF OR CXCR4 AND WITHOUT CAUSING BONE PAIN OR THROMBOCYTOPENIA

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Stem Cell Therapy and Gene Therapy rely on stem cell generation such as bone marrow derived CD34+ cells. G-CSF is the standard of care for CD34+ cell mobilization from bone marrow, but has limitations. Firstly, CD34+ cell yield can be too low. Secondly, G-CSF causes bone pain in the majority of patients (pts), as well as thrombocytopenia. Thirdly, a regimen of daily injections with short-lasting filgrastim, may impose inconvenience to patients. Plinabulin (Plin) is a novel small molecule immune-enhancer, given as one single infusion for 30 minutes and is in Phase (Ph) 3 trials for the prevention of chemotherapy-induced neutropenia (CIN). Plin boosts the number of progenitor stem cells in bone marrow with a MOA different from G-CSF and CXCR4, and has a faster onset of action for CIN, and does not cause bone pain or thrombocytopenia. To-date, Plin has been administered to > 600 pts worldwide and exerts a favorable tolerability and safety profile. We previously reported from the CIN Ph2 study BPI-2358-105 (NCT03102606) in Lung Cancer pts that single agent Plin increased CD34+ counts to clinically and statistically ($P<0.001$) significant levels, under myelosuppressive conditions, since all patients also received the chemotherapy Docetaxel (Blayney ASH 2018). In contrast to Peg, Plin did not cause bone pain or thrombocytopenia. Since Plin and G-CSF have a different MOA, we also evaluated the effects on CD34+ cell mobilization with combining Plin with pegfilgrastim (Peg, at 6 mg (full) dose, and at lower doses of 3mg and 1.5 mg) in study BPI-2358-106 (NCT03294577), a Ph2 trial in Breast Cancer pts receiving taxotere, doxorubicin, cyclophosphamide (TAC). The data shows that combining Plin with low dose Peg produces statistically significant CD34+ cell count increases ($P<0.03$) to values that are comparable or numerically higher than with full dose 6mg Peg alone under severe myelosuppressive conditions (TAC use). Bone pain in the Plin/Peg combinations was almost eradicated compared to the full dose Peg alone group. In each comparison we had 7 to 9 patients with CD34+ data per treatment arm. CD34+ counts were obtained by centralized FACS analysis (Covance Laboratories). Thus, Plin as single agent may represent a safe, potent, convenient and well-tolerated alternative to G-CSF for CD34+ cell mobilization, or could be combined with Peg.

Keywords: Bone Marrow CD34+ mobilization, Gene Therapy, Stem Cell Therapy

TSC356

A DYNAMIC HEMATOPOIETIC MICROENVIRONMENT: LIVE IMAGING OF STEM CELL EXPANSION AND BONE MARROW VASCULAR ARCHITECTURE IN ASSOCIATION WITH BONE REMODELING

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Endosteal and vascular niches have been considered as two major compartments that regulate fate determination of normal and leukemic stem cells. Based on intravital tracking of the newly developed MDS1GFP/+ FLT3-Cre transgenic mouse model, our recent work confirmed the dual endosteal-vascular localization of native long-term hematopoietic stem cells (LT-HSCs). However, although sharing similar niches, their clonal proliferation behaviors are heterogeneous and only occur within a subset of bone marrow cavities while other LT-HSCs remain as singlets. Given that bone formation precedes hematopoiesis, and the endosteal surface is constantly undergoing remodeling, we reason that different stages of bone turnover may impose a degree of heterogeneity on the bone marrow microenvironment that has so far been overlooked. To visualize bone remodeling in vivo, we have developed an intravital labeling technique and identified distinct bone marrow cavities based on the features of local bone deposition and resorption. It relies on sequential administrations of spectrally-distinct dyes that label the bone fronts 48 hours apart and intravital imaging immediately after the second dye injection. The dye administered at 48 hours prior to imaging serves to delineate where the old bone front has been partially eroded via bone resorption while the dye administered on the day of imaging marks the new bone surface. Therefore, 3D quantification of Dye1/Dye2 ratio enabled us to classify the bone marrow cavities into deposition (D), mixed (M), and resorptive (R) types. With this strategy, we observed that both LT-HSCs and HoxA9-Meis1 leukemic cells expanded almost exclusively in a subset of M-type cavities. To further determine the remodeling dynamics over time, we added a third dye after an additional 48-hour interval. Longitudinal analysis of the Dye1/Dye2 and Dye2/Dye3 ratios from the same animal showed that a majority of bone marrow cavities underwent active turnover that resulted in substantial changes in dye ratios. Moreover, the spatial organization of arteriole vessels was found to be closely associated with such remodeling dynamics. These findings point

to the importance of live imaging techniques for characterizing the spatially heterogeneous and temporally dynamic aspects of the bone marrow microenvironment.

Keywords: Intravital imaging, Bone remodeling, Hematopoietic stem cells

TSC362

3 NOVEL RODENT ES CELL LINES INCLUDING FEEDER-INDEPENDENT, GERMLINE TRANSMISSIBLE MOUSE NOD ES CELLS AND THE FIRST ES CELLS FROM ACOMYS, A REGENERATIVE RODENT

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Embryonic stem (ES) cells remain central to genome engineering strategies in rodents. Yet ES cell lines are not available for important mouse genetic backgrounds or emerging model systems. We established three feeder-independent ES cell lines that will be broadly useful to the scientific community. Obviating the need for feeder cells, our new ES cell lines allow for simplified transfection, selection, and subcloning. The first ES cell line was derived from a F1 (C57BL/6J x Black Swiss) cross, which combines a desired genotype (C57BL/6J) with a strain that displays robust fecundity (Black Swiss) and is compatible with CD1 4-8-cell injections. This F1 line is extremely robust. For example, on average, for every 10 CD1 embryos injected and transferred, we obtained 2 100% chimeras and subsequent germline transmission of more than 50 mutant alleles. The second ES cell line is a stable, feeder-free NOD ES cell line. As a model of Type I diabetes, the NOD mouse strain has enabled the study of immune and environmental factors influencing the onset of diabetes. However, genetic analysis has been hampered by the absence of genetically pure (non F1) NOD ES cells. To date, we have also transmitted two targeted alleles from this line. The third ES cell line is derived was from Acomys cahirinus - the first ES cell line established for this rodent model. Acomys is an emerging rodent model of mammalian regeneration and are the only mammal known to retain extensive regenerative, non-fibrotic wound healing as adults. This Acomys ES cell line shares multiple transcriptomic features with the robust mouse F1 (C57BL/6J x Black Swiss) line. Experiments are ongoing to generate both mouseß à Acomys interspecific chimeras and Acomys chimeras.

Funding source: NIH R21OD023838 and the Keck Foundation

Keywords: stem cells, acomys, NOD

TSC414

A GENETIC/EPIGENETIC-BASED METHOD FOR HUMAN IPSC/ESC LINE AND SUB-LINE AUTHENTICATION TO IMPROVING THE STANDARD FOR DATA SHARING AND REPRODUCIBILITY

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Reproducibility (here understood as consistency in the results obtained after repeating experiments using the same procedures/analytical methods and the same cell line) is a major concern in the field of human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs). Sources of reproducibility failure include mislabeling and cross-contamination (which can occur when growing multiple lines side by side), and the intrinsic instability and heterogenicity of these lines (which may lead to the adoption of distinct cell populational features when a single cell or a small subpopulation is intentionally or unintentionally segregated from the parental hiPSC/hESC culture during genome editing or sub-culturing, respectively). The gold-standard analysis for cell authentication is genetic profiling of polymorphic short-tandem repeat (STR) loci, combined with selective PCR genotyping when using genome-edited lines. Rarely, however, these analyses are performed on cultures of the same hiPSC/hESC line over time (i.e., repeatedly), or contemporarily to experiments included in publications. Critically, also, STR/PCR analyses cannot distinguish clonal/sister cultures generated in the same reprogramming or CRISPR-editing event, which are often used to support data reproducibility. Here, we describe a method based on H3K4me3 chromatin immunoprecipitation sequencing (ChIP-seq) for robust hiPSC/hESC (sub)line authentication. It relies on the integration of genetic variation (single-nucleotide variants, SNVs) and stable epigenetic features (H3K4me3). This approach can also be incompletely implemented with RNA-seq, ATAC-seq, and non-H3K4me3 ChIP-seq data. A major advantage of our method is that it can be applied retroactively to provide (sub) line authentication contemporary to next-generation sequencing (NGS) data generated in the past. We propose that our method to authenticating hiPSC/hESC cultures could be a requirement for depositing NGS datasets in public repositories (GEO-NCBI, SRA-ENA, or DRA-DDBJ), at least as a quality test for datasets that then become suitable for integration in meta-analyses; or when hiPSCs are aimed for disease modeling, drug screening, or regenerative medicine purposes, with the goal of improving the standard for data sharing and reproducibility.

Funding source: Department of Defense (DoD), Convergence Science Research Award, W81XWH1910315

Keywords: hiPSC/hESC, Cell authentication, Reproducibility

PLACENTA AND UMBILICAL CORD DERIVED CELLS

TSC363

EPITHELIAL CELL DIFFERENTIATION OF HUMAN MESENCHYMAL STROMAL CELLS IN DECELLULARIZED DERMIS SCAFFOLDS

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Mesenchymal stem cells (MSC) represent an attractive tool to improve engineered skin constructs as therapeutic strategy for cutaneous lesions, given their cell plasticity and production of beneficial paracrine factors. The ability of MSC to differentiate into epithelial lineage following culture under epithelial induction conditions has been previously reported. However, little is known about the impact on epithelial cell differentiation when MSC are combined with skin scaffolds. Here, we studied the ability of MSC from Wharton jelly (WJ-MSC) to repopulate and differentiate into epithelial-like cells after culture on human acellular dermis. Acellular skin scaffolds were generated from human skin grafts obtained at the institutional Tissue Bank. After decellularization, green fluorescent protein (GFP)-expressing WJ-MSC and wild type WJ-MSC were seeded on skin scaffolds using an air–liquid interface cell system. Viability, cell proliferation and epithelial differentiation was monitored by fluorescence microscopy. WJ-MSC attached and proliferated on the dermis scaffold. Cells were able to differentiate towards epithelial-like cells as shown by expression of the epithelial markers plakoglobin, filaggrin and involucrin. Moreover, WJ-MSC displayed keratinocyte-like morphology, consistent with morphology patterns observed for HaCaT control cells. Finally, WJ-MSCs showed significant reduction of the mesenchymal marker vimentin. Together this data points to the potential capacity of WJ-MSC to differentiate toward epithelial-like phenotype and opens a new venue to use MSC as cell resource for skin tissue engineering.

Funding source: Departamento Administrativo de Ciencia, Tecnología e Innovación Code: 681580763539

Keywords: Mesenchymal stromal cells, Ephitelial differentiation, Acellular skin scaffolds

TSC371

SECRETED RESPONSES OF UMBILICAL CORD MESENCHYMAL STROMAL CELLS TO FULL THICKNESS SWINE BURNS ARE REFINED BY THE ZONE OF INJURY

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Wood, Catherine - *Aurora BioSolutions Inc., Medicine Hat, AB, Canada*
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Primary treatment of battlefield burns is often inadequate due to delayed field evacuation or limited care in mass casualty incidents. Untreated burns expand in depth and surface area and elicit a hyper-immune response until the damaged tissue is removed. Third degree (full-thickness) burns penetrate the dermis and comprise 3 concentric zones of injury. The central zone of coagulation is irrevocably damaged while the surrounding zone of stasis is at risk. Tissue in the outermost zone of hyperemia can be saved despite an acute inflammatory response and increased perfusion. Mesenchymal stromal cells (MSCs) reportedly improve outcomes as post-surgical burn treatments. We assessed in vitro whether MSCs may also benefit burn sequelae if applied prior to surgical intervention. Nine anaesthetized swine received 3rd degree burns on the inner flank by a heated brass bar. After 30 minutes, the burn was excised ~1 cm outside the zone of hyperemia. Control (unburned) samples were also excised. The burn center (zones of coagulation and stasis) and margin (zone of hyperemia) were isolated by dissection. Human umbilical cord MSCs (UC-MSCs), were co-incubated with 0.4 +/- 0.005g of tissue using a 6-well Transwell system for 24, 48 or 72 hours (h) in xeno-free media. Secreted proteins were assessed by multiplex ELISA of 115 analytes. Within 24h of exposure to any burn tissue, UC-MSCs increased output of inflammation mediators including fractalkine, GCP-2, GRO-β, I-309, IL-19, MCP-3, MIP3-α, MMP-2 and TSLP. IL-2Ra and MIG were specifically elevated in response to the necrotic center tissue, followed by β-NGF and IL-3 at 48h and SCF and SCGF-β at 72h. Intriguingly, the burn margin stimulated production of wound healing molecules in addition to inflammation mediators. G-CSF, HGF, IL-1β, IL-1RA, LIF and TECK increased significantly at 24 hours, followed by IL-20, IL-35, MDC, sCD163 and sTNF-R1 at 72h. Thus, UC-MSCs exhibit refined soluble responses that may improve outcomes for severe burn patients that initially receive sub-optimal treatment and delayed definitive care. The observed zone-specific responses suggest that the molecular mechanisms of MSC treatments may differ if the cells are applied before or after surgical debridement and depending on their localization within the wound.

Keywords: umbilical cord MSC, full-thickness (3rd degree) burn, paracrine response

POSTER SESSION II
22:00 – 23:59

Theme: Cellular Identity

CARDIAC

CI105

CELL-TYPE-SPECIFIC TRANSCRIPTOME AND CHROMATIN ACCESSIBILITY DYNAMICS IN A MODEL OF HUMAN HEART DEVELOPMENT AND MATURATION

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Human pluripotent stem cells (hPSCs) have the potential to generate virtually any cell type, as such, can be used to study human development, model diseases and even hold the promise of enabling cell replacement therapies. However, further development and application of human PSC-based technologies have been severely hampered by the heterogeneity and immature nature of differentiated cells derived from these stem cells. To chart the cell-type-specific regulatory network underlying human heart development and maturation, we generated single-cell transcriptome and chromatin accessibility profiling along directed cardiac differentiation in vitro. We sampled from pluripotent states to terminal differentiated cultures over 9 months. Active chromatin regions and enriched transcription factors (TFs) expression identified in human primary heart tissues were observed in different cell types at different developmental stages. We used this resource to map genetic risk for congenital heart disease at cardiac progenitor stages. Moreover, we integrated chromatin accessibility with transcriptomics to identify putative enhancer-gene linkages and transcription factors that regulate human heart development and maturation. Overall, this resource provides molecular insights into gene-regulatory dynamics at previously inaccessible stages of human heart development, including epigenomic signatures of congenital heart disease.

Keywords: Single cell RNA-seq, Single cell ATAC-seq, Cardiac differentiation

CI109

DO MITOCHONDRIAL DYNAMICS CONTROL MATURATION OF CARDIOMYOCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS?

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Current protocols to differentiate cardiomyocytes from human induced pluripotent stem cells (iPSCs) inadequately recapitulate cardiomyocyte maturation during development. We recently identified that inhibition of the mechanistic target of rapamycin (mTOR) pathway with Torin1 can enhance maturation of iPSC-derived cardiomyocytes (iPSC-CMs) via induction of cellular quiescence. Unexpectedly, we had found that although oxygen consumption rate is increased, indicative of metabolic maturity, we found a decrease in the mitochondrial to nuclear DNA ratio, suggesting that the more mature cardiomyocytes have fewer or smaller mitochondria. Although mitochondria are best known for their role in energy production, changes in mitochondrial morphology can also control stem cell fate and the quiescent state. We sought to investigate whether Torin1 alters mitochondrial morphology and function and whether this affects quiescence. We performed transmission electron microscopy to quantify mitochondrial size. We evaluated expression of mitochondrial fission and fusion proteins via western blotting. We quantified mitochondrial membrane potential with MitoTracker Red CMXRos dye. Vehicle-(DMSO)-treated iPSC-CMs had hyperelongation of mitochondria, which was prevented with Torin1 treatment (200 nM x 7 days), with significantly decreased mitochondrial width, length, and area versus control ($p<0.001$ for each). We observed that Torin1 increased expression of Parkin and decreased protein expression of mitofusins 1 and 2, suggesting increased mitochondrial fission and decreased mitochondrial fusion, respectively. We found that Torin1-treated iPSC-CMs had a significantly increased mitochondrial membrane potential ($p<0.05$), suggesting removal of dysfunctional mitochondria. Torin1-treatment significantly decreased superoxide and total reactive oxygen species production compared to vehicle control ($p<0.01$), consistent with a quiescent phenotype. These results suggest that Torin1-treatment inhibits mitochondrial fusion and promotes mitochondrial fission, with retention of more mature mitochondria. Ongoing work will quantify whether Torin1-induced mitochondrial quality control mechanisms are responsible for supporting a quiescent state and directing cardiomyocyte maturation.

Funding source: J.C.G. is funded by an NIH T32 fellowship (T32HL007572).

Keywords: cardiomyocyte, maturation, mitochondria

EARLY EMBRYO

CI142

DERIVATION OF FORMATIVE-LIKE PLURIPOTENT STEM CELLS FROM MAMMALIAN EMBRYOS

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Two phases of pluripotency, naïve and primed, have been captured in vitro and studied in detail. A third "formative phase" was recently proposed to exist between naïve and primed phases. Formative pluripotency entails permissiveness for direct primordial germ cell (PGC) induction and chimera competency, and is characterized by molecular features intermediate of naïve and primed pluripotency. To date, however, stable pluripotent stem cells (PSCs) harboring formative features have not been derived from early mammalian embryos. Here, we developed a method that enabled the derivation and long-term culture of stable formative-like embryonic stem cells (ESCs) from mouse blastocysts, which not only shared transcriptional similarity with E5-6 epiblast and formative epiblast like cells (EpiLCs), but were also capable of direct entry into the germline and contributing to blastocyst chimeras. The same culture system supported the generation of embryonic stem cells (ESCs) and transgene-free induced pluripotent stem cells (iPSCs) from a large domestic mammal, the horse, which lacks stable PSCs. Formative-like horse PSCs (ESCs/iPSCs) could efficiently chimerize horse, mouse, goat, sheep and pig early embryos. Stable formative-like PSCs will be invaluable for studying mammalian pluripotency and early PGC development, and our method may be broadly applicable for the derivation of analogous stem cells from other mammalian species.

Keywords: Pluripotent stem cells, Formative state, PGC-LC induction

CI144

ESTABLISHING INTEGRIN ALPHA6 (CD49F) AS A RELIABLE BIOMARKER THAT DEFINES FUNCTIONAL MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are isolated from many tissues and can be derived from differentiation of human embryonic stem cells (hESCs). MSCs are characterized by their self-renewal potential and multi-lineage differentiation into bone, fat and cartilage. Success of MSC-based therapies has been confounded by population heterogeneity and by use of non-standardized methods for their definition and characterization. Integrin α6, also known as CD49f, is a biomarker that has been identified in all stem cell populations, including MSCs. We and others have shown that CD49f plays an important role in self-renewal of different stem cell populations. We hypothesized that the expression of CD49f can be used to identify a functional MSCs. For this, we derived MSCs from H1 and H9 hESCs, and we cultured these cells from passage 1 through 8, and perform characterization of these cells in term of expression of biomarkers by flow cytometry, capacity to in vitro differentiation, and self-renewal by colony forming units (CFU) assays. While we observed that the expression of commonly used biomarkers such as CD70, CD90 and CD0105 remained highly expressed from early to late passages, the expression of CD49f remained high until passage 3 and declined in subsequent passages. At early passages, the cells were functionally active- defined by active osteogenic and adipogenic differentiation capability, and CFU; these characteristics declined drastically at late passages. Subsequently, we used magnetic beads sorting to separate passage 3 MSCs into CD49f positive and negative cells. These populations were analyzed for their functional characteristics based on CFU, osteogenic and adipogenic differentiation potentiality. Our results show that the CD49f+ cells have significantly higher capabilities for CFU and osteogenic and adipogenic differentiation. In conclusion, our results indicate that integrin α6 is a reliable biomarker to identify and define functional MSCs.

Funding source: OU's CBR REF

Keywords: Mesenchymal Stem Cells, Integrin Alpha6, Biomarkers

CI147

DETERMINING THE ROLE OF FOCAL ADHESION KINASE IN THE NUCLEUS OF HUMAN PLURIPOTENT STEM CELLS

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Focal Adhesion Kinase (FAK) is a protein tyrosine kinase that regulates proliferation and migration of cells. In somatic cells, FAK resides in the cytoplasm and focal adhesion sites, while we have demonstrated that in undifferentiated pluripotent stem cells (PSCs) FAK resides in the nucleus, and is translocated to the cytoplasm once phosphorylated during differentiation. Interestingly, in the nucleus of PSCs, FAK interacts with the transcription factors Oct4 and Sox2, suggesting involvement in regulation of PSC self-renewal. Our aim is to determine the role of FAK in the nucleus of PSCs. We hypothesize that nuclear FAK acts as scaffold promoting the physical interaction between p53 and the ubiquitinase MDM2, which results in p53 degradation. To test this, PSCs are treated with 0.5mM of manganese chloride (Mn2+) to promote the nuclear exportation of FAK, while control cells are treated with 0.50 mL of PBS. Immunocytochemistry (ICC), Western Blot analysis, PCR analysis, and ELISA tests are performed to test the activity of MDM2, FAK, and the expression and activity of p53. Based on current analyses, Mn2+ treatment induces FAK nuclear exportation, which led to the accumulation of p53 in the nucleus, expression of its downstream target -p21-, and to decrease of MDM2 and Oct4 nuclear expression. Using ImageJ analysis on ICC images, we were able to observe almost a 50% increase in p53 accumulation in the nucleus while there was approximately a 40% decrease in MDM2 nuclear expression. We have verified that there is an increase in the expression of

p53 and a decrease in MDM2 expression using Western Blot analysis. After analyzing the Western Blot films using ImageJ, we have observed the increased expression of p21, and double expression of p53 after treatment, while a 40% reduction in MDM2 expression and 50% reduction of Oct4. We will perform co-IP assays to demonstrate the direct binding between FAK/p53/MDM2. Thus far, our results support our hypothesis and will shed light in novel mechanisms by which p53 is regulated in PSCs.

Funding source: OU's CBR REF

Keywords: human pluripotent stem cells, Self-renewal, FAK

CI153

CONTIGUOUS EROSION OF THE INACTIVE X CONCLUDES WITH AN IMPAIRED EPIGENOME IN HUMAN PLURIPOTENCY

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Bansal, Prakhar - *Genetics and Genome Sciences, University of Connecticut, UConn Health, Farmington, CT, USA*

Human pluripotent stem cells (hPSCs) tend to exhibit instability of DNA methylation in specific imprinted genes, as well as the inactive X chromosome (Xi). This process of X chromosome erosion (XCE), typically initiates with repression of XIST, the long non-coding RNA responsible for silencing the Xi, followed by irreversible loss of key epigenetic features of the Xi that culminates in the reactivation of previously silenced genes. While XCE has been suggested to be driven by upregulation of specific Xi-linked genes that provide a selective advantage for female hPSCs in culture, and has been described as ranging from sporadic to chromosome-wide, the scale, dynamics and key transition events of this process have remained almost entirely unclear. To address this knowledge gap, provide guidance on staging XCE in female hPSCs, and assess how this progressive X reactivation phenomenon may impact female-specific hPSC epigenome instability, we performed an integrated DNA methylation and expression analysis across ~ 400 published hPSC lines. Differential methylation across the eroding X (Xe) enables ordering female hPSCs across a trajectory of XCE from initiation to terminal stages. Our analysis incriminates cis-regulatory sites that reflect XIST repression, trace contiguously growing domains of reactivation to a few euchromatic origins on the Xi, and suggest the late-stage Xe may act as an epigenetic contagion that impairs DNA methylation genome-wide. These results are relevant to understanding and staging the epigenetic fidelity of female hPSC disease models, and implicate species-specific differences that may predispose the human Xi to reactivation.

Funding source: This work was supported by NIH grant R35GM123926 to SP.

Keywords: X chromosome inactivation, DNA methylation, Sex-specific gene dosage

CI175

CHANGING THE WADDINGTON LANDSCAPE TO CONTROL MESENDODERM COMPETENCE

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As pluripotent human embryonic stem cells progress along the developmental trajectory towards one differentiated fate, they lose competence to adopt other fates. This process can be pictured as a classical Waddington landscape in which a differentiating cell rolls down a hill and enters one of several "valleys" that constrain its fate choice. Here, we show that the loss of competence for alternative fates occurs at a specific point along the developmental trajectory along this landscape and that this point can be moved using genetic perturbations. We first show that competence to adopt mesendoderm-derived fates in response to BMP4 and Activin A signal exposure is lost during ectoderm-directed differentiation. By monitoring each cell's progression along its developmental trajectory, we can prospectively predict that cell's mesendoderm competence. We then exploit this predictive ability using RNA-seq and ATAC-seq to identify and validate candidate transcription factors that can modulate mesendoderm competence. These factors exert their effects by controlling the cell's progression along the developmental trajectory, by tuning its competence to form mesendoderm at any given point along that trajectory, or by altering both of these aspects. In the Waddington landscape, these effects correspond to changing the cell's location on the landscape and altering the location of the barrier between fates, respectively. The ability of the underlying gene regulatory network to modulate these two aspects of the developmental landscape could allow separate control of the dynamics of differentiation and tissue size proportions.

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Keywords: cell fate decisions, mesendoderm competence, Waddington landscape

CI177

HEPARAN SULFATE REGULATES CELL FATE DECISIONS OF HUMAN EMBRYONIC STEM CELLS

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All mammalian cells, including human embryonic stem (hES) cells, are decorated with heparan sulfate, a highly sulfated polysaccharide. Heparan sulfate can influence communication between stem cells and their microenvironment by modulating growth factor assembly and interactions. Although studies in mouse embryos indicate that heparan sulfate is essential for mammalian development, the biological roles of heparan sulfate in human cell fate commitment are largely unknown. To address this knowledge gap, we engineered a heparan sulfate-deficient hES cell line by CRISPR-mediated targeting of EXT1, a glycosyltransferase required for heparan sulfate biosynthesis. Using RNA-seq analysis, embryoid body formation assays, and directed differentiation assays, we found that EXT1-/- cells can self-renew but their abilities to differentiate into mesoderm and endoderm lineages are severely compromised. We also identified defects in the Nodal signaling pathway, a critical developmental process, which is a plausible cause of the impaired primitive streak formation. EXT1-/- cells can differentiate into ectoderm lineage, yet the resulting neural cells have arrested axonal extensions, suggesting a role for heparan sulfate in proper neurodevelopment as well. In summary, our findings highlight the significance of cell surface glycans in human development and diseases.

Keywords: Heparan sulfate, Primitive streak, EXT1

CI331

PRPF6 SAFEGUARDS GENOME STABILITY AND SELF-RENEWAL OF HUMAN EMBRYONIC STEM CELLS THROUGH RNA M6A MODIFICATION AND ALTERNATIVE SPLICING

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Human embryonic stem cells (hESCs) are widely used for basic research and hold great potential for regenerative medicine due to their capacities to self-renew unlimitedly and to differentiate into all cell types of the human body. However, the potential genomic instability hampers the maintenance and full application of hESCs. Understanding molecular mechanisms underlying hESC genomic stability is critical to achieve a more qualified hESC culture for further applications. Here, we report that PRPF6, a member of the basal spliceosome, is essential for the maintenance of hESC self-renewal. PRPF6 knockdown (KD) by RNA interference reduces hESC survival, while cell cycle and proliferation remained unchanged. Concomitantly, we observe DNA damage enhancement, as indicated by increased gamma H2A.X level and longer comet tail at an early time point after PRPF6 KD, and DNA damage-induced apoptosis, as evidenced by elevated cleaved Caspase-3 and Caspase-9 levels. These results suggest that PRPF6 may play a genome stability-safeguarding role in hESCs. Our RNA-seq analysis demonstrates that many genome stability-related genes are aberrantly spliced in PRPF6 KD hESCs, suggesting that PRPF6 may function through an alternative splicing mechanism to maintain hESC genome stability and self-renewal. Moreover, our IP/MS data analysis shows that PRPF6 interacts with RNA m6A modification writers, consistent with our finding that PRPF6 KD decreases RNA m6A level in hESCs. Furthermore, functional assays show that KD of either RNA m6A modification writers or one RNA m6A reader also induces DNA damage in hESCs. Collectively, RNA m6A modification may be involved in the regulation of genomic stability of hESCs by PRPF6. Multi-omics analyses are being performed to study how PRPF6 regulates the alternative splicing of genomic stability- related genes through RNA m6A modification, thereby stabilizing hESC genome stability. Our finding provides novel insights into how hESC genome stability and self-renewal are maintained.

Keywords: human embryonic stem cell, genome stability, RNA m6A

CI335

COPPER SUPPLEMENTATION DURING IN VITRO MATURATION OF PORCINE OOCYTE AFFECT TRANSCRIPT LEVELS RELATED TO APOPTOSIS AND DEVELOPMENTAL COMPETENCE

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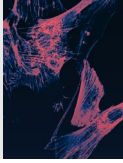
Copper acts as a cofactor of enzymes related to respiration, radical, detoxification, iron metabolism since it has a redox activity. However, excess copper can be toxic to cells due to accumulation of hydrogen peroxide. In this study, we investigated the effect of copper treatment during in vitro maturation (IVM) of porcine oocytes on various transcript levels related to apoptosis and developmental competence. Copper chloride was supplemented to maturation media (TCM199-PVA) and its concentrations were 0, 0.7, 1.4, and 2.8ug/ml each group. After 40-42 h of IVM, we evaluated transcript levels of apoptosis, proliferative, developmental and copper transport related genes in oocyte and cumulus cells by using real-time PCR (polymerase chain reaction). As a first result, Nrf2 and Bax levels in cumulus cells were significantly decreased in all copper treatment groups compared to control. On the other hands, copper supplementation increased Has2 level in cumulus cells. In oocytes, PCNA mRNA level increased in 0.7ug/mL Cu group compared to control. In addition, there were significant increases of apoptosis related genes, Bax and Bcl-2, in 0.7 and 2.8ug/mL Cu group. However, the ratio of Bax/Bcl-2 mRNA expression shows significant decrease apoptosis rate in 0.7 and 1.4ug/mL Cu group. Copper supplementation also improved maternal effect genes, Zar1 and NPM2 in 0.7 and 2.8ug/mL Cu group compared to control. In case of copper transport related genes, there was only a decrease of Ctr1 gene as copper concentration increased in cumulus cells. CTR1 is a protein involved in Cu1+ transport process However, the mRNA levels of Sod1 which known as a antioxidative enzyme were significantly high in 0.7 and 1.4ug/



mL Cu group than that of control. Although further studies are required to evaluate nuclear and cytoplasmic maturation rate, these results demonstrate that copper supplementation during IVM decrease apoptosis rate in both cumulus cells and oocytes. In addition, copper treatment increases developmental related genes.

Funding source: This work was supported by "Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries(IPET) (grant number: 819029-2)"

Keywords: copper, porcine oocyte, in vitro maturation

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CI184

CYTOSKELETAL REGULATION OF HUMAN PANCREATIC CELL FATE

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Recent progress has been made in the differentiation of pancreatic β cells from human pluripotent stem cells, providing a potentially unlimited supply of insulin-producing cells for treating diabetes. This differentiation methodology relies on the stepwise addition of growth factors and small molecules to recreate stages of in vivo development, though physical cues from the cells' microenvironment have often been overlooked during this process. Here, we report that the state of the actin cytoskeleton in pancreatic progenitors is critical to cell fate selection during this directed differentiation protocol. By manipulating cytoskeletal state via cell arrangement (two- vs. three-dimensional), substrate stiffness, or directly with chemical treatment, we demonstrate that a polymerized cytoskeleton in pancreatic progenitors inhibited NEUROG3-induced endocrine differentiation. In particular, the high stiffness of tissue culture polystyrene increased actin polymerization enough to prevent premature endocrine induction in planar culture, facilitating the expression of the crucial β cell transcription factor NKX6-1. Subsequent differentiation of these NKX6-1+ pancreatic progenitors to stem cell-derived beta (SC-β) cells in planar culture, however, required timed cytoskeletal depolymerization with the compound latrunculin A. Inappropriate cytoskeletal signaling promoted by either omitting latrunculin A during endocrine induction or adding it at the incorrect time led to drastic increases in markers associated with other endodermal lineages, including liver, intestine, and pancreatic exocrine. These results highlight how physical culture conditions such as substrate stiffness and dimensionality can influence endodermal cell fate choice during the SC-β cell protocol and indicate why all successful protocols have included some form of three-dimensional cell aggregation during the endocrine induction step. These new insights into how the microenvironment and cytoskeletal signaling influence endodermal fate selection facilitated adaptation of suspension-based protocols for generating highly functional SC-β cells to a planar format, providing an easier methodology that enhances differentiation reproducibility and SC-β cell function across multiple human cell lines.

Funding source: This work was funded by the NIH (5R01DK114233, T32DK108742), JDRF (5-CDA-2017-391-A-N), and the Washington University-Centene Personalized Medicine Initiative.

Keywords: stem cell-derived beta cells, endodermal differentiation, mechanotransduction

CI193

CHARTING AND NAVIGATING HUMAN IN VITRO BETA CELL DIFFERENTIATION USING SINGLE-CELL RNA SEQUENCING AND GENOME-WIDE CRISPR PERTURBATION SCREENS

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In vitro directed differentiation of human beta cells promises to cure Type I diabetes by replacing endogenous cells lost to autoimmune injury. Current protocols modulate cellular signaling pathways in a stepwise manner, starting from human pluripotent stem cells to first produce pancreatic progenitors and next glucose-responsive stem-cell-derived (SC-) beta cells. We first apply single-cell RNA sequencing to comprehensively map cell fates produced during SC-beta differentiation and, in turn, apply whole-genome CRISPR-based perturbation screening to identify genes that can modulate this process. Through sequencing of >100,000 cells from various stages of the protocol, we identify progenitor states and two other endocrine cells types produced alongside beta cells in vitro: glucagon-producing alpha and serotonin-producing enterochromaffin cells. Leveraging 3D culture conditions that scale to >1 billion cells, we carried out a whole-genome CRISPR knock-out screen to identify genetic perturbations that control the balance between these stem-cell derived endocrine cell types. Notably, numerous transcription factors (TFs) are amongst the top screen hits. Among others, we readily recapitulate the known importance of TFs such as PDX1, NEUROG3, TCF7L2 and PAX4. Our unbiased genome-wide screen also identifies novel regulators of beta cell identity, ranging from ATOH1, a known regulator of neuroendocrine differentiation not previously connected to pancreatic islets, to currently uncharacterized zinc-finger TFs. Correlating with our single-cell map, we note that while many hits are dynamically expressed during SC-beta differentiation, many significant hits are instead relatively stable. This observation highlights the continued importance of perturbational experiments to complement expression profiling of developmental processes. Our work paves the way to creating bespoke human cell

lines whose pluripotency is intentionally curtailed by targeted modification in order to restrict the emergence of undesired fates.

Funding source: Harvard Stem Cell Institute, Helmsley Charitable Trust, JDR, JPB Foundation, Human Islet Research Network (HIRN, RRID:SCR_014393; UC4 DK104165-04 and UC4 DK104159-03), NIH T32GM007226

Keywords: beta cells, diabetes, directed differentiation

EPITHELIAL

CI204

EPIGENETIC MECHANISMS TRIGGERING DIFFERENTIATION AND PLASTICITY IN THE DEVELOPING MOUSE ORGAN OF CORTI

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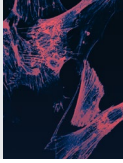
Sensory hair cell loss caused by loud noise, ototoxins or other environmental insults is the primary cause of hearing loss. While sensory hair cells in non-mammalian vertebrates can be readily regenerated from adjacent supporting cells, mammalian supporting cells lose regenerative potential during early development. The molecular mechanisms underlying the limited plasticity of nascent supporting cells remain unknown. By interrogating genome-wide histone modification changes during the differentiation of hair cells and supporting cells, we identified an intermediate cell type that has yet to undergo fate selection as a hair cell and supporting cell, but which is “epigenetically primed” to be permissive for hair cell and supporting cell differentiation. In progenitors, both hair cell and supporting cell gene regulatory networks are repressed by histone 3 lysine 27 trimethylation (H3K27me3); while the primed progenitor has lost much of its H3K27me3 character, and acquired the epigenetic mark typical of primed enhancers, H3K4me1. The reduction of H3K27me3 occurs at both hair cell and supporting cell-specific regulatory elements in the transition from de facto progenitor (E13) to the primed progenitor intermediate (E14.5). Meanwhile, low-level cell type-specific transcriptional activation at both hair cell and supporting cell-specific genes occurs in all the intermediate cells, and this plastic state is subsequently resolved by the onset of Notch-mediated lateral inhibition. Transition to the hair cell fate is driven by acquisition of cell type-specific H3K27 acetylation (H3K27ac). Our findings suggest that the intermediate primed progenitor may represent a required metastable state, that can subsequently be resolved by Notch-mediated lateral inhibition. Understanding the epigenetic basis for the transdifferentiation potential of nascent supporting cells will provide insights into hearing restoration attempts through hair cell regeneration.



Keywords: Inner ear, Differentiation, Transdifferentiation

EYE AND RETINA

CI297

GENERATION OF A PITX2-EGFP REPORTER LINE OF HUMAN INDUCED PLURIPOTENT STEM CELLS ENABLES ISOLATION OF PERIOCULAR MESENCHYMAL CELLS.

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PITX2 (Paired-like homeodomain transcription factor 2) plays important roles in asymmetric development of the internal organs and symmetric development of eye tissues. During eye development, cranial neural crest cells migrate from the neural tube and form the periocular mesenchyme (POM). POM cells differentiate into several ocular cell types, such as corneal endothelial cells, keratocytes, and some ocular mesenchymal cells. In this study, we used transcription activator-like effector nuclease technology to establish a human induced pluripotent stem cell (hiPSC) line expressing a fluorescent reporter gene from the PITX2 promoter. Using homologous recombination, we heterozygously inserted a PITX2-IRES2-EGFP sequence downstream of the stop codon in exon 8 of PITX2 Cellular pluripotency was monitored with alkaline phosphatase and immunofluorescence staining of pluripotency markers, and the hiPSC line formed normal self-formed ectodermal autonomous multizones. Using a combination of previously reported methods, we induced PITX2 in the hiPSC line and observed simultaneous EGFP and PITX2 expression, as indicated by immunoblotting and immunofluorescence staining. PITX2 mRNA levels were increased in EGFP-positive cells, which were collected by cell sorting, and marker gene expression analysis of EGFP-positive cells induced in self-formed ectodermal autonomous multizones revealed that they were genuine POM cells. Moreover, after 2 days of culture, EGFP-positive cells expressed the PITX2 protein, which co-localized with forkhead box C1 (FOXC1) protein in the nucleus. We anticipate that the PITX2-EGFP hiPSC reporter cell line established and validated here can be utilized to isolate POM cells and to analyze PITX2 expression during POM cell induction.

Funding source: This work was supported in part by the Project for the Realization of Regenerative Medicine of the Japan Agency for Medical Research and Development.

Keywords: Periocular mesenchyme (POM), PITX2, TALEN

GERMLINE

CI296

IDENTIFICATION OF MEIOTIC GERM CELL-SPECIFIC MGA SPLICE VARIANT THAT FUNCTIONS AS A NEGATIVE REGULATOR OF NON-CANONICAL PRC1 LEADING TO THE PROMOTION OF MEIOTIC ONSET

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Non-canonical Polycomb Suppressor Complex 1 (PRC1.6) has been shown to suppress precocious meiotic entry in germ cells. Therefore, it is conceivable that germ cells disrupt PRC1.6 function at the timing or prior to their true meiotic onset. However, how germ cells inactivate this complex remains largely obscure. Our careful inspection of publicly available RNA-sequence data of genes encoding either one of 14 components of PRC1.6 complex led to the identification of a splice variant of Mga with extra-sequence derived from a portion of 18th intron. Interestingly, further analyses revealed that this variant is generated rather specifically in germ cells that are either in process of or immediately after the meiosis. MGA is a large scaffolding protein and constitutes a DNA binding domain of the complex by dimerizing with MAX (also a component of PRC1.6) through their common bHLH-LZ domains. However, the MGA isoform derived from this variant lacks this domain due to premature termination codon (PTC) in the newly inserted sequence. Like Mga splice variant, mRNAs with PTC are in general subjected to rapid degradation with the RNA surveillance mechanism known as non-sense-mediated mRNA decay (NMD). However, since it is known that germ cells at meiotic stage are rather defective in NMD, we hypothesized that Mga variant mRNA exists rather stably in meiotic germ cells and is translated into a mutant MGA protein that lacks bHLH-LZ domain. Then, the translated mutant MGA protein functions as a dominant negative factor against the construction of PRC1.6 complex. In line with this hypothesis, we found that Mga variant levels in spermatocytes were not influenced by the treatment of NMD inhibitor, i.e., cycloheximide and we could detect MGA mutant protein with nuclear extract from testis. Next, we examined the consequence of forced

expression of Mga variant in embryonic stem cells (ESCs), since disruption of PRC1.6 in ESCs has been shown to provoke meiotic onset ectopically. In accordance with our expectation, our analyses revealed that overexpressed Mga variant led to the induction of ectopic meiosis in ESCs that is assessed by upregulation of meiosis-related genes. Taken together, our data indicate that germ cells generate dominant negative MGA mutant against PRC1.6 with exquisite use of special situation of NMD-impaired background.

Keywords: meiosis, alternative splicing, PRC1.6

HEMATOPOIETIC SYSTEM

CI213

CDX4 REGULATES THE EXPRESSION OF HEMATOPOIETIC AND CARDIOGENIC GENES WITHIN EARLY MESODERM

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To achieve efficient, reproducible directed differentiation of human pluripotent stem cells (hPSCs) towards specific mesodermal cell types, we must not only understand the origins of each lineage, but also the underlying genetic processes regulating their specification. In turn, this will enable the development of a unique, powerful platform for disease modeling and future cell replacement strategies. To this end, our lab aims to generate hematopoietic stem cells (HSCs) from hPSCs. By utilizing stage-specific WNT signal modulation to obtain exclusively extra-embryonic-like or exclusively intra-embryonic-like (definitive) hematopoietic progenitors from hPSCs, we recently identified that CDX4, expressed within definitive hemogenic mesoderm, is a critical regulator of definitive hemogenic endothelium specification. In order to mechanistically study CDX4-mediated hematopoietic development, we utilized an engineered iCDX4;CDX4Y/- hPSC line to precisely identify its transcriptional targets. Paired RNA/ChIP sequencing revealed that CDX4 was both binding and upregulating 73 gene targets, including the hematopoietic regulators HOXA3/5/7 and LMO2, consistent with a role in definitive hematopoietic development. Interestingly, CDX4 also bound and repressed the expression of 37 genes, including TBX20, suggesting it may also simultaneously play role in suppressing cardiomyocyte development. Critically, we found that CDX4 expression inversely correlated with cardiogenic capacity. In the presence of stage-specific CDX4 expression, definitive hematopoietic specification occurred normally, but when placed under cardiogenic-promoting differentiation conditions, cultures yielded very few (~12%) CD90negcTNT+ cells. Conversely, in the absence of CDX4 expression, definitive hematopoietic specification is reduced, but cardiac-promoting conditions exhibited increases (~2-fold) in GATA4, TBX5, and TBX20 expression and led to a significant increase (~3-fold) in CD90negcTNT+ cells. Collectively, these results suggest that CDX4 rapidly promotes hematopoietic development, while simultaneously repressing cardiomyocyte specification, within early mesoderm. These insights will ultimately improve hPSC-derived regenerative medicine applications.

Keywords: Hematopoiesis, Cardiogenesis, Mesoderm

CI215

CELL SIZE DETERMINES STEM CELL POTENTIAL DURING AGING

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A remarkable feature of stem cells is their small size, with human hematopoietic stem cells (HSCs) measuring a mere 7 µm in diameter. Whether a small size is important for stem cell function was not known. We show that HSCs increase in size under conditions known to decrease their function such as irradiation. Remarkably, inhibiting cellular enlargement during irradiation is radioprotective. Naturally large HSCs and intestinal stem cells also exhibit decreased stem cell potential indicating that large size is a characteristic of exhausted stem cells under physiological conditions. We further show that the accumulation of cell cycle inhibitors prevents large HSCs from proliferating. Finally, we demonstrate that murine and human HSCs enlarge during aging; preventing this enlargement preserves their proliferative capacity. We conclude that small cell size is critical for stem cell function and propose that increased stem cell size contributes to the functional decline observed during damage and aging.

Funding source: Howard Hughes Medical Institute, Jane Coffin Childs Memorial Fund, Swiss National Science Foundation, Glenn Foundation for Medical Research

Keywords: Cell size, mTOR, Aging

IMMUNE SYSTEM

CI223

GENE EDITING FOR COMMON VARIABLE IMMUNE DEFICIENCY

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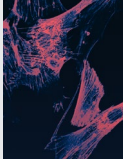
Common variable immune deficiency (CVID) is the most common primary immune deficiency in humans. This disorder usually presents in later childhood, adolescence, or adulthood. CVID results in a wide spectrum of disease that varies greatly in severity, organ involvement, and requirement for therapy. TNFRSF13B, a gene that encodes the B-cell surface receptor TACI, is mutated in ~10-15% of cases of CVID, though there is incomplete penetrance of disease within cohorts of families with TNFRSF13B mutations. In our study, we will silence TNFRSF13B in hematopoietic stem cells through lentiviral-driven short hairpin RNA (shRNA), which



we predict will decrease B-cell TACI expression. Then, we will introduce corrective codon-optimized cDNA into TACI-deficient CD34+ cells using CRISPR/Cas9, which will result in B-cell TACI expression, as the codon optimized sequence will differ at the DNA and RNA levels from endogenous TACI, while the amino acid sequences will be identical to the endogenous protein. Our work has demonstrated suppression of TACI expression through the use of a 10 hairpin construct driven by a MNDU3 promoter. Additionally, flow cytometry demonstrated expression of site-specific integrated mCitrine when using an exon 1 donor with ribonucleoprotein targeting Crispr/Cas9-induced double strand breaks within exon 1. These preliminary efforts target the creation of a gene therapy modality for common variable immune deficiency

Funding source: Supported by California Institute for Regenerative (CIRM) CSUN-UCLA Bridges to Stem Cell Research Program (CIRM TB1-01183).

Keywords: Common Variable Immune Deficiency, TACI expression, gene editing

CI224

EPIGENETIC CROSSTALK THROUGH KDM4A AND KDM4C SUSTAIN AN ONCOGENIC TRANSCRIPTIONAL AND SIGNALING STATES

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Cell fate specification is critically dependent on the coordination between cellular signaling and transcriptional regulation, processes frequently misregulated in cancers. Regulators of chromatin structure and function are mutated, misexpressed, amplified or deleted in several cancers. Here we show that cancer cells rely on the prototypical histone H3 Lysine 9 (H3K9me2/3) demethylases— KDM4A and KDM4C for their in vitro and in vivo maintenance. Mechanistically, inhibition of KDM4A and KDM4C cells results in a preferential increase of H3K9me2/3 over endogenous retro-elements (EREs), which are repressed by these modifications. Incidentally, these EREs are enriched for motifs recognized by Wnt-beta catenin effector transcription factors (e.g. LEF1, ETV6, and ETS2) and lineage-specific TFs (IRF8 and IRF4). Local increases in H3K9me2/3 represses H3K9me2/3 methyltransferases (SETDB1, SUV39H1, and SUV39H2) and the H3K9me2/3-dependent repressor TRIM28/KAP1. Consequently, there is global hyper-acetylation which results in the activation of inflammatory transcription factors, IRF1, IRF7, and IRF9. Importantly, analysis of transcriptomic data from cancer patients revealed that a small subset of genes, identified based on epigenetic and transcriptional mechanisms uncovered here, can risk stratify DLBCL and colorectal patients. Consistently, dual loss of KDM4A/4C reduces the viability of colorectal cancer lines suggesting a tissue-agnostic regulation of Wnt/beta-catenin signaling by KDM4A/KDM4C through epigenetic modulation of Wnt effector TFs. Our results highlight the benefit of targeting mechanisms that regulate global transcriptional networks and establish KDM4A and KDM4C as viable therapeutic targets in lymphomas and Wnt-driven tumors.

Keywords: epigenetics, histone modifications, cancer

CI226

DETERMINING CORRELATES OF REPROGRAMMING EFFICIENCIES IN T CELLS THROUGH FLOW CYTOMETRIC PROFILING

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iPSCs can be derived from a range of different somatic cell types, however iPSC derived from T-cells (T-iPSC) are unique in that they will retain the gene encoding the specific T-cell receptor. This means that upon differentiation of T-iPSC to T-cells, they will express that same TCR. Thus, T-iPSCs can be used to generate an endless supply of antigen specific T-cells which could be used as treatment against cancers and viral infections. Alternatively, for other cell types derived from T-iPSC used in regenerative medicine, this unique TCR sequence can be used as means of cell tracking post-engraftment. T cells can be

difficult to reprogram successfully, which is compounded by the large amount of variability from donor to donor that is dependent on a variety of factors including age, genetics, and disease status. The ability to predict upfront which donor's cells can be successfully reprogrammed would save much time and effort. Here, we have developed a means of predicting reprogramming efficiency of T-cells, based on the expression signature of a set of markers that are typically associated with T-cell exhaustion. This predictive method was consistent across multiple donors tested and allowed us to optimize the reprogramming workflow to maximize chances of generating T-iPSCs.

Keywords: Reprogramming, iPSC, T-Cells

MUSCULOSKELETAL

CI228

EPIGENOME EDITING FOR ENDOGENOUS ACTIVATION OF TENDON TRANSCRIPTION FACTORS

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Tendon trauma, accounting for about half of the 33 million musculoskeletal injuries in the U.S., does not spontaneously heal. Scar-like tissue is formed with high cellularity and disarrayed collagen fibers, failing to restore structural integrity, mechanical properties or functionality. Enhanced tendon regeneration using stem cell/progenitor cells remains an attractive approach. Proper lineage programming into tenocytes or tendon stem/progenitor cells has been elusive and will likely require multi-gene transcriptional regulation to achieve a sufficient lineage populations. Transcription factors Scleraxis (Scx) and Mohawk (Mkx), are extensively involved in tendon development, identity and healing. We take a multiplexed approach to precisely target and endogenously activate both tendon master regulators using genomic epigenetic editing with a dCas9-VPR CRISPR activation system. We hypothesize that robust endogenous activation of Scx and Mkx will activate downstream lineage pathways toward tenogenic differentiation. Four gRNAs per gene were designed, targeting the upstream region of each transcription factor, and then screened for the best performing gRNAs with HEK-293T cells. We observed sustained and increased gene expression at 24 and 48 hours and the best performing gRNAs targeting Scleraxis and Mohawk, produced a >100 and 4 fold increase in the target gene expression, respectively. Best gRNAs were then multiplexed together (10 ng) to achieve endogenous activation of both transcription factors concurrently, achieving a 43 and 2.6 fold increase of Scx and Mkx respectively (n=3). To further improve Mkx upregulation, we applied increasing gRNA concentrations and improved the Mkx expression up to 10 fold, suggesting a modular capability of our epigenetic activators. Future experiments with biologically relevant iPS or bone marrow derived mesenchymal stem cells include single-cell transcriptomics to characterize the lineage programming. In summary, we have established a cell fate engineering approach to epigenetically edit multiple tendon transcription factors using dCas9-VPR. To achieve proper lineage programming, a transcriptional network approach endogenously regulating multiple genes simultaneously may be necessary for enhanced tendon regeneration and future therapeutics.

Funding source: This project is supported by NIH/NIAMS 5R01AR071316-02 & 5R01AR065023-05 to C.H.L

Keywords: CRISPRa, Epigenome, Tendon

CI230

EPIGENETIC REGULATIONS IMPLICATE STEM CELL BEHAVORS DURING MUSCLE INJURY AND REPAIR

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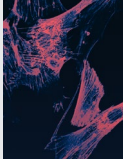
Injuries result in micro-environmental changes and influences the release of niche factors that govern resident stem cell behavior during muscle healing. Our previous study suggests that cellular reprogramming can be initiated by strong stimuli in injured skeletal muscles. In injured muscles, we discovered cells fundamentally different from the normal muscle satellite cells and termed then injury-induced muscle-derived stem cells (iMuSCs). After isolating them and running experiments, we discovered that they had multipotent behaviors such as the increased capacity for migration and differentiation. However, the mechanism behind the reprogramming of muscle cells to iMuSCs is largely unknown. This prevents our understanding of the basic biology behind injury and limits our therapeutic strategies in repairing injured muscles. Studies have shown that epigenetic modifications such as histone methylation can control the fate and behavior of stem cells during development and regulate stem cell-mediated regeneration of adult tissues. Therefore, we hypothesize that the injured milieu influences cellular reprogramming through epigenetic pathways and regulates the process of muscle healing. During our analysis of iMuSCs, we discovered that Msh homeobox (Msx1, a developmental musculoskeletal gene) is upregulated in iMuSCs compared to regular muscle satellite cells. Msx1 also is a histone methyltransferase involved in the locating of the transcriptionally repressive H3K27me3 at target sites. After initiating a hypoxic environment simulating the injured milieu, we discovered that global levels of H3K27me3 increased relative to the control. Additionally, the increased expression of Msx1 suggests that the injury environment initiated the reprogramming of muscle cells.



These findings are significant as they implicate epigenetics in the reprogramming of muscle cells during injury. This can lead to further research studying the involvement of epigenetics in maintaining stem cell homeostasis and function, thus regulating muscle healing.

Funding source: NIH

Keywords: Epigenetic regulations, Muscle injury, Stem cells

NEURAL

CI233

GENERATING CORTICAL NEURONS BY DIRECT INDUCTION OF INDUCED PLURIPOTENT STEM CELLS

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While previous studies have shown that specific neuron subtypes can be produced from induced pluripotent stem cells (iPSCs), efficient methods for producing excitatory glutamatergic neurons positively stained for transcription factor TBR1 remain to be developed. The production of TBR1+ neurons could be useful in developing potential therapeutic strategies for neuronal replacement in disorders such as stroke or Alzheimer's disease (AD); in which, entorhinal cortex (EC) layer 2 neurons degenerate at early stages. Previously, TBR1+ neurons have been generated from human iPSCs via Neuroepithelial-like cells (NES) cells. However, alternative approaches may streamline development of these neurons for patient treatment. Recently, a direct induction protocol has been reported to generate cortical neurons from iPSCs, though it is not clear if TBR1 is expressed. To determine if TBR1+ neurons can be generated more efficiently, we used direct induction and evaluated the genesis of TBR1+ neurons, then we compared the efficiency with the NES cell differentiation method. In the direct induction protocol, mouse or human feeder free iPSCs were infected with lentivirus-Neurogenin 2 using a doxycycline and puromycin selection system and cultured on astrocyte feeder layers. For comparison, we used an embryoid body and rosette formation protocol to produce NES from iPSCs, followed by differentiation into cortical neurons using Wnt3a, BMP4, and cyclopamine. All generated neurons were analyzed by immunocytochemistry, Western blot, and qPCR to examine markers for cortex-specific progenitor and neurons. We also screened these cells for EC-specific markers because our ultimate goal is generation of specific neurons for transplantation. Then, we used single nuclei RNA sequencing for transcriptome-wide gene expression analysis of individual neurons. Our data will lay a foundation for efficiently generating cortical neurons of TBR1+ fate and provide a starting point for optimization of generating EC neuron subtypes. We believe that the production of specific neuron types will be an important feature of future cell therapies, particularly if the generated neurons can integrate into host cortex and reconstitute neuronal circuits.

Funding source: California Institute for Regenerative Medicine

Keywords: induced pluripotent stem cell, neuronal induction, Tbr1

CI236

THE ANALYSIS OF GENE EXPRESSION LEVELS FROM PRIMARY BRAIN TUMOR CELL LINES

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Glioblastoma multiforme (GBM) represents the most common and most aggressive primary brain tumor despite a multimodal therapy including a radio-chemotherapy and surgical removal of the tumor, the overall survival time is still only 12 to 15 months. Glioma stem cells (GSCs) are responsible for the formation, expansion, recurrence, and the treatment resistance of GBM that the identification of specific GSC markers may help to develop targeted therapies. We were isolated primary GBM cell from surgical removed tumor tissues and performed the expression analysis of stem cell marker such as CD133 and CD44. And transcriptome was analysis with commercial cell line, U87-MG. From result, we've got dataset (2,246/20,809 gene expression, $P<0.05$) which consist with up-regulate and down-regulate 1,108 genes (5.32%) and 1,138 genes (5.47%), each. In GBM, brain tumor related gene was elevated such as NF2, TRAF7, IDH1, ATRX, Sparc, ELF4, CD44, and Nestin with oncogene, SMO and AKT1. whereas GFAP expression was not changed. This expression was correlated with PI3K-Akt Signaling Pathway, Signaling Pathways in Glioblastoma, Focal Adhesion-PI3K-Akt-mTOR-signaling pathway, Ras Signaling, Integrated Breast Cancer Pathway, TGF-beta Signaling Pathway, VEGFA-VEGFR2 Signaling Pathway, EGF/EGFR Signaling Pathway, and Oxidative Damage. In summary, we found that several potential stem cell and differentiation markers in stem-like GBM cells which is their expression levels correlated with patients' variation that need to further studied in different subtype.

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Keywords: Brain tumor, GBM, Transcriptome

CI244

COMPREHENSIVE TRANSCRIPTOMICS AND PROTEOMICS OF HUMAN-IPSC DERIVED NEURONS FOR DRUG DISCOVERY

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To build a credible chain of translation in which the in vitro model captures the molecular mechanism of the clinical condition, it is necessary to have human physiologically relevant cell models that can be produced in a robust and reproducible manner. Literature reports have shown that over-expression of critical transcription factor, neurogenin 2 (NGN2), can reset the epigenetic state of induced pluripotent stem cells (iPSC) to rapidly generate highly pure populations of cortical excitatory glutamatergic neurons. This method allowed us to produce neurons with high yield and minimal batch-to-batch variation. A healthy control iPSC was engineered to inducibly express NGN2, a transcription factor critical for driving endogenous neuronal differentiation. 7189L-NGN2-H2 iPSCs were differentiated into neurons for three days and cryopreserved. Five batches of neurons were thawed, matured, and characterized for consistency by gene expression (RNA-seq), protein expression (nLC-MS/MS) and function by microelectrode array technology (MEA). Both proteomics and RNA-seq analyses showed that the batch-to-batch variation was minimal, and the neurons became more mature with time. More than 11,000 proteins were identified in proteomics study, spanning an abundance range of over seven orders of magnitude. Proteomics data showed that upregulation of proteins associated with neuronal function such as synapse formation and neurotransmitter production throughout the time course. RNA-seq study showed that axonal guidance and synaptogenesis were most enriched gene sets in a time-dependent manner. MEA studies showed robust network activity of the neurons after 28 days of maturation and confirmed the presence of AMPA dependent network bursting, consistent with excitatory neurons. We have established a robust, reproducible, scalable protocol to generate human cortical neurons which were fully characterized by proteomics and transcriptomics for disease modeling and assay development.

Keywords: Human iPSC-derived neurons, Transcriptomics, Proteomics

CI317

REGULATION OF NEURAL DIFFERENTIATION THROUGH MRNA METHYLATION IN HUMAN STEM CELLS

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Stem cells are able to differentiate into any cell type in the body, a property known as pluripotency that enables them to initiate early growth and development. In order to maintain a pluripotent state, yet rapidly differentiate in response to external signals, stem cells need to exert tight control over expression of lineage-specific factors. However, the mechanisms by which this occurs are poorly characterized. N6-methyladenosine (m6A) RNA methylation is an abundant post-transcriptional modification that influences RNA structure and association of RNA-binding proteins and miRNAs. RNA methylation is important for the establishment and maintenance of pluripotency, but the role of m6A-binding proteins is less clear. In this study, we show the RNA-binding protein YTHDF2 contributes to pluripotency by targeting a group of mRNAs encoding factors important for neural development. The down-regulation of YTHDF2 during neural differentiation is consistent with increased expression of neural factors during this time. Based on our results, we propose that stem cells are primed for rapid differentiation by transcribing low levels of mRNAs encoding neural factors that are subsequently targeted for degradation, in part by YTHDF2, until differentiation is induced. This model illuminates the role of another factor involved in the complex regulatory pathways utilized to tightly control gene expression in stem cells. Our study expands the knowledge of how stem cells regulate gene expression, and identifies avenues to optimize use of stem cells in personalized medicine.

Keywords: RNA decay, m6A methylation, induced pluripotent stem cells

NEW TECHNOLOGIES

CI291

ELUCIDATING DYNAMIC GENE NETWORK REGULATION FROM SINGLE-CELL TRANSCRIPTOMIC DATA VIA EPOCH

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Elucidating regulatory relationships between transcription factors (TFs) and target genes is fundamental to understanding how cellular identity is established and maintained. Computational gene regulatory network (GRN) reconstruction methods aim to map this control by inferring relationships from transcriptomic data. However, such methods suffer from several drawbacks including high false positive rate and low sensitivity, which results in imprecise networks. Moreover, many methods are computationally burdensome, which limits a users' ability to hone analysis through iterative application. Finally, the networks reconstructed by these methods are typically static, and it may not be apparent how they transition from one topology to another. Here we present Epoch, a computational GRN reconstruction tool that leverages single-cell transcriptomics to efficiently infer dynamic network structures. Epoch leverages pseudotemporal ordering to extract dynamically expressed genes for reconstruction. Epoch groups genes by temporal expression patterns, making it possible to trace dynamic changes in network topology. Finally, Epoch optionally refines networks by weighting interactions by lag time (peak-time distance) between temporal expression profiles of TFs and target genes. We benchmarked Epoch against commonly used reconstruction methods using single cell data from a developmental system with well characterized regulatory programs and synthetic single cell data generated using the Dyngen package. Based on area under the precision-recall curve, Epoch out performs original versions of CLR and GENIE3 and is on par with GENIE3 reconstruction limited to dynamically expressed genes. Importantly, Epoch has the additional benefit of running substantially faster than GENIE3. We applied Epoch to reconstruct the dynamic networks guiding early cell fate decisions, including germ layer specification, in day 0 through day 8 of in vitro directed differentiation of mouse ESCs across 4 protocols. Our analysis identified both novel and previously reported regulatory relationships, such as Pou3f1 and Sox11 in promoting neural fate commitment, and revealed the dynamic topological changes from a pluripotency network through in vitro equivalents of primitive streak, endoderm, mesoderm, and neurectoderm.

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Keywords: gene regulatory networks, single-cell transcriptomics, directed differentiation

CI310

ENHANCING CELL FATE REPROGRAMMING WITH AN ARTIFICIALLY EVOLVED POU FACTOR

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Cellular reprogramming technologies hold the promise that personalized disease models, tailored drug evaluation for individual patients, and regenerative therapies could become a reality in clinical settings for a range of diseases. To reach this goal cell fate conversions need to be efficient, fast, scalable, and can reproducibly produce fully functional cells. Our group has utilized protein engineering and directed molecular evolution in mammalian cells to identify potent, artificially evolved transcription factors (eTFs) based on the scaffolds of endogenous proteins. To benchmark screens with pooled eTF libraries and phenotypic selection, we used the scaffold of Pit-Oct-Unc (POU) factors and the generation of induced pluripotent stem cells (iPSC). Using an iterative screening approach we identified an enhanced POU factor (ePOU) that substantially outperform wild-type Oct4 in terms of speed, efficiency, synergizes with re-engineered Sox factors, and remains active in two-factor cocktails. To reveal the molecular mechanism that endows the ePOU with its enhanced capacity we performed a detailed analysis of its biochemical as well as biophysical properties and studied genome-wide binding, regulation, and chromatin dynamics. Here, I will show how DNA binding, chromatin association and opening, gene regulation, protein stability, and partner factor interactions can contribute to the enhanced capacity of the ePOU in reprogramming. We anticipate that the conceptual framework provided by this work will open avenues for the engineering of factors in more challenging direct transdifferentiation systems and for the tailoring of cellular phenotypes with potential applications in vitro as well as in vivo.

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Keywords: Oct4, Pluripotency Reprogramming, Protein Engineering

CI327

BIOPHYSICAL REGULATION OF IN-VITRO GASTRULATION USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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In-vivo embryogenesis demonstrates a flawless example of a self-organized system which uses a combination of diffusible morphogens and the effect of physical stress of the endometrial constraint as inducers for germ layer differentiation and their organization. Modulating various physical environmental factors has supported the growth and development of a pre-gastrulation mouse embryo in in-vitro studies. Although a lot of information is available on how diffusible morphogens affect gene expression and signalling dynamics for germ layer differentiation, the mechanistic role of the physical environment in inducing these events is poorly understood. Due to ethical and physiological limitations of studying human embryos, in-vitro models present a better platform to understand the contribution of mechanical stress towards the differentiation and self-organization capacity of pluripotent stem cells. Previous studies have demonstrated a role of colony geometry in germ layer organization of embryonic stem cells in response to exogenous BMP4 induction. Here, we present an in-vitro gastrulation model, which uses polyacrylamide hydrogels to toggle and combine physical factors like substrate stiffness and geometry and create a self-sufficient system for differentiation and spatial organization using induced pluripotent stem cells, without the need of using exogenous induction. We demonstrate that in response to substrate stiffness and colony geometry, iPS cells differentiate into and organize a SOX17+ endodermal population along with the appearance of a T/Brachyury+ population which represents primitive streak/ mesoderm without using any exogenous chemical morphogens. These micro-colonies control the orientation of cell packing and the confinement causes cytoskeleton mediated nuclear shape alterations, inducing the signalling dynamics to create these gastrulation-like events. This model presents a simple approach to understand how pluripotent cells interpret the intricacies of their physical microenvironment to alter cell identity and mediate spatial organization, which better represents the in-vivo environment.

Funding source: This work was supported by the Australian Research council Grant # FT180100417

Keywords: Micropatterning, mechanics, hydrogel, in-vitro gastruation, spatial organization, iPSCs, tri-lineage, primitive streak

Theme: Clinical Applications

ADIPOSE AND CONNECTIVE TISSUE

CA105

MACHINE LEARNING MESENCHYMAL STEM CELL EFFICACY FOR CARTILAGE REGENERATION

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Cartilage injury is unfortunately common due to tears, accidents and arthritis. It often leads to joint pain, stiffness, and inflammation. Cartilage disorders affect millions of people worldwide, including 52.2 million adults in US, and more than 10 million in UK. In particular, osteoarthritis alone affects more than 200 mil- lion people globally. The inconsistency of mesenchymal stem cell's (MSC) efficacies for regenerative medicine has been seen in many clinical trials. Precise prediction on the therapeutic outcome of a MSC therapy based on the patient's conditions would provide valuable references for clinicians to decide the treatment strategies. In this article, we perform a meta-analysis on MSC therapies for cartilage repair using machine learning. The unique features of our machine learning model in handling missing data and computing prediction uncertainties have enabled precise prediction of post-treatment cartilage repair scores with the coefficient of determination of 0.637 ± 0.005, using a small database generated from published in vivo and clinical studies. From this model, we identify defect area percentage, defect depth percentage, implantation cell number, body weight, tissue source, and the type of cartilage damage as critical properties that would significant impact cartilage repair. A dose of 17-25 million MSCs has been identified to achieve optimal cartilage repair in human. Further, critical thresholds at 6% and 64% of cartilage damage in area, and 22% and 56% in depth have been identified to significantly diminish the efficacy of MSC therapy. This study demonstrates a machine learning approach to predict patient-specific cartilage repair post MSC therapy. This machine learning model can be applied to identify and investigate additional critical factors involved in MSC-induced cartilage repair, and adapted for other clinical indications.

Funding source: Agency for Science Technology and Research (A*STAR)

Keywords: Machine Learning, Cell Therapies, Mesenchymal Stem Cells

EARLY EMBRYO

CA116

CHEMICAL CYTOPROTECTION AS A NOVEL STRATEGY FOR EFFICIENT AND SAFE GENOME EDITING IN HUMAN PLURIPOTENT STEM CELLS

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The CRISPR-Cas9 and induced pluripotent stem cell (iPSC) technologies are among the most important scientific breakthroughs and hold great promise to transform disease modeling, drug discovery, and development of new cell and gene therapies. However, low editing efficiencies and poor cell survival of dissociated iPSCs has limited the full potential of both technologies. Here, we report improved single cell cloning as well as significantly increased gene editing efficiency in human iPSCs by using a newly developed small molecule cocktail termed “CEPT”. First, using advanced microfluidics-based cell dispensing technologies, we demonstrated that single cell cloning of iPSCs was significantly enhanced in the presence of CEPT versus the commonly used ROCK inhibitor Y-27632. Hence, identification of correctly edited clones was dramatically improved in comparison to current strategies. Second, we discovered that ordinary single cell dissociation of iPSCs caused DNA double-strand breaks as shown by the Comet assay and Western blotting against gamma-H2AX and other marker proteins. Importantly, the use of the CEPT cocktail supported the structural integrity of dissociated cells and prevented DNA damage as compared to Y-27632 or the commercially available reagent CloneR. Third, taking advantage of the cytoprotective effects conferred by CEPT, we could demonstrate increased gene editing efficiencies using various genetic and biochemical assays, thereby providing novel insights into DNA repair mechanisms in human pluripotent cells. In summary, we propose that the chemically defined CEPT cocktail will become an essential tool for improved and safer genome editing for basic research and clinical applications.

Funding source: NIH Common Fund Regenerative Medicine Program

Keywords: CRISPR, Gene editing, human pluripotent stem cell

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CA118

CELL THERAPY IMMUNOPROTECTION TESTED IN SPONTANEOUSLY DIABETIC DOGS

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Diabetes mellitus is a chronic metabolic disorder affecting humans as well as several species of domestic pets. Among the veterinary species, spontaneous diabetes is a very common disorder diagnosed in dogs and cats. Diabetes in dogs most closely matches human Type 1 diabetes with very similar clinical signs and pathophysiology. The long-term clinical management of the condition is also similar between species with the need for insulin injections. One of most commonly prescribed formulation of insulin in dogs is the human form (Humulin). Traditionally research dogs served as an important translational model for the research and management of diabetes in humans. Dogs with spontaneous diabetes can provide a better match to human disease and offer a very useful pre-clinical translational model of therapies on the path to humans. We developed a novel technology for microencapsulation of cells using biocompatible hydrogels including polyethylene glycol and hyaluronic acid. We have completed safety and efficacy studies in diabetic research dogs, and safety studies in spontaneously diabetic dogs using allogeneic canine islets transplantation. Without immunosuppression, the islet cells were protected from a foreign body response in research dogs. Insulin requirements prior to the transplant ranged from 10 - 17 units of insulin per day and dropped to insulin independence following the microencapsulated islet transplant. Normoglycemia was maintained through the study, while blood and urine chemistry were within reference ranges. A subsequent safety study in spontaneously diabetic dogs was conducted at sub-threshold cell doses. Like in the research dogs, the animals with spontaneous disease had no adverse events and blood chemistry was normal, with the exception of blood glucose. Following the transplants, some spontaneously diabetic dogs showed insulin independence for a short period of time, even with the sub-threshold dosing, although most dogs required a lower dose of exogenous insulin. We are now working on encapsulation of a scalable source of insulin-producing cells derived from induced pluripotent stem cells. The data demonstrate the use of spontaneous animal diseases in a manner that can benefit translation of preclinical studies for human therapies.

Keywords: Diabetes, Encapsulation, Islet

CA127

BLASTOCYST COMPLEMENTATION RESTORES LIVER DEVELOPMENT IN HHEX KNOCKOUT MICE AND PIGS

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There are thousands of patients currently waiting to receive liver transplants and demand is growing due to increasing rates of chronic liver disease and declining availability of suitable donors. Blastocyst complementation is one emerging methodology of regenerative medicine with the potential to meet this urgent need. The purpose of the present study is to investigate the potential to generate exogenic liver tissue in mice and pigs through complementation of blastocysts edited to knockout HHEX, a gene necessary for proper liver development. CRISPR/Cas9-mediated knockout (KO) of HHEX was accomplished by electroporation delivery of one-gRNA in mouse zygotes or microinjection delivery of two-gRNAs in pig zygotes, resulting in an efficient and large deletion in exon 2 in both species. Transfer of KO embryos into surrogate mice or pigs resulted in a severe, embryonic lethal HHEX KO phenotype with an absence of liver tissue. Transcriptomic and histological analyses of mouse and pig HHEX KO fetuses demonstrated significant developmental defects particularly in liver-specific expression of alpha-Fetoprotein, Transthyretin, and Albumin. We next investigated whether intraspecies blastocyst complementation of mouse and pig HHEX KO embryos with pluripotent stem cells (PSCs) could restore liver formation. Intraspecies complementation of mouse HHEX KO embryos with GFP+ mouse iPSCs resulted in chimeric fetuses surviving past the stage of HHEX KO embryonic lethality. Histological analysis indicated the presence of donor cell-derived liver tissue co-expressing GFP and albumin. Similarly, intraspecies complementation of pig HHEX KO morula with GFP+ pig blastomeres resulted in chimeric fetuses with GFP+ liver cells expressing liver-specific proteins. Finally, we investigated interspecies complementation of pig HHEX KO embryos with GFP+ marmoset ESCs overexpressing pro-survival protein BCL2. We observed several interspecies chimeras with donor derived GFP+ cells surviving and proliferating in different fetal tissues. This work demonstrates that loss of liver development in the HHEX KO can be rescued through intraspecies blastocyst complementation in mice and pigs, although additional studies are still needed to enhance contribution to liver formation by xenogeneic cells.

Funding source: NIDDK R01 DK117286

Keywords: Blastocyst complementation, Gene editing, Liver development

EPITHELIAL

CA131

DIFFERENTIATION AND TRANSPLANTATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OTIC EPITHELIAL PROGENITORS INTO THE DAMAGED COCHLEA

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Neurosensory hearing loss is associated with degeneration of hair cells (HCs) in the inner ear. In mammals, this degenerative process is irreversible. Cell therapy for the inner ear would require a better understanding of factors and signals that direct differentiation of stem cells into HCs. The work presented here proposes a rapid and efficient in vitro strategy to generate HCs from human induced pluripotent stem cells (hiPSCs). We also present results of transplantation of hiPSC-derived otic progenitors into an in vivo model of neurosensory hearing loss. Our findings provide a new tool for cell-based therapies in the inner ear. Our goals were to generate human otic progenitor cells (hOPCs) from hiPSCs and to explore their in vivo engraftment after injection in the adult ototoxin-damaged cochlea.The hOPCs were generated from the induction of hiPSCs in a monolayer culture system as previously reported [Lahlou et al., 2018]. The differentiation of hOPCs was monitored by the expression of a comprehensive panel of otic lineage markers by qPCR and immunocytochemistry. Partially differentiated hOPCs were labeled with the fluorescent lipophilic tracer-DiI and transplanted into the cochlea of ototoxin-treated guinea pigs through a cochleostomy. The hOPCs migrated throughout the cochlea, engrafted in non-sensory regions and survived up to four weeks post-engraftment. Some of the engrafted hOPCs responded to environmental cues within the damaged cochlear sensory epithelium and displayed molecular markers of HCs and supporting cells. We further extended these results by delivering HC progenitors derived from Atoh1-GFP mice [Lopez-Juarez et al., 2019]. The ability of otic progenitors to migrate, engraft, and initiate sensory cell differentiation in the damaged auditory epithelium is a key step towards developing stem cell-based cell therapies for the treatment of neurosensory hearing loss.

Funding source: Supported by the European Commission, under the FP7-health-innovation-otostem project

Keywords: Sensorineural hearing loss, Human otic progenitors, Cell transplantation

CA132

GENERATION AND PURIFICATION OF MOUSE PLURIPOTENT STEM CELL DERIVED DISTAL TIP-LIKE CELLS FOR IN VIVO TRANSPLANTATION AND FUNCTIONAL ANALYSIS

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Recent work suggests that mouse Sox9+ embryonic lung epithelial tip cells cultured in vitro can engraft and differentiate in injured mouse lungs, providing a potential method for cell-based therapy of lung injury. However, the use of primary embryonic donor cells severely limits the clinical applicability of this approach. Directed differentiation of mouse pluripotent stem cells (mPSCs) may provide an alternative source of donor cells, but current protocols do not produce Sox9+ progenitor cells comparable to those generated by primary culture. Here we describe an optimized protocol for the directed differentiation of mPSCs into distal tip-like cells. The resulting cells express high levels of distal tip markers, maintain low levels of mature alveolar markers, and can be expanded in culture, similar to cultured primary embryonic tip cells. Through optimized serum-free directed differentiation of mPSCs we prospectively isolate the Nkx2.1+/EpCAM+ source cells that give rise to these tip-like progenitors, define their global transcriptomic programs through RNA-Seq, and develop conditions for their differentiation and expansion as “epithelial-only” spheres in 3D culture. In parallel, we have successfully transplanted cultured primary Sox9+ tip-like progenitor cells into injured mouse lungs, where these cells give rise to both Sftpc+ and Pdpn+ cells. In order to perform similar transplant experiments using mPSC-derived cells, we have generated an isogenic iPSC line marked with ubiquitous luciferase and GFP. Having developed these tools, we can now directly compare the in vivo functionality of primary and mPSC-derived tip-like progenitors through isogenic competitive lung transplantation assays.

Funding source: 2 T32 HL 7035-41 A1 1 F32 HL149263-01

Keywords: Lung, Cell Transplantation, Directed Differentiation

EYE AND RETINA

CA147

EFFECT OF ECM SUBSTRATES AND CELL CULTURE SURFACE ON RABBIT ORAL MUCOSAL EPITHELIAL CELLS ATTACHMENT AND GROWTH

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Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) have proven to be an effective cell source for the treatment of limbal stem cell deficiency. CAOMECS was produced to reconstruct the epithelium of the ocular surface. However, cell culture conditions/reagents used to produce CAOMECS were not xeno-free and feeder free nor approved for clinical grade application. The aim of the present research was to produce a multilayered cell sheet grown with GMP-grade surface and extracellular matrix. Rabbit oral mucosal epithelial cells (OMECS) were isolated and seeded on GMP-certified 6 multi-well plate (6MWP), coated with clinical grade extracellular matrix, CTS™ CELLstart™ and Biolaminin™ 521-CTG to examine their effect on cell attachment and growth to form a multilayered cell sheet. We also evaluated the effect of a newly designed cell culture medium that is xeno-free and clinical grade to culture OMECS and differentiate it toward corneal epithelial cells. Both humanized substrates, CELLstart™ and Biolaminin™ 521 promoted OMECS attachment to treated 6MWP surface. CELLstart™ substrate showed higher cell attachment and confluence as compared to Biolaminin™ 521 for OMECS, as shown by live imaging during microscopic examination. Cells showed typical epithelial morphology with several colony forming units that helped the cells grow and form a monolayer in less than 10 days, and a multilayered cell sheet in less than 3 weeks. Cell sheets were harvested using GMP grade collagenase treatment supplemented with calcium chloride. Western blot analysis showed stronger expression of deltaNp63 (a progenitor stem cell marker), integrin beta 4 and keratin 3 in cell sheets grown with CELLstart™, compared to cell sheets cultured with Biolaminin™ 521. These results suggest that our designed clinical grade cell culture conditions successfully produced oral mucosal epithelial cell sheets that qualify for safe grafting onto the ocular surface of patients with limbal stem cell deficiency.

Funding source: Support by Emmaus Medical, Inc.

Keywords: Oral mucosal epithelial cell sheet, Cornea, Limbal Stem Cell Deficiency, Clinical grade cell culture conditions

HEMATOPOIETIC SYSTEM

CA156

GENERATION OF T CELLS USING IPSC-DERIVED HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Induced pluripotent stem cells (iPSCs) can be differentiated to form all key cell types (beta cells, hematopoietic stem and progenitor cells (HSPCs), and thymic epithelial cells) that mediate the initiation and progression of type I diabetes (T1D), and therefore hold great promise for the modeling and mechanistic studies of T1D. However, the generation of engraftable HSPCs that are capable of reconstituting the immune system has proven difficult. T cells can be differentiated from iPSCs in vitro, but the process is similarly inefficient, and the function and gene expression profile of iPSC-derived T cells are not identical to those of mature peripheral blood T cells. To explore how to improve efficiency and T cell function, we have generated HSPCs and T cells from human iPSCs via two different strategies. In the first strategy, iPSCs were directly differentiated into hemogenic endothelium, followed by cell fate conversion to HPSCs through the treatment of a combination of small molecules/growth factors that recapitulate the endothelial-to-hematopoietic transition (EHT). During EHT the cells were transduced with a cocktail of 5 transcription factors (RUNX1, ERG, LCOR, HOXA5, HOXA9) to further enhance the production of definitive, adult-type HSPCs with the capacity of engraftment and lymphoid repopulation in irradiated mice. In a second strategy, iPSCs were first differentiated into committed CD34+CD45+ myelo-erythroid precursors that can be stably propagated in vitro by virtue of conditional immortalization with a distinct cocktail of 5 doxycycline-inducible transcription factors (HOXA9, ERG, RORA, SOX4, and MYB). These precursors were then differentiated to T cells via co-culture on OP9 stroma expressing Notch ligands. After 5 weeks of culture, CD4/CD8 single positive T cells that express CD3 and TCRαβ were detected. Additionally, in order to further facilitate the generation and maturation of T cells, iPSCs were differentiated to form thymic epithelial cells (iPSC-TECs) and co-cultured with iPSC-derived T progenitors. Our result showed that co-culture with iPSC-TECs significantly enhanced the production of CD3+ T cells. In conclusion, we have developed new platforms that allow us to more efficiently generate functional HSPCs and T cells from iPSCs.

Keywords: iPSC, T cell differentiation, Hematopoietic stem and progenitor cell

CA248

GENERATION OF T-LYMPHOCYTE PROGENITORS FROM HUMAN PSC FOR IMMUNE SYSTEM RECONSTITUTION

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Autologous modified T-cell progenitors looks attractive sours for a treatment of immunodeficiencies. To date, T-cell progenitors have been successfully obtained from umbilical cord blood. The use of hiPSCs to produce T-cell progenitors is promising strategy, since these cells will be autologous to the patient that will remove the immunocompetence barrier, also hiPSC can be successfully edited and tested for offtargeted effects. We have developed a protocol for obtaining T-lymphocyte progenitors from hPSCs. The Protocol includes two stages. In the first stage, we obtain hematopoietic progenitors under fully characterized serum- and feerder-free conditions. The second stage of differentiation involves specialization in T-lymphocytes. We described in detail the dynamics of early T-cell differentiation and specialization and identified pre-T, pro-T1 and pro-T2 stages, characterized by expression of CDRA45+CD34+CD7+, CDRA45+CD34-CD7+CD5-, CDRA45+CD34-CD7+CD5+ respectively. We showed that under in vitro conditions, our progenitors can reach DP CD4+CD8+CD3+ stage and successfully rearrange TCRab giving rise to polyclonal CD8ab cells. We have selected conditions that allow us to obtain T-progenitors in quantities that allow us to use these cells for transplantation experiments in animals and for RNA sequencing, which will further help us to check their thymus homing activity and compare them with T-cell progenitors from umbilical cord blood.

Keywords: T-lymphocyte progenitors, Fully defined differentiation conditions, Homing to thymus

NEURAL

CA180

GENE EXPRESSION PROFILE OF MULTIPLE SCLEROSIS DONOR-DERIVED MESENCHYMAL STEM CELL-NEURAL PROGENITORS (MSC-NP) REVEALS UPREGULATION OF TROPHIC AND IMMUNOMODULATORY GENES

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Multiple sclerosis (MS) is an autoimmune-mediated demyelinating disease of the CNS. Patients with progressive MS experience a steady worsening of neurologic function attributed to chronic demyelination and axonal loss. A novel regenerative therapy utilizing autologous mesenchymal stem cell-derived neural progenitors (MSC-NP) is currently under clinical investigation in patients with progressive MS. MSC-NPs are thought to promote neural repair though the paracrine release of trophic and immuno-modulatory factors. Recent results from a phase I trial demonstrated reversal of established disability after repeated intrathecal MSC-NP injections into patients with MS. A phase II

randomized double-blind placebo-controlled trial is underway to confirm the efficacy of this approach. As this autologous cell therapy moves into clinical use, there is a need to better define and characterize MSC-NPs in order to better understand the mechanisms underlying therapeutic potency. The objective of this study was to define the transcriptional profile of MSC-NPs from secondary progressive MS (SPMS), primary progressive MS (PPMS), and non-MS donors in order to better understand their functional characteristics and therapeutic potential. Bone marrow MSCs were derived from 10 donors (SPMS n=4; PPMS n=4; non-MS n=2). MSCs were transferred to neural progenitor maintenance medium to generate MSC-NPs. The transcriptional profile of each MSC/MSC-NP pair was determined by RNA sequencing analysis. MSCs derived from SPMS, PPMS, or controls demonstrated minimal differential gene expression despite differences in disease type, duration, and donor age. Compared to MSCs, MSC-NPs exhibited significant differential gene expression with 1,924 and 1,310 genes upregulated and downregulated, respectively. Upregulation of novel gene candidates of trophic/immunoregulatory mechanisms of action of MSC-NPs were identified and validated, including HGF, TGFß, TIMP1, MMP14, SERPINF1, and SPP1. Characterization of the transcriptional profile of MSC-NPs has revealed potential pathways that mediate therapeutic mechanisms of this novel cell therapy in MS. These studies form the basis of marker-based potency assays that may be used to better predict the therapeutic efficacy of individual batches of autologous MSC-NPs.

Keywords: mesenchymal stem cells, multiple sclerosis, cell therapy

CA193

EFFICACY OF FRESH AND CRYOPRESERVED HUMAN IPSC-DERIVED MIDBRAIN DOPAMINERGIC PROGENITORS FOR AUTOLOGOUS CELL REPLACEMENT THERAPY OF PARKINSON'S DISEASE

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The use of autologous human iPSC-derived midbrain dopaminergic progenitors (mDAPs) for cell replacement therapy of Parkinson's disease (PD) is a promising therapeutic strategy. Toward this goal, we established an efficient platform to generate clinical grade hiPSCs and to differentiate them into authentic and functional mDAPs free of undifferentiated cells. For the successful clinical application of this cell manufacturing approach we sought to establish that cryopreserved mDAPs are as effective as freshly harvested one. In order to assess this, we used the 6-OHDA hemi lesioned athymic rat models. Using clinical grade freshly isolated and cryopreserved mDAPs we transplanted cells into the striatum of the lesioned animals and assessed the impact on graft survival and behavior recovery using a battery of motor tests such as drug-induced rotation as well as corridor, cylinder and stepping tests. Here, we present transplantation results of our hiPSC-derived mDAPs showing robust and long-term restoration of motor dysfunction and reinnervation of host brains. Our data strongly suggest that using cryopreserved mDAPs is suitable for the successful implementation of personalized autologous cell replacement therapy for PD.

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Keywords: Human iPSC-derived dopaminergic progenitors, Graft survival and behavior recovery, Parkinson's disease

CA196

COMPARISON OF MITOCHONDRIAL FUNCTION IN EXCITATORY NEURONS FROM SUBJECTS WITH THE 22Q11.2 DELETION SYNDROME WITH AND WITHOUT SCHIZOPHRENIA

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Schizophrenia is a highly heterogeneous disorder in both its symptoms and risk factors, but metabolic compromise affecting the development and function of particular brain circuits has been repeatedly implicated. One of the strongest of the genetic risk factors for schizophrenia (SZ) is the hemizygous microdeletion at chromosome 22q11.2 (22q11DS), that confers a 25-fold increased risk. Six of the roughly 40 genes directly disrupted in 22q11DS encode for mitochondrial-localizing proteins. Recently, we tested the hypothesis that stem cell-derived neurons from subjects with the 22q11DS and schizophrenia have mitochondrial deficits relative to typically developing controls (Li et al., 2019). Human iPSCs from 4 lines of affected subjects and 5 lines of controls, were differentiated into forebrain-like excitatory neurons (iNrns) by transient overexpression of Neurogenin 2. In the patient group, we found significant reductions of ATP levels that appear to be secondary to reduced activity in oxidative phosphorylation complexes I and IV. Protein products of mitochondrial-encoded genes were also reduced. As one of the genes deleted in the 22q11.2 region is MRPL40, a component of the mitochondrial ribosome, we generated a heterozygous mutation of MRPL40 in a healthy control iPSC line. Relative to its isogeneic control, this line showed similar deficits in mitochondrial DNA-encoded proteins, ATP level, and complex I and IV activity. These results suggested that in the 22q11DS MRPL40 heterozygosity leads to reduced mitochondria ATP production secondary to altered mitochondrial protein translation. We are now extending these results to new set of IPSCs lines, this time including those from

22q11DS both with and without SZ. Initial studies show similar reductions in ATP levels between iNrns from 22q11DS+SZ relative to controls. Remarkably, iNrns from 22qDS (-) SZ do not appear to show this deficit. Studies to understand the mechanisms underlying these differences are ongoing.

Keywords: 22q11.2 deletion syndrome, iPSC, schizophrenia, mitochondria, excitatory neuron

CA267

A TRANSCRIPTOMIC APPROACH TO IDENTIFY PREDICTIVE MARKERS FOR DIFFERENTIATION POTENTIAL OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO NEURAL PROGENITOR CELLS

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Human induced pluripotent stem cells (hiPSCs) have the ability to differentiate into a variety of cells and to self-renew in vitro. Because of these two characteristics, hiPSCs have been expected to provide new applications for regenerative medicine/cell therapy. Although various in vitro differentiation protocols have been developed for efficient derivation of specific cell types, hiPSC lines vary in their ability to differentiate into specific lineages. Therefore, surrogate markers that accurately predict the differentiation propensity of hiPSCs could be helpful for the development and manufacture of hiPSC-derived cells for therapies or in vitro assays. In the previous study, we have identified the SALL3 gene as a marker predictive of differentiation propensity, using the rank correlation method and analysis of ten hiPSC lines. The SALL3 expression correlates positively with ectoderm differentiation and negatively with mesoderm/endoderm differentiation. In this study, we tried identifying the genes that potentially predict the differentiation propensity of hiPSCs into neural progenitor cell (NPC). Using the same 10 hiPSC lines as in the previous study, we performed NPC-differentiation by two types of differentiation methods (suspension and adhesion culture). Subsequently, genes showing a significant correlation between the gene expression profile of undifferentiated hiPSCs obtained in the previous study and the propensity for differentiation into neural progenitor cells were extracted. As a result, we identified 37 genes and 198 genes significantly correlated with differentiation using the suspension and adhesion culture methods, respectively. Furthermore, some genes identified in common between the two differentiation methods could be functionally involved in induction of neural differentiation. Our findings provide a practical method for selecting appropriate hiPSC lines in clinical-grade cell banks, allowing for the prediction of differentiation capacity toward desired cells.

Keywords: Human induced pluripotent stem cell, Neural progenitor cell, Differentiation propensity

NEW TECHNOLOGIES

CA199

FLOWFECT(TM) TRANSFECTION OF IPSCS MAINTAINS CELL PLURIPOTENCY

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Induced pluripotent stem cells (iPSCs) represent an opportunity for stem cell therapy to move from the lab to the clinic with clinical trials ranging from cancer to neurological. In both Japan (allogeneic) and in the US (autologous) iPSC-based therapy is becoming a reality. The ability of iPSCs to differentiate into numerous cell types and to proliferate indefinitely make these cells ideal candidates for gene therapy. However, hurdles in transitioning iPSC-derived therapies to the clinic can include high toxicity and loss of pluripotency after editing. To date both chemical and physical non-viral editing methods have been used in iPSC-based research. However, the use of physical techniques has become more prevalent in order to reduce the cytotoxicity associated with chemical techniques such as liposomes, non-viral vectors, and inorganic nanoparticles. We developed Flowfect™, a non-viral process that combines continuous fluid flow with electric fields, to transfect iPSCs. Flowfect™ transfection of eGFP-mRNA in adherent iPSCs resulted in highly functional cells which maintained stem cell lineage markers and differentiative properties. Flowfect™ transfection resulted in 83% eGFP expression in live cells. Additionally, the pluripotency markers Nanog and TRA-1-60 were co-expressed in 85% of cells two weeks after Flowfect™ transfection, demonstrating cell state retention. These cells were then differentiated using specialized media into endoderm or ectoderm, positive differentiation was based on lineage markers CXCR4 or Nestin, respectively. Rate of differentiation between non-transfected (NT) cells and Flowfect™ transfected (FF) cells differed by less than 5% in endoderm, NT=57.9% compared to FF=62.5%, and less than 10% in ectoderm, NT=52.3% compared to FF=62.2%. This data strongly suggests that Flowfect™ technology can be used to transfect iPSCs with high efficiency while maintaining cell state and function. Flowfect™ can be used to translate discovery in iPSCs to therapeutic applications due to seamless scalability between 25 µl samples in an automated liquid handling system to 10 mL in our large volume setting. Flowfect™ technology will allow the field to increase edited cell survival and ultimately help drive iPSC-based discovery research to increase the therapeutic potential.

Funding source: MassVentures - START program NSF - SBIR Phase II (1853194)

Keywords: Cell manufacturing, Non-viral delivery, Cell therapy

CA208

CULTIVATED MEAT: HOW CREATING ANIMAL MEAT FROM STEM CELLS CAN UPEND A TRILLION DOLLAR GLOBAL MEAT MARKET AND INFLUENCE THE REGENERATIVE MEDICINE INDUSTRY

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Cultivated meat is genuine animal meat grown directly from animal cells rather than by raising and slaughtering an animal. Since 2015, over 50 companies aimed at commercializing cultivated meat have formed across the globe. Armed with over $350 million in funding, cultivated meat producers aim to affordably manufacture stem cells at massive scales. While there are key differences in bringing cultivated meat and regenerative medicine therapies to market—regulatory landscapes, requirements for function vs. structure, and notable differences in the ultimate scale and price regime—many of the fundamental production techniques are shared. Here, we describe how companies and a growing body of academic researchers are developing low-cost medium formulations, large-scale bioprocessing strategies, continuous manufacturing techniques, automation implementations, scaffolding biomaterials, and other novel technologies with translatability to the cell therapy and regenerative medicine industries. Current estimates for future stem cell therapies are as high as $1 million per patient, and this may limit their use due to costs while putting an additional burden on the healthcare system. The development of cultivated meat thus provides an opportunity for cross-industry collaborations that bolster synergistic research, prevent redundancy, and unlock new growth opportunities in fledgling industries while potentially saving time and costs for the broader access to future stem cell therapies.

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Keywords: cultivated meat, cultured meat, clean meat

CA213

DEVELOPMENT OF SCALABLE MANUFACTURING PROCESS PLATFORMS FOR PSC- AND MSC-BASED THERAPEUTIC PRODUCTS USING LOW-SHEAR, VERTICAL-WHEEL BIOREACTORS

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Despite the remarkable progress of stem cell based therapeutic development, several challenges remain for the commercialization of cellular products. One of the bottlenecks is in vitro expansion of cells to achieve target doses, which vary between 10E6 to 10E9 cells/dose, with desirable cell qualities for clinical trials. Stirred tank bioreactors (STRs) with horizontal impellers are widely used for the mass production of mammalian cells in the bioprocessing industry owing to their advantages over two dimensional platforms such as reduced labor and cost, controlled culturing environment, and increased cell density per medium volume. However, the hydrodynamic flow of traditional STRs provides harsh conditions for shear sensitive cells because STRs require relatively high agitation power input. Moreover, shear generated from the gradient of local fluid velocity of STRs can influence the quantity and quality of therapeutic products. Such drawbacks of STRs make manufacturing process more challenging, often resulting in insufficient cell growth or undesirable cell product quality. A novel single-use Vertical-Wheel bioreactor offers a low-shear environment by providing homogeneous energy dissipation distribution via an innovative vertical mixing system. In particular, it provides advantageous culture conditions for aggregate cultures and microcarrier-based cultures. In the aggregate cultures of pluripotent stem cells (PSC) using Vertical-Wheel bioreactors, over 30-fold expansion in 6 days was achieved with a desirable size and a narrower distribution of aggregates that maintain high pluripotent cell quality. Moreover, in the microcarrier-incorporated dynamic cultures of mesenchymal stromal cells (MSC) via the Vertical-Wheel bioreactor, over one million cells/mL were attained, achieving more than a 20-fold expansion in 5 days with desirable cell qualities. Throughout several runs of bioreactors, consistent results of cell expansion were achieved. Furthermore, cell growth kinetics remained constant across different sizes of Vertical-Wheel bioreactors. Since the efficient vertical mixing system is constant across a full range of vessel sizes from 0.1 to 80L, robust and consistent manufacturing process platforms for PSCs and MSCs can be achieved via single-use Vertical-Wheel bioreactors.

Keywords: Single-use bioreactor, Vertical-Wheel, Stirred-tank, Low-shear, Homogeneous energy dissipation distribution, Therapeutic cell products, Pluripotent stem cell, Mesenchymal stromal cell, Cell aggregates, Microcarriers, Culture conditions, Shear sensitive, C

CA218

COMPOUNDS IDENTIFIED IN A HIGH-CONTENT SCREEN PROMOTE MATURATION OF HIPSC-DERIVED MEGAKARYOCYTES, ENABLING CLINICAL-SCALE PRODUCTION OF FUNCTIONAL PLATELETS

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hiPSC derived platelets (PLTs) promise to overcome limitations and adverse effects associated with conventional donor PLTs, such as supply shortages, variable and unpredictable activity, risk of contamination, and adverse immune reactions. However, current in vitro PLT production systems are inefficient, requiring large culture volumes to produce a suitable yield for clinical use (≥1x1011 PLTS, 1 unit). Conditionally immortalized megakaryocytic cell lines (imMKCLs)1 expand exponentially in suspension culture, but when induced to terminally differentiate yield only 3-10 PLTs/MK, requiring up to 50 liters to produce one unit. We sought to identify novel chemical compounds and associated pathways to increase PLT yields. We developed a confocal imaging platform to assess the rate and extent of MK-maturation and pro-PLT production in a high-throughput fashion and used it to screen chemical libraries. The screen identified two structurally related compounds (targeting the same pathway) that robustly induce PLT production from imMKCLs and cord blood derived MKs when added during the later stages of terminal differentiation. Combining this treatment with another compound that promotes maturation when added earlier during terminal differentiation enabled production of ≥1x1011 Calcein+/ AnnexinV-/CD41+/CD42B+ PLTs per 10 liters, thereby facilitating production for clinical testing. These imMKCL-derived PLTs are structurally and functionally similar to normal human PLTs as judged by size, morphology and ultrastructure; resting and agonist-stimulated surface expression of activation biomarkers; and PLT aggregation. Furthermore, bioreactor-produced PLTs reduce bleeding times in thrombocytopenic mice and are incorporated into developing thrombus formation after laser-induced vascular injury in non-thrombocytopenic mice at rates similar to those of normal human donor PLTs. Identification of novel chemical enhancers of PLT production from imMKCLs is another step closer to cost-efficient clinical scale production of highly functional PLTs.

Funding source: National Blood Foundation, Early Career Research Grant

Keywords: immortalized megakaryocytic cell lines, hiPSC derived Platelets, Clinical scale production of platelets

CA230

DISCOVERY OF EXTRAGENIC GENOMIC SAFE HARBORS FOR EFFECTIVE CAR T CELL ENGINEERING

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Adoptive immunotherapy using CAR T cells is one of the most promising new therapies to treat cancer. CARs are typically introduced into T cells using gamma retroviral or lentiviral vectors, resulting in variable transgene expression and effectiveness of the resultant CAR T cells. As we previously established, integration of a CAR cDNA in the TCR alpha locus provides consistent regulated expression of CD19 CARs and superior CAR T cell efficacy in a mouse model of B-cell acute lymphoblastic leukemia (B-ALL). Here, we set out to identify alternate loci that could be reliably targeted for safe and predictable expression of CARs or other immunomodulatory transgenes to potentiate adoptive immunotherapy. We searched for genomic regions devoid of any known function, remote from endogenous genes and supportive of durable transgene function aka 'genomic safe harbors' (GSHs). For efficient transgene integration, a GSH locus must be cleaved at a high efficiency by CRISPR-Cas9 in the target cell type. We hypothesized that cleavage efficiency would be governed by chromatin accessibility and thus screened for accessible GSHs as measured by ATAC-seq. In human primary T cells, we identified 379 such sites. The 6 highest ranking GSHs showed high cleavage efficiency and allowed for CAR cDNA targeted integration and expression. However, CAR expression and CAR T cell cytotoxicity diminished within a week at most sites. To rescue the GSHs from possible heterochromatinization, we incorporated chromatin insulator elements with barrier activity flanking the CAR transcription unit. This variably extended CAR expression, maintaining antigen-specific cytotoxicity and proliferation until CAR expression could be preserved. One of the GSHs, which maintained long term CAR expression, supported potent anti-leukemic CAR T cell efficacy in the B-ALL mouse model. Two other sites which silenced over time, afforded initial tumor control but failed to prevent subsequent relapse. In conclusion, CAR expression was found to be exquisitely dependent on the site of integration, with many sites allowing for initial expression but silencing over time. We identified a GSH site that can be used for effective therapeutic engineering. Our study provides a model for identifying GSHs that provide safe and predictable transgene expression in any given cell type.

Keywords: Gene editing CRISPR-Cas9, Cell engineering, Genomic safe harbors

PLACENTA AND UMBILICAL CORD DERIVED CELLS

CA240

EVALUATION OF HUMAN UMBILICAL CORD TISSUE MESENCHYMAL STROMAL CELL EXPANSION CULTURE ON 3D MICROCARRIERS IN A SCALABLE, FED-BATCH BIOREACTOR SYSTEM

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In recognition of the potential therapeutic value of human umbilical cord-derived mesenchymal stromal cells (UCMSCs), the banking of umbilical cord tissue by newborn stem cell banks is now commonplace. While UCMSCs can be easily isolated from the thawed tissue, scalable approaches for efficiently expanding clinically-significant quantities of cells are still necessary. We evaluated the mid-scale expansion of UCMSCs grown on suspended microcarriers in a 500mL-capacity fed-batch, stirred bioreactor. UCMSCs were derived by explant culture from research donated cord tissue previously cryopreserved as a composite material and expanded to create a master bank. For each trial, one vial of $1x10^6$ cells was thawed and expanded in monolayer to $10.5x10^6$ cells which were seeded onto 6.25g of microcarriers and cultured in 450mL RoosterNourish™-MSC-XF medium (RoosterBio, Inc) in a 0.5L capacity PBS Mini bioreactor for one week. Media were supplemented on Day 3 with 10mL RoosterReplenish™ -MSC-XF. Solohill (SH) and Corning Synthemax II (SM) microcarriers were evaluated. One week culture on SH and SM microcarriers yielded $1.15x10^8$ and $1.26x10^8$ cells, respectively, equating to 11.0 and 12.0 fold increases in cell number. Following harvest, cells expanded on either microcarrier exhibited fibroblastic morphology, adherence to culture plastic, and proliferation in standard monolayer culture. Cells grown on SM carriers exhibited 98.6% and 94.1% positive expression of CD73 and CD90, respectively. Compared to 2D culture in T150 flasks, use of the bioreactor system required approximately 20% of the incubator space, while consuming an equivalent or smaller volume of medium. In addition there was significantly less technician and open-culture time required for media supplementation compared to the media exchanges typical of 2D culture. A commonly referenced dose for potential UCMSCs therapies is $1x10^6$ cells/kg. Given a 100kg patient, the bioreactor expansion method explored here demonstrates cell quantities in the clinically relevant range in a single, seven-day expansion and prior to method optimization for maximum cell density. Including the initial explant derivation, this suggests that clinically-relevant UCMSCs doses can be achieved from cryopreserved umbilical cord tissue by this method in less than 4 weeks.

Funding source: All authors are paid employees of Generate Life Sciences.

Keywords: UCMSCs, Bioreactor, Stem Cell Bank

Theme: Modeling Development and Disease

CARDIAC

MDD109

FUNCTIONAL PHENOTYPING THE QT GWAS LOCUS 16Q21 IN HUMAN EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTES IDENTIFIED GINS3 AS THE CAUSAL VARIANT REGULATING CARDIAC REPOLARIZATION

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Prolongation of the QT interval on the electrocardiogram is an indicator of abnormal cardiac repolarization and is a heritable high risk factor for ventricular arrhythmia and sudden cardiac death. Genome-wide association studies of the QT interval have identified more than 35 common variant loci encoding hundreds of candidate genes whose roles in cardiac repolarization are largely unclear. We performed functional validation of three candidate genes NDRG4, CNOT1 and GINS3 at 16q21 in a human embryonic stem cell (hESC) derived cardiomyocyte model to determine the causal genes responsible for QT interval prolongation at this locus. We first performed loss-of-function studies using the short hairpin RNA (shRNA)-mediated knockdown approach and whole-cell patch-clamp techniques. hESC derived cardiomyocytes were transduced with lentivirus carrying shRNAs targeting NDRG4, CNOT1, GINS3 or a scrambled CTL. Knockdown of NDRG4 or CNOT1 had no effects on cardiomyocyte action potential durations (APD). GINS3 knockdown significantly shortened APD at 90% repolarization (APD90, 187 ± 25 ms) compared with shRNA CTL (264 ± 19 ms). We further evaluated the role of GINS3 on cardiac repolarization by overexpressing GINS3 via a lentiviral vector in hESC-derived cardiomyocytes. GINS3 overexpression significantly prolonged cardiomyocyte APD90 (363 ± 42 ms) compared to CTL (247 ± 19 ms). qPCR analysis of KCNQ1 and KCNH2, major cardiac repolarization genes, showed no changes of KCNQ1 but reduced KCNH2 mRNA levels in GINS3 overexpressing cardiomyocytes. Both KCNQ1 and KCNH2 expressions were not altered in GINS3 knockdown cells. Our results show that GINS3 can regulate cardiac repolarization through regulation of hERG and is likely the causal variant at 16q21 locus that contributes to QT prolongation.

Keywords: human stem cell derived cardiomyocyte model, regulation of cardiac repolarization, functional genomics

MDD112

HCM CAUSING ALPHA-ACTININ 2 MISSENSE MUTATION HIGHLIGHTS ALTERED PROTEIN FUNCTION AND DEGRADATION AS CELLULAR PATHOLOGIES IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Hypertrophic cardiomyopathy (HCM) is the most common genetic myocardial disease. By now, numerous mutations in genes encoding for proteins of the cardiac sarcomere are reported to cause HCM. We previously identified a rare missense mutation (c.740C>T; p.T247M) in ACTN2, encoding for alpha-actinin 2, and were able to relieve accompanied pathophysiology in a personalized medicine approach. The aim of this study was to gain insights to molecular alterations leading to the development of HCM using human induced-pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) and live cell imaging. In addition to previously characterized hiPSCs from the index patient (HCM) and its isogenic control (HCMrep), we developed a homozygous mutant cell line (HCMmut) using CRISPR/Cas9 gene editing. All hiPSC lines were differentiated to hiPSC-CMs, plated in 2D and cultured as engineered heart tissue (EHT) for 30 days. Furthermore, hiPSC-CMs were transduced with an AAV6 carrying either wild-type (WT) or mutant (Mut)-ACTN2 fused to a HaloTag® and cultured in 2D. Analysis of HCMmut-EHTs presented lower force and higher relaxation when compared to HCMrep- and HCM-EHTs. 2D-cultured HCMmut-CMs showed a pronounced cellular HCM phenotype than HCMrep-CMs, observed as a deficiency in forming regular sarcomeres, alpha-actinin 2 aggregates and higher cell area displayed by immunofluorescence (IF) analysis. Western blot experiments showed no difference in alpha-actinin 2 protein levels between 2D-cultured HCM- and HCMrep-CMs but were markedly lower in HCMmut-CMs. Furthermore, gene expression analysis revealed lower expression of sarcomere-related genes in HCMmut-CMs in contrast to HCM-CMs. Confocal microscopy of living AAV6-transduced hiPSC-CMs revealed integration of exogenous alpha-actinin 2 in ~ 80% and ~ 20% of all hiPSC-CMs transduced with WT- and Mut-ACTN2, respectively. Finally, a markedly higher autophagic flux was detectible in HCMmut-CMs in comparison to HCMrep-CMs, which might explain lower baseline levels of alpha-actinin 2 in HCMmut-CMs despite detection of alpha-actinin 2 aggregates. This study reveals that mutant alpha-actinin 2 is unstable in living hiPSC-CMs suggesting altered function and activated protein degradation pathways to play crucial roles in HCM disease progression on cellular level.

Keywords: Hypertrophic cardiomyopathy, Alpha-actinin 2, Proteotoxicity

MDD121

BIOWIRE II HUMAN ENGINEERED CARDIAC TISSUES: A PLATFORM FOR MODELING HYPERTROPHIC CARDIOMYOPATHY

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The Biowire™ II platform was used to generate 3D engineered cardiac tissues (ECTs) from patient derived iPSC-CMs with a beta myosin mutation known to cause hypertrophic cardiomyopathy generated by Cellular Dynamics ("MYH7-R403Q") and its CRISPR-corrected isogenic control ("R403QCorr"). Over the course of seven weeks, ECTs were subjected to external stimulation to induce mature cardiac phenotypes. Wild-type tissue characteristics include: (1) lack of spontaneous beating, (2) post-rest potentiation and (3) the presence of a positive force-frequency response from 1-4 Hz, while differences were observed in the force-frequency relationship and post-rest potentiation in R403Q tissues compared to its isogenic control. The "MYH7-R403Q" ECTs demonstrated a 4-fold increase in active force amplitude over the "R403QCorr" ECTs and those generated from a healthy donor wild-type line (CDI iCell Cardiomyocytes2) at each measured time point over the culture period. Pharmacology studies performed in these ECTs confirm the presence and functionality of pathways that modulate cardiac contractility in humans. Inotropic responses were evaluated for drugs that act via β-adrenergic and cAMP-mediated pathways, modulate the activity of cardiac ion channels or the activity of cardiac sarcomere proteins. Differences in contractile responses to a subset of these agents were seen between the "MYH7-R403Q" and isogenic control tissues. Additionally, preliminary immunohistochemical data suggests structural differences between the "MYH7-R403Q" and "R403QCorr" tissues.The development of hallmarks of functional cardiac maturity and the establishment of phenotypes consistent with cardiac hypertrophy

in “MYH7-R403Q” tissues in addition to the resulting canonical responses to clinically utilized inotropes demonstrate that the ECTs developed on the Biowire™ II platform are well-suited to enable phenotypic-based drug discovery and development in a human-relevant disease model.

Keywords: cardiac disease modeling, cardiomyopathy, Tissue engineering

MDD129

DEVELOPMENT OF A FUNCTIONAL READOUT FOR ASSESSMENT OF EXCITATION-CONTRACTION COUPLING OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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The process of cardiomyocyte excitation-contraction coupling involves the intricate coordination of the integrated ion channel activity at the plasma membrane with calcium release through the sarcoplasmic reticulum culminating in contraction of cardiomyocytes through the interaction of myosin motor proteins with actin microfilaments and other accessory proteins. The temporal nature of these process coupled with any compound or inherited genetic mutation that disrupts the timing of these events can lead to pathophysiological conditions such as dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM) and drug-induced arrhythmias. In order to study this intricate process, we utilized the Real-Time Cell Analysis system that can simultaneously measure cardiomyocyte integrated ion-channel activity, contractility and viability. We utilized human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) as a model system. Using field potential recording of hiPSC-CM we demonstrated a spontaneous basal beating rate of 35 ±10 bpm as well as field potential duration of 432 ± 30 ms. In order to measure the timing between the integrated electrophysiological activity and contractility we came up with a parameter referred to as electro-mechanical window (EMW). This parameter remained stable during the period of recording. Additionally, we developed parameters such as impedance amplitude (IAMP) to measure the extent of contraction of these hiPSC-CM. The measured IAMP of the hiPSC-CM was 0.25 ±0.1 CI under basal conditions. The treatment of 100 nM isoproterenol increased IAMP by 52 ± 14% and profoundly decreased FPD in maturation enhanced hiPSC-CM via long-term electrical pacing. Interestingly, the compound blebbistatin, which is an inhibitor of myosin heavy chain ATPase, significantly decreased the IAMP at 1 µM without affecting the field potential readout. We applied the same parameters for quantitative assessment of hiPSC-CM derived from a DCM patient and we show significant differences in all measured parameters, especially contractility. Development of these quantitative parameters will aid in discerning the impact of compounds and inherited mutations on cardiomyocyte function and excitation-contraction coupling.

Keywords: real-time functional assay for cardiomyocyte, contractility and viability, cardiac disease model

EARLY EMBRYO

MDD140

CONTROLLED, EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO SELF-RENEWING TROPHECTODERM

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The in vivo embryonic counterpart of cultured human pluripotent stem cells (hPSC) are presumably the cells of the post-implantation epiblast, which is thought to give rise to the embryo proper and no longer able to develop into trophectoderm (TE). Previous studies that reported TE differentiation from hPSC remain controversial, in part due to incomplete differentiation or the use of undefined culture conditions. Here, we describe a novel method in chemically defined conditions resulting in highly efficient TE differentiation, followed by fusion of a subset of the cells into multi-nucleated syncytiotrophoblast. Extensive analyses including immunocytochemistry, RNA sequencing, single cell analysis, microRNA expression profiling, and epigenetic mapping confirmed robust induction of genes associated with trophectoderm and placental development including GATA3, IGFBP3, KRT7, CDX2, CGA, DAB2, TEAD3, DLX3 and others. In addition, we developed another chemically defined medium that supported stable self-renewal of TE cells while maintaining their molecular features. Taken together, our data clarifies that cultured hPSCs can indeed generate extra-embryonic cell types and the subsequent derivation of self-renewing human TE cells represents a novel paradigm for modeling diseases of the placenta, drug screening, and cell-based therapies.

Funding source: NIH Common Fund

Keywords: trophectoderm, human pluripotent stem cells, self-renewal

MDD478

BONE DEVELOPMENT DEFECTS IN WERNER SYNDROME CAUSED BY SHOX DYSREGULATION

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Werner Syndrome (WS) is a rare progressive disorder, which is characterized by the appearance of unusually accelerated aging. Around 90% WS patients are caused by mutations in WRN gene, the only gene currently connected to WS. Affected individuals usually have problems with cataracts, type 2 diabetes, skin ulcer, etc. However, the first symptoms of WS is short stature. Patients with WS have a very slow growth rate and stop grow at the very beginning of puberty. However, the answer is still not very clear. In order to better understand the mechanisms, we chose a chondrogenesis protocol, which directly differentiates human embryonic stem cells towards chondrocytes. Then, by transcriptome analysis, we found SHOX gene. SHOX (short stature homeobox) is an important player during chondrogenesis. But the role of SHOX in WS patients is not known. So we checked the expression pattern of WRN and SHOX during chondrogenesis and found that they showed the similar expression pattern, which may indicated that they may both participate in chondrogenesis. Then we blocked the WRN expression and SHOX expression was inhibited, however, when we blocked SHOX expression, WRN expression slightly changed. This hinted that WRN might as upstream target of SHOX during chondrogenesis in vitro.

Keywords: human embryonic stem cell, aging, chondrogenesis

MDD495

TRANSIENT INHIBITION OF MTOR IN HUMAN PLURIPOTENT STEM CELLS ENABLES ROBUST FORMATION OF MOUSE-HUMAN CHIMERIC EMBRYOS

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It has not been possible to generate naive human pluripotent stem cells (hPSCs) that significantly contribute to mouse embryos. We found that a brief (3 hr) inhibition of mTOR with Torin1 converted hPSCs from primed to naive pluripotency. The naive hPSCs were maintained in essentially the same condition as mouse embryonic stem cells and exhibited high clonogenicity, rapid proliferation, mitochondrial respiration, X chromosome reactivation, DNA hypomethylation, and transcriptomes sharing similarities to those of human blastocysts. When transferred to mouse blastocysts, naive hPSCs generated 0.1-4% human cells, of all three germ layers, including large amounts of enucleated red blood cells and photoreceptors in E17.5 mouse embryos. The results suggest a significant acceleration of hPSC development in mouse embryos. Torin1 induced nuclear translocation of TFE3; TFE3 with mutated nuclear localization signal blocked the primed-to-naive conversion. The generation of chimera-competent naive hPSCs unifies some common features of naive pluripotency in mammals and may enable applications such as human organ generation in animals.

Funding source: The work is supported by NYSTEM contracts C028129, C029556, C30290GG, and Buffalo Blue Sky Initiative (J.F.).

Keywords: naive state human pluripotent stem cells, mouse-human chimeric embryo, mTOR

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

MDD149

CIRCADIAN CONTROL OF ORGANOID PHYSIOLOGY

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Organoids that recap tissue physiology could transform disease research and therapy, yet most methods generate products that resemble fetal, not adult organs. Organoids are usually grown in constant environments, whereas our tissues mature in presence of daily physiological rhythms. We recently showed that circadian rhythms can further maturation of human stem cell-derived pancreatic islet organoids. Inducing islet organoid clocks, by entrainment to daily feeding cycles, triggers rhythmic metabolism and insulin responses with a raised glucose threshold, a hallmark

of islet maturity, and renders organoids functional within days of transplant. How entrainment leads to these effects is unclear. Systematic maturation studies are limited by scarcity of tools for organoid-level mapping of cellular activities without disrupting tissue architecture, function, and cell-cell interactions. We have advanced tools to implant and distribute nanoelectronics across whole organoid bodies, enabling detection of membrane potential changes as integrative signal readouts of cellular activities. Soft, stretchable mesh sensor arrays are embedded and reconfigured by cell-cell attraction forces during organogenesis, resulting in their global distribution across spherical "cyborg" organoids. This enables non-invasive, chronic electrophysiology recordings with single-cell and millisecond resolution over substantial time windows. Using islet and cardiac cyborg organoids, we demonstrate the utility of our approach for tracing the evolution, propagation, and synchronization of electrophysiological activities during functional maturation. Nonuniform activities with clear time latency reveal tissue-wide propagation of local field potentials, supporting existence of pacemaker-like cells. Using STARmap (spatially-resolved transcript amplicon readout mapping), we paint a molecular picture of these cells within their 3D environment. Finally, we show that maturation by feeding entrainment is marked by increases in the amplitude of membrane depolarization and by synchronization of bursting phases without changes in burst duration or frequency. The approach and results offer a general framework for understanding the interplay between feeding rhythms, cellular activities, and organoid physiology.

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Keywords: Organoids, Maturation, Circadian rhythms

MDD155

DEVELOPMENT OF A NOTCH AGONIST AND PATIENT IPSC MODELS FOR TREATMENT OF ALAGILLE SYNDROME

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Alagille syndrome (ALGS) is a complex, autosomal dominant disorder that affects multiple organ systems including the liver, heart, skeleton, eyes, kidneys, and central nervous system. One of every 30,000 babies is born with ALGS each year, with a 60% survival rate by age 20. There is an urgent need for a cure because current treatments only target specific symptoms of ALGS, rather than the cause. ALGS is caused by heterozygous mutations in primarily JAGGED1 (~95%) and NOTCH2 (~2%), resulting in impairment of the Notch pathway. In the liver, the Notch pathway plays a critical role in duct cell formation and maintenance, thus, impairment of the Notch pathway in ALGS patients leads to ductal cell paucity. In addition, Notch also plays a key role in heart formation, leading to malformations, such as the Tetralogy of Fallot (TOF), when the pathway is disrupted in ALGS. Therefore, to broadly target the many pathologies of ALGS, our strategy is to identify new therapies that could restore normal Notch activity in patients, particularly by screening for functional Notch agonists. We have identified a small molecule, SBPz1, that we found to be a direct Notch agonist in cell types affected by ALGS, including liver, kidney, and heart cells. Importantly, our data show that after only 3 hours of SBPz1 treatment of ALGS patient liver fibroblast, Notch receptor activation and expression of Notch downstream genes, HES1 and SOX9, are significantly increased. In the light of these exciting results, we next aim to derive Alagille patient-derived iPSCs into cardiomyocytes and hepatocytes, as ALGS models, to further assess the potential of SBPz1 as a therapeutic that specifically targets the pathway affected in ALGS, Jagged/Notch. In summary, we have found a Notch agonist and have been working towards establishing an iPSC disease model for ALGS.

Funding source: California Institute for Regenerative Medicine (CIRM)

Keywords: Alagille Syndrome, Jagged-Notch, iPSC disease model

MDD156

AUTOMATED PLATFORM FOR LARGE-SCALE PRODUCTION AND PHENOTYPIC PROFILING OF STEM CELL-DERIVED BETA CELLS

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Directed differentiation of patient-derived pluripotent stem cells represents a powerful tool for studying genotype-phenotype relationships in vitro. In severe monogenic diseases, this relationship is often pronounced and can be demonstrated experimentally using cell lines from a low number of affected individuals. For genetically complex diseases such as type 2 diabetes (T2D), however, large cohorts of patient-derived cells are required to effectively model disease phenotypes. Yet large-scale production of pancreatic beta cells from multiple donors faces significant technical challenges, including line-to-line variation in differentiation efficiency and experimental variation introduced by manual procedures. Current procedures for large-batch beta cell production, such as those using spinner flasks, are not amenable to population-scale disease modeling. To address these limitations, we leveraged the NYSCF Global Stem Cell Array®, our automated platform for iPSC derivation, to develop a fully automated, high-throughput platform for the directed differentiation of human pluripotent stem cells into functional pancreatic beta cells. Using Design of Experiment (DoE) principles, we generated a pipeline to iteratively optimize and adapt the differentiation protocol for automation. This approach successfully improved the molecular and functional properties of differentiated tissues. We also developed automated procedures for assessing organoid morphology, developmental marker expression profiling by intracellular flow cytometry, and functional evaluations with Glucose Stimulated Insulin Secretion (GSIS) assays. Finally, we developed fully automated procedures for thawing, expansion, and seeding of large cohorts of pluripotent stem cells, which importantly minimizes manual error and experimental variation. Our automated platform for the derivation of functional pancreatic beta cell organoids is optimized for population-based disease modeling of diabetes and high-fidelity drug screening.

Keywords: Automated platform for iPSC derivation, Stem cell-derived beta cells, Type 2 diabetes

MDD158

DIFFERENTIATION OF PARATHYROID CELLS IN VITRO FROM HUMAN PLURIPOTENT STEM CELLS

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The parathyroids are endocrine glands that control calcium homeostasis through secretion of the parathyroid hormone (PTH). Loss of parathyroid function, a common complication of thyroid surgery, causes hypoparathyroidism. Current medical management of hypoparathyroidism requires daily treatment and is of only limited success. Hypoparathyroidism is an attractive target for cell replacement therapy as successfully producing a single cell type in vitro could provide a cure, yet no protocols have been reported to efficiently differentiate parathyroid cells in vitro. Starting with human pluripotent stem cells (hPSCs), we set out to identify cellular signaling modulators and transcription factors that drive differentiation of parathyroid cells or their pharyngeal endoderm progenitor. To screen signaling factors, we differentiated hPSCs to anterior foregut endoderm (AFE) progenitors, treated these cells with gradients of activators and inhibitors of eight signaling pathways, and assessed differentiation by 33 marker genes using a Nanostring assay. Markers of the parathyroid and pharyngeal endoderm are induced by multiple FGFs and by inhibition of BMP. The effects of BMP inhibition are consistent with the known role of the BMP inhibitor Noggin in mouse parathyroid development, while the role of FGFs is novel. Concurrently, we are testing combinations of ten transcription factors (TFs) with roles in pharyngeal and parathyroid development for capacity to induce parathyroid differentiation from AFE. As this work matures, we hope to combine cellular signaling modulation with direct TF expression to establish a differentiation protocol that paves the way to a stem cell therapy for hypoparathyroidism.

Funding source: NIH T32GM007226

Keywords: directed differentiation, transdifferentiation, parathyroid

MDD167

DEVELOPMENT OF NOVEL HIPSC-DERIVED HEPATOCYTES AND INTESTINAL EPITHELIAL CELLS TO ADVANCE DISEASE MODELING AND DRUG DISCOVERY

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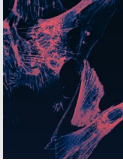

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Reliable and relevant in vitro cell models are crucial for advancing disease modeling and drug discovery. Primary cells and immortalized cell lines have long been the gold standard models, but they have several shortcomings. While primary cells mimic the functionalities of cells in vivo, their utility is significantly limited by their rapid loss of function when cultured in vitro, large inter-donor variability, and finite number of cells harvestable from each donor. Immortalized cell lines provide an inexpensive, readily available source of cells, but are often derived from tumors and, therefore, may not provide physiologically-relevant results or accurately reflect in vivo cell function. Human induced pluripotent stem cell (hiPSC)-derived cells address several of these limitations. hiPSC-derived cells provide a renewable source of cells that can be used for a variety of downstream applications to study and treat disease. We developed a robust protocol for highly efficient differentiation of hiPSCs to definitive endoderm (DE) cells and further differentiation into disease-relevant cell types. We used this protocol to generate mature and functional hiPSC-derived hepatocytes that express genes important to drug metabolism (such as CYPs, phase II enzymes, and transporters). We exposed the hiPSC-derived hepatocytes to known hepatotoxins for up to 14 days and found they respond appropriately, demonstrating their utility for chronic toxicology studies. hiPSC-derived hepatocytes also respond to insulin, and can take up and store low-density lipoproteins and fatty acids. We also developed a novel protocol for differentiation of DE cells into small intestinal epithelial cells (IECs). These cells express key IEC markers as well as important enzymes and transporters that are involved in drug metabolism at levels similar to primary intestinal cells. Additionally, hiPSC-derived IECs form a functional barrier for intestinal permeability and absorption studies. Taken together, we have developed differentiation protocols to produce mature, functional, hiPSC-derived cells with improved functionality and relevance compared to the current gold standards. These cells provide researchers with readily available, more accurate models to advance their disease modeling and drug discovery research.

Keywords: disease model, drug metabolism, hepatocyte, intestinal epithelium

MDD465

SCPRED-RNA VELOCITY MODELLING DERIVED FROM HUMAN KIDNEY SINGLE CELL DATA APPLIED TO PREDICT CELLULAR IDENTITY IN STEM CELL-DERIVED ORGANOIDS

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Single cell sequencing is a useful tool for understanding the developing human kidney, which, based on current definitions, contains over 25 distinct cell types arising from two distinctly patterned populations. Like many systems, the classification of cellular identify from cell transcriptomics has relied upon selected markers frequently predicted from murine data, rather than unbiased identity classification. This is particularly challenging in the organoid settings, where the synthetically derived tissues lack spatial information and may not completely represent a genuine embryonic state. Hence, there is a need for unbiased computational prediction tools. Here we use multiple human scRNA-seq data from both fetal and adult kidneys to form a benchmark transcriptional profile from which a hierarchical system of machine learning-derived predictive models was generated. These models can compare unknown populations, such as those derived in vitro from hiPSCs, with bona fide kidney cell types. This approach to unbiased cell identity prediction provided a high degree of accuracy when applied back to bona fide human kidney samples at both fetal and postnatal stages. When applying these models to organoid data we show an overall decrease in the degree of similarity, although the model was able to identify the cell types predicted in prior analyses and provide a quantitative comparator. The greatest challenge to cell identity prediction in developing systems is that the cells are in a state of developmental flux. To evaluate this, we tested the dynamics of expression information using RNA velocity, allowing us to position cells on a trajectory between cell states. This approach provides an additional layer of specification to identity, decreasing the requirement for cut off points when classifying cell populations. RNA velocity predictions also provide a framework for identifying gene expression changes resulting from disease or changes to culture protocols. In conclusion, this work represents an important step forward in standardising the methods for cellular identity predictions for kidney populations. While applied to developing kidney, this unbiased methodology for cell identity prediction can be applied to other biological systems, facilitating comparability between data and experiments.

Keywords: Single cell RNA-seq, Classification, kidney organoids

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

MDD189

CONVERTING HUMAN PLURIPOTENT STEM CELLS INTO BLOOD VESSELS - THE SUPERHIGHWAY OF THE BODY

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Blood vessels are of paramount importance to life as they transport nutrients and oxygen to organs, thus performing a critical role as the superhighway of the body. Blood vessels are lined by endothelial cells which act as barriers regulating the exchange of molecules (e.g., nutrients, wastes) and cells (e.g., infiltrating immune cells) between the vessel lumen and surrounding tissues. A large supply of human endothelial cells would drive diverse applications, enabling the creation of new in vitro models of human cardiovascular diseases and also enabling us to vascularize organoids or engineered tissues for regenerative medicine. However, it has been challenging to obtain a large supply of endothelial cells in a dish for the following three major reasons. First, primary human endothelial cells cannot be expanded long-term as they quickly lose normal markers and karyotype. Second, current methods to differentiate ESCs into endothelial cells generate mostly impure cell populations (with 93% pure SOX17-mCherry+ artery endothelial cells within 3 days of ESC differentiation, as assessed using a SOX17-mCherry reporter ESC line to track artery emergence. Moreover, artery differentiation is efficient and reproducible across 5 ESC and iPSC lines. Along the other developmental path, we can generate >83% pure NR2F2-GFP+ vein cells within 4 days of ESC differentiation, as assessed using a NR2F2-GFP reporter ESC line. These highly pure ESC-derived artery and vein endothelial cells develop respective networks of elongated cells in vitro and after in vivo transplantation into mice. In sum, a large supply of highly pure ESC-derived artery or vein endothelial cells hold promise for disease modeling and regenerative medicine.

Funding source: California Institute for Regenerative Medicine Thomas and Stacey Siebel Foundation

Keywords: Pluripotent stem cells, Blood vessel, Differentiation

MDD190

GENERATION OF HUMAN VASCULARIZED NEURO-ORGANOIDS FROM IPSC

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Human neuro-organoids (hNOs), derived from human pluripotent cells (hPSCs), provide a platform for studying human brain development and diseases in vitro. However, current hPSC-derived organoid models all lack the accompanying vasculature, resulting in limited oxygen and nutrient delivery to the inner-most parts of the organoid. Utilizing key endothelial transcription factors ETV2, ERG, and FLI1, we generate endothelial cells from hPSCs which transcriptomically and functionally resemble bona-fide endothelium (EEF-ECs). We demonstrate EEF-ECs have the ability to form vessels in vitro and in vivo while also responding appropriately to inflammatory and angiogenic stimuli. Here, we demonstrate a method of using hPSC derived endothelial cells to functionally vascularize hNOs. These vascularized organoids could become a robust model to study brain disease in vitro.

Keywords: Organoid, Endothelial Cell, Blood Brain Barrier.

EPITHELIAL

MDD193

BIPHENOTYPIC IPSC-DERIVED KIDNEY ORGANOIDS SUPPORT LOSS-OF-HETEROZYGOSITY AS THE PATHOGENIC MECHANISM DRIVING DEVELOPMENT OF RENAL DISEASES IN TUBEROUS SCLEROSIS COMPLEX

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Organoids derived from induced pluripotent stem cells (iPSCs) offer unique potential for modeling rare diseases that cannot be adequately recapitulated by animal models. Tuberous Sclerosis Complex (TSC) is an incurable rare disease primarily characterized by neurological and renal manifestations (RMs). RMs include angiomyolipomas, epithelial cysts and renal cell carcinoma (RCC). While the developmental mechanisms giving rise to TSC-associated RMs are poorly understood, the widely supported "Two-Hit" hypothesis establishes that a second, loss-of-heterozygosity mutation, taking place in a pre-existent heterozygous TSC locus, can lead to loss of protein function and aberrant tissue growth due to dysregulation of mTOR. These proposed mechanisms have been difficult to validate in experimental models. Here we generated kidney organoids from patient-derived TSC2+/- and isogenic TSC2-/- iPSC lines, to study the development of kidney RMs. Our data show that while differentiation of TSC2+/- iPSCs resulted in nephron-containing 2D and 3D organoids without an observable phenotype, differentiation of a "two-hit" TSC2-/- iPSC line generated by gene editing, resulted in kidney organoids containing both AML-like tissues and tubular cysts. Cells in AML-like domains co-expressed the tubular epithelial cell marker CDH1 and the melanocyte marker PMEL, a melanocyte marker expressed by AML tumor cells that is indicative of epithelioid cell phenotype. Within the same organoids, tubular cysts expressed either CDH1 or stained positive for LTL, indicating cystic proximal or distal tubules, respectively. In summary, our TSC kidney organoid model supports the hypothesis of a second, loss-of-function mutation in a heterozygous TSC locus, as a pathogenic trigger of TSC-associated kidney diseases.

Keywords: kidney tuberorganoids, kidney angiomyolipoma, Tuberous Sclerosis Complex

MDD200

GENERATING HAIRY HUMAN SKIN FROM PLURIPOTENT STEM CELLS

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The skin is the largest organ of our body, which is essential for blocking external stresses and infections, controlling body temperature and fluid, and transmitting sensations of touch and pain. The skin develops from coordinated interactions between multiple cell lineages into a multi-layered tissue forming appendages (e.g. hair follicles and sebaceous glands) that are difficult to be reconstructed once damaged. Worldwide, over one hundred million people suffer from loss of skin or injuries from burns, surgical resections, diseases, or genetic defects. Despite abundant studies on skin regeneration for decades, however, a robust technique for generating appendage-bearing skin in a dish or in bioengineered skin equivalents remains elusive. Here, we report an organoid culture system that produces hairy human skin from pluripotent stem cells and recapitulates key features of skin development. During the first week of differentiation, we modulate TGFβ and FGF signaling pathways to co-induce epithelial cells and cranial neural crest cells in a single organoid unit. This matures into a skin organoid that is comparable to ~25-weeks of human fetal facial skin development through stratification of epidermis, development of fat-rich dermis, and formation of pigmented hair follicles that contain hair-bulge and sebaceous glands. Interestingly, the skin organoid also consists of sensory neurons with processes that interweave between hair follicles and target mechano-sensing Merkel cells in hair follicles, mimicking human touch circuitry. Furthermore, the skin organoid is capable of incorporating into and reconstructing hairy skin in a xenograft mouse model. Together, our results demonstrate that skin resembling human fetal facial tissue can be generated in our in vitro organoid culture system and used for skin reconstruction in vivo. We anticipate that our skin organoid system will be a foundation for studying human skin development, disease modeling, or a cell source for skin transplantation.

Funding source: NIH-R01AR075018

Keywords: Skin organoid, Hair follicles, Skin development

MDD202

GENERATION OF MATURE LUNG ORGANOIDS FROM CRYOPRESERVED HUMAN IPSC DERIVED ANTERIOR FOREGUT ENDODERM CELLS

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We previuously describe a stepwise protocol to efficiently differentiate human iPSCs to mature branching & alveolar lung bud organoids (LBOs). After 40-50 days of differentiation, LBOs express high levels of mature lung markers including surfactant-producing type II alveolar epithelial marker-SFTPB/SFTPC along with other airway, cilia and pulmonary markers. In an efffort to streamline the long differentiation protocol, cells at various stages of differentation were cryopreserved and subsequently thawed and tested for their ability to resume differentiation into mature branching LBOs. Cryopreserved LBOs from Day 20-25 exhibited very low cell viablity upon thawing. In contrast, anterior foregut endoderm (AFE) cells cryopreserved at day 8 exhibited high viablity upon thawing and could readily differentiated into mature LBOs. We show that large numbers of highly pure Pax9+EPCAM+ and Pax9+Sox2+ double positive AFE cells can be efficiently generated without the need for cell sorting. Along from the lung, AFE cells have the potential to give rise to other organ lineages including the thyroid, pharynx, thymus, esophagus and trachea. The ability to generate and cryopreserve large numbers of AFE cells from human iPSCs should facilitate and further streamline efforts to obtain lineage-specific organoids.

Keywords: Organoids, Human Lung, Anterior Foregut endoderm

EYE AND RETINA

MDD225

GENERATION OF PATIENT-DERIVED STEM CELL MODELS FOR NOVEL NONCODING DISEASE MODELING

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Inherited eye diseases are responsible for a wide variety of congenital malformations and degenerative conditions, with effects ranging from reduced or absent visual acuity at birth to progressive childhood or late-onset blindness. While coding mutations in critical eye development genes are commonly implicated as the cause of ocular malformations, causal variants for many ocular diseases remain unresolved, suggesting that non-coding genomic regions might be harboring the associated disease variants. We used whole genome sequencing to evaluate families with congenital ocular malformations without causal variants in protein coding regions and identified a novel, rare ocular disorder termed X-linked Foveal Dysplasia (XLFD) that is characterized by macular lesions that lead to the loss of retinal tissue and retinal pigment epithelium (RPE) and degeneration of the choroid. We linked XLFD to an insertional translocation of a 339 kb gene-less DNA fragment from chromosome 6q14 into chromosome Xq27. Further examination of this genomic region using ATAC-seq assay in RPE cells revealed multiple open chromatin sites with presumed enhancer-like activities. Similarly, publicly available DNase I hypersensitivity data show multiple open chromatin sites within the XLFD region coinciding with specific periods of retinal development. To further understand how XLFD affects the genomic regulation of the developing retina, we are developing “disease-in-a-dish” models using patient-derived induced pluripotent stem cells (iPSC) to generate RPE and 3D retinal organoids. Combining the techniques of epigenetic and transcriptomic regulatory mapping with an accessible, patient-specific disease model, we will dissect the biological and functional connections between the non-coding insertion and the XLFD locus to gain further insight into the mechanism of the disease.

Keywords: noncoding mutation, RPE and retinal organoids, macula, fovea

MDD443

USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO MODEL PRIMARY OPEN-ANGLE GLAUCOMA (POAG)

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Primary open-angle glaucoma (POAG) is an optic neuropathy characterized by gradual degeneration of retinal ganglion cells (RGCs) leading to irreversible vision loss and, if untreated, blindness. Upon diagnosis treatment options are limited and often fail to halt the progression of vision loss. This is due to the incomplete understanding of the disease. The molecular profiling of human RGCs in normal and diseased tissue is hindered by the lack of non-invasive means for obtaining RGCs from living donors. This can now be circumvented by the use of induced pluripotent stem cells (iPSCs) as a source of RGCs. With the use of an automated platform (TECAN Freedom EVO 200), we generated 305 human iPSC lines from glaucoma patients (n = 143) and healthy controls (n = 162). All lines underwent quality control analysis consisting of virtual karyotyping and assessment of pluripotency markers OCT-4 and TRA-1-60. Subsequently, 183 lines were differentiated into RGCs for 49 days via retinal organoids and then subjected to single cell RNA sequencing (scRNA-seq). Following the quality control assays, 258,071 cells from 114 individuals (57 POAG, 57 healthy) underwent further analysis. Using Louvain clustering from Seurat, we identified 23 subpopulations that were evenly distributed across cell lines from all patients. Cells were assigned to different classes based on expression of characteristic markers identified by previous studies. RGCs represented over 16% of all cells and were localised across 3 subpopulations. We also identified retinal progenitors, retinal pigment epithelium, photoreceptors, interneurons (amacrine/ horizontal cells) and lens cells. scRNA-seq allowed us to gain in-depth information about transcriptomic differences between healthy controls and glaucoma patient samples. Understanding mechanisms underlying RGC function, maintenance of homeostasis and those conferring susceptibility to POAG is crucial to discover new therapeutic targets and commence the process of drug discovery.

Funding source: Clemenger Foundation, Phillip Neal bequest (AH, AP), Research Training Program (MD), Stem Cells Australia (AH, AP, JP), NHMRC (AH), Australian Research Council (AP)

Keywords: Glaucoma, Induced pluripotent stem cells, single-cell RNA sequencing

HEMATOPOIETIC SYSTEM

MDD243

CHARACTERIZATION AND ENRICHMENT OF DEFINITIVE HEMATOPOIETIC STEM/ PROGENITOR CELLS FROM HUMAN EMBRYONIC STEM CELLS USING FEEDER-/SERUM-FREE ORGANOID-INDUCED DIFFERENTIATION PROTOCOL

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Ex vivo production of hematopoietic stem/precursor cells (HSPCs) represents a promising approach for blood disorders. To derive true HSPCs from ESCs, we sought to derive definitive hematopoiesis capable of both adult hemoglobin production among erythroid progeny and a diverse TCR repertoire among lymphoid progeny. In this regard, we differentiated mesordemally specified EBs onto gelatin-coated dishes to obtain HSPC generating organoid-like structures in feeder/ serum-free conditions. Our preliminary work showed that 7-day EB maturation followed by 8-day 2D differentiation provided the highest definitive (CD235a-CD34+, 69%, $p<0.01$) and lowest primitive (CD235a+CD34-, 1.55%, $p<0.01$) erythroid precursor cells along with the highest colony forming units (149.8±11.6, $p<0.01$). Flow cytometry analysis showed that the HSPC fraction (CD34+CD38-CD45RA-CD49f+CD90+) reached to 7.6-8.9% after 10 days of differentiation with 14.5% adult β-globin production followed by RBC differentiation. To further enrich HSPC fraction, spherical cells-derived from 3D structures were sorted for CD34 and CD43 markers. Myeloid and erythroid colonies were strictly restricted to CD34+CD43+ fraction (370.5±65.7, $p<0.01$) while only CD34-CD43+ fraction produced a small number of colonies (21.6±11.9), consistent with RBC differentiation results. To further determine the definitive potential of this population, we differentiated the sorted cells towards lymphocytes using the OP9/DLL1 co-culture system. By day 22 of differentiation, double-positive cells (CD4+CD8+) were detected along with CD3+ expression. DNA sequencing further confirmed broad TCR repertoire in ESC-derived CD34+CD43+ cells comparable to peripheral blood mononuclear cells-derived T-cells. Confocal imaging of antibody stained and fluorescently tagged organoid-like structures presented an accumulation of spherical CD34+CD43+ cells in sac-like compartments. In addition, a close association of CD31+ and CD144+ cells with CD34+CD43+ cells suggests a potential emergence of HSPCs through endothelial to hematopoietic transition. The ability to derive HSPCs that give rise to RBCs with β-globin and T-cells with broad TCR repertoire associated with definitive hematopoiesis has prompted us to test engraftment of these HSPCs in immunodeficient mice.

Keywords: pluripotent stem cells, definitive hematopoiesis, β-globin

MDD247

DEVELOPING A PANEL OF HUMAN PLURIPOTENT STEM CELL DERIVED MICROGLIA TO MODEL NEURODEGENERATIVE DISEASES

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Microglia, the resident immune cells of the brain, are key players in neuroinflammation and manifestation of neurodegenerative diseases. Recent studies have identified genes that are highly expressed in microglia associated with increased risk of developing Alzheimer's disease (AD), Parkinson's disease (PD), Frontotemporal Dementia, or Amyotrophic Lateral Sclerosis, propelling microglia to the forefront of neurodegenerative disease progression and emphasizing the critical need of model systems to study microglia. Since primary human microglia from living donors are not accessible for research, human induced pluripotent stem cell (iPSC)-derived microglia emerge as an authentic human, preclinical tool to mimic neurodegeneration and enable drug screening applications in vitro, as well as co-culture with other neural cell types for drug candidate validation. Recent GWAS studies have shown that genetically inherited variants apolipoprotein E4 (APOE4), presenilin 1 (PSEN1), presenilin 2 (PSEN2), or amyloid precursor protein (APP), the presence of specific Single Nucleotide Polymorphisms (SNPs) within triggering receptor expressed on myeloid cells 2 (TREM2), CD33 or Siglec 3, ATP-binding cassette transporter A7 (ABCA7), have a strong association with increased disease risk of AD. A panel of 12 human iPSCs from apparently healthy and diseased donors harboring many inherited mutations were selected and successfully differentiated into cryopreserved hematopoietic progenitor cells and microglia. In addition, microglia were also derived from engineered and non-engineered iPSCs to create isogenic pairs with mutations in TREM2, A53T and MeCP2. End stage microglia retained the presence of cell surface (CD45, CD11b and CD33) and intracellular (P2RY12, TREM-2, CX3CR1, IBA) microglia-specific antigens, gene expression patterns and exhibited phagocytic function. In addition, cryopreserved microglia retained the ability to be polarized towards an inflammatory or anti-inflammatory subtype by specific stimuli. Thus, this panel of both normal and disease associated microglia can be used to investigate the role of gene variants in human microglia, develop coculture applications with other neural cell types to create predictive in vitro models for neurodegenerative diseases.

Keywords: Disease Associated Microglia, Induced Pluripotent Stem Cells, Neurodegenerative Diseases

MDD249

GENERATION OF ISOGENIC CRYOPRESERVABLE MACROPHAGES FROM NORMAL AND ENGINEERED HUMAN PLURIPOTENT STEM CELLS FOR DISEASE MODELLING

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Macrophages are innate immune cells that play critical roles in tissue homeostasis, inflammation and immune oncology, and hence have a potential utility for cell therapy. Human peripheral blood monocyte-derived macrophages (PBMACs) have been used widely used to characterize macrophages. PBMACs lack proliferative capacity offering limited scalability options and they are terminally differentiated. Tumor-derived monocytic cell lines often tend to have abnormal karyotypes, increased proliferation and exhibit poor function. Induced pluripotent stem cell (iPSC)-derived macrophages (iMACs) can be generated in large quantities and cryopreserved in large numbers. In addition, these cells can be used to study human macrophage biology, disease modeling, functional genomic analysis, drug screening and cell therapy applications. The present study describes the generation and characterization of macrophages from iPSCs via a defined serum free method amenable to scale up to generate a large batch of macrophages from apparently healthy (iCell Macrophages 2.0) and disease associated donors. Cryopreserved Macrophages retained purity and expressed CD68, CD33, CD11c, CD11b, CD1a, HLA-DR, CD86, CD64, CD80, CD206 and CD169. They revealed a classic morphology by wright stain, responded to stimuli and secreted analytes to mimic inflammatory or anti-inflammatory responses. The cells displayed robust phagocytic function coming right out of cryopreservation. In addition, cryopreserved end stage macrophages were also derived from isogenic iPSCs engineered to mimic Rett's syndrome (MECP2), Parkinson's disease (A53T) and Frontotemporal dementia mutations (GRN2). End stage macrophages from isogenic engineered clones expressed macrophage-specific purity markers, gene expression patterns, exhibited phagocytic function and elicited response to specific stimuli. Thus, the macrophage panel containing a collection of normal and disease associated macrophages can be used to set up high throughput screening, multi-cell co-culture and immune oncology applications

Keywords: Macrophages, Induced pluripotent stem cells (iPSC), Disease modelling

MDD251

GENETIC LANDSCAPE OF HUMAN BM-MSCS AND DIFFERENTIATED LINEAGES: RNA AND SMALL RNA INTEGRATED ANALYSIS

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Adult stem cells have a remarkable capacity of differentiating into various cell types necessary for tissue and organ regeneration. Multiple studies have focused on the differentiation potential of mesenchymal stem cells (MSCs). However, little is known about the molecular characteristics of human MSCs and their progenies obtained from donors of different ages. In this study, we analyzed publicly available sequencing data obtained from young (~22-year-old, n=8) and old (~65.5-year-old, n=8) human donors of MSCs and their differentiated counterparts: osteocytes, chondrocytes and tenocytes. The raw mRNA and small RNA (non-coding RNA) sequencing data was downloaded from NIH BioProjects and systematically analyzed in order to identify uniquely expressed genes in MSC-derived osteocytes, chondrocytes and tenocytes. We identified similar up- and down-regulated gene patterns in both age groups. However, the young group displayed a greater variety of expressed genes across all three differentiated groups. This discrepancy in gene expression profiles may indicate a greater differentiation potential of younger MSCs. miRNA analysis of osteocytes, found 21 microRNAs in the young group (5 upregulated and 16 downregulated) and 97 in old group (16 upregulated and 81 downregulated), chondrocytes, 232 differentially expressed microRNAs in the young group (one upregulated and remaining downregulated) and 285 in the old group (two upregulated and remaining downregulated) and tenocytes, 63 microRNAs differentially expressed in the young group (five upregulated and remaining downregulated) and 228 microRNAs in the old group compared to stem cells (four upregulated and remaining downregulated). miRNA and mRNA integrated analysis using miDIP, showed key miRNA regulation of mRNAs in both age groups across three differentiated lineages. Our analysis provides additional data which is important for the identification of MSC markers of plasticity. Additionally, our data may shed light upon the molecular mechanisms of age-associated musculoskeletal dysfunction caused by the decreased capacity of MSCs regeneration in elderly people.

Keywords: Genomics, miRNA, mRNA, big data, BM-MSCs

IMMUNE SYSTEM

MDD261

FUNCTIONAL CHARACTERIZATION OF ALZHEIMER'S DISEASE GENETIC RISK USING HUMAN IPS-DERIVED MICROGLIA

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More than 40 genomic loci have been linked to late onset Alzheimer's disease (AD) containing hundreds of potential risk genes. While the impact of some genes, such as APOE and TREM2, has been widely studied, it is unknown how most other risk genes are linked to AD pathogenesis. Interestingly, transcriptomic and epigenetic data have shown that a significant number of these genes are expressed in microglia, the immune cells of the brain. Microglia are essential for the maintenance of a healthy brain, and their role in synapse loss caused by abnormal phagocytosis leading to cognitive decline has been demonstrated in multiple mouse models. To understand how AD risk genes affect phagocytosis we turned toward human iPSC-induced microglia. Using single cell transcriptomics, we have characterized how AD risk gene expression changes during engulfment of various substrates. Also, we characterized how basic microglial functions are changed by the presence of AD risk gene variants. Together, our data identified key phenotypes that characterize individuals with high genetic risk of developing AD and those phenotypes will be further pursued in in vivo models.

Keywords: Alzheimer's disease, microglia, GWAS

MUSCULOSKELETAL

MDD263

CO-CULTURE OF HUMAN IPSC DERIVED MOTOR NEURONS AND SKELETON MUSCLES PROVIDES A PHYSIOLOGICAL NEUROMUSCULAR JUNCTION MODEL IN-A-DISH

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Neuromuscular junction NMJ is a highly organized synapse which are formed between the axon of a motor neuron and a muscle fiber. Motor neurons transmit efferent signals to control muscle fiber contraction. The functional integrity of NMJ is tightly associated with the etiology, pathophysiology and progression of neuromuscular diseases. NMJ models have been well established using rodent cells, however, there is still a need to establish a human model due to the physiology difference between mouse and human. Here, we have re-established

the NMJ models by co-culturing human motor neurons and skeleton muscles differentiated from the same human iPSC line. The formation of functional NMJ connections are confirmed by imaging muscle fiber calcium transients and the contraction in response to glutamate-induced motor neuron firing. In the future, the application of this model using patient derived iPSC cells as well as high through-put small molecule screen assay will deepen our research on the etiology and provide targeting molecule candidates that can improve the physiological function of NMJ.

Keywords: Neuromuscular junction NMJ, Human iPSC differentiated motor neuron, Human iPSC derived skeleton muscle cells

MDD264

ELUCIDATING HEPARAN SULFATE ROLES IN OSTEOCHONDROMA PATHOGENESIS USING IPS CELLS DERIVED FROM A HME PATIENT

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Hereditary multiple exostoses (HME) is a pediatric disorder in which benign tumors called osteochondromas form along the growth plates. Osteochondromas lead to skeletal deformities, growth retardation, and early onset osteoarthritis. Current treatments include surgical removal of osteochondromas but patients continue to experience physical difficulties and chronic pain. HME is caused by heterozygous mutations in EXT1 or EXT2, two members of the exostosin (EXT) family encoding glycosyltransferases that are required for heparan sulfate biosynthesis. Heparan sulfate is an anionic polysaccharide that is essential for mammalian development. It is unknown how defects in heparan sulfate biosynthesis leads to osteochondroma pathogenesis in HME patients. To address this knowledge gap, we developed an in vitro disease model by deriving induced pluripotent stem (iPS) cells reprogrammed from fibroblasts of a HME patient. To determine how a heterozygous mutation in EXT1 alters heparan sulfate biosynthesis, we employed a quantitative mass spectrometry-based method to determine the compositions and molecular weights of heparan sulfate of the iPS cells. The data indicate that changes in heparan sulfate length leads to aberrations in growth factor signaling pathways during chondrogenesis that may result in osteochondroma formation. In summary, our HME-patient derived iPS cell line is an excellent tool for modeling HME in vitro. Moreover, our findings highlight the significance of glycans in disease pathogenesis and tissue development. Findings from our study will elucidate the underlying molecular mechanisms and advance the development of therapeutics that prevent osteochondroma formation.

Keywords: Hereditary multiple exostoses, Heparan sulfate, Osteochondroma

MDD270

DEVELOPMENT OF A NEUROMUSCULAR JUNCTION-ON-A-CHIP MODEL USING HUMAN IPSC-DERIVED MOTOR NEURONS AND SKELETAL MUSCLE

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The lack of effective therapies for degenerative neuromuscular disorders (NMD) such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis represents a significant unmet medical need. Development of therapies to treat NMD have been hampered by the challenges of modeling motor neurons, skeletal muscle and their interactions. To address the need for a more physiologically relevant human model that captures the biological complexity of two distinct human cell types, we adapted a previously published neuromuscular junction (NMJ)-on-a-chip model. The chip design was optimized to improve handling and increase throughput. Human iPSC-derived motor neuron spheres and skeletal muscle progenitor cells were generated from SMA and healthy control samples and introduced into the chip in various combinations. Axonal processes migrated out from the motor neuron spheres to form neuromuscular junctions with the skeletal muscle microbundles. NMJ functionality as assessed by activities such as muscle bundle contractile response to glutamate stimulation of neurons or acetylcholine were evaluated in the absence or presence of therapeutic treatments. These studies establish the NMJ-on-a-chip model as a platform for evaluating future therapies for NMD.

Keywords: neuromuscular junction, muscle, motor neurons

NEURAL

MDD296

DEVELOPING HUMAN CELLULAR TOOLS FOR STUDYING AND UNDERSTANDING JORDAN'S SYNDROME

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Jordan's Syndrome (JS), also known as PPP2R5D-related intellectual disability (ID), is a neurodevelopmental disorder that is caused primarily by de novo missense mutations in the PPP2R5D gene. PPP2R5D encodes a 602-amino acid protein PPP2R5D (also known as the B56δ subunit), which is a regulatory subunit of the heterotrimeric enzyme serine/threonine-protein phosphatase 2A (PP2A). Mutations in phosphatases are known to cause human overgrowth due to their involvement in cellular regulation. With the recent identification of Jordan's Syndrome using Whole Exome Sequencing, PPP2R5D mutations specifically have been associated with various patient intellectual disabilities (IDs), malformations, autism spectrum disorders, seizures, and behavioral challenges. The wide range of the severity of patient IDs, along with the association of PPP2R5D to Alzheimer's, autism, Parkinson's-like symptoms, and cancer, has generated an impetus for further research to elucidate the specific cellular pathways that are affected by PPP2R5D. In order to study JS in vitro, various biological tools need to be generated including neural cellular models. Induced pluripotent stem cells (iPSC) are highly proliferative cells that can differentiate into all three germ layers in vitro. Reprogramming patient somatic cells in to iPSC offers a means of generating difficult to source neural stem cells and neurons to study PPP2R5D mutations. Our team has generated five chromosomally stable iPSC clones from JS patient-derived skin fibroblasts representing four of the most common PPP2R5D disease-causing mutations. We have also generated chromosomally stable isogenic clones of these iPSC lines using precision CRISPR editing to provide genetically matched unmutated cells as controls. Neural stem cell (NSC) lines and neurons have been derived from each line to help study disease biology and novel therapeutic agents in a disease relevant cell type, as well. iPSC and NSC provide a theoretically unlimited supply of disease-specific cells that can be used to study JS. They are important and necessary tools for advancing the field of JS research and developing therapies for this debilitating disorder.

Keywords: Jordan's Syndrome (PPP2R5D), Neural Stem Cells, Induced Pluripotent Stem Cells

MDD309

CHARACTERIZATION OF DOPAMINERGIC NEURONS WITH COPY NUMBER VARIATIONS ASSOCIATED TO AUTISM AND SCHIZOPHRENIA

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Several copy number variations (CNVs) have previously been associated with development of neuropsychiatric and neurodevelopmental disorders. One of the CNVs is the duplication or deletion of the 16p11.2 gene region. Clinical studies have shown that deletion of 16p11.2 leads to severe developmental deficits, increased head size, intellectual disability and autism, whereas duplication of 16p11.2 is highly associated with schizophrenia and bipolar disorder. Currently, treatment options for these disorders are limited, and commonly used medications can cause severe side effects for patients. To develop more efficient and specific medications it is important to find the key molecular pathways regulating the neuronal maturation and formation of network connections during brain development. To address this need, we differentiated the human iPSCs with CNVs of 16p11.2 duplication and 16p11.2 deletion into dopaminergic (DA) neurons in vitro and we characterized their molecular and functional deficits compared to control neurons. For assessment of network activity and synchronization we used the high-density micro electrode array (MEA) platform and for analyses of excitability and excitatory post-synaptic currents we used the conventional patch clamping method. We also analyzed the transcriptional gene expression levels of these DA neurons to identify genes that are affected by 16p11.2 CNVs. We detected that the cells carrying 16p11.2 CNVs had abnormal synaptic marker expression and altered synaptic activity and network function compared to healthy control DA neurons. We also characterized the downstream pathways affected by the 16p11.2 CNVs. This data implicated that these copy number variations can cause different physiological and psychological symptoms in patients due to deficient dopaminergic neuron function. In the future these cell culturing platforms can be utilized for disease phenotyping and drug screening assays in vitro to find novel compounds for treatment of these neuropsychiatric disorders.

Funding source: Tommy Fuss Center for Neuropsychiatric Disease Research at Boston Children's Hospital, and Translational Neuroscience Center at Boston Children's Hospital.

Keywords: Dopaminergic neurons, High-density micro-electrode array, Copy number variations of 16p11.2.

MDD312

DISSECTING MECHANISMS OF SYNAPTIC MATURATION USING HUMAN CELLULAR MODELS

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The synapse is a fundamental point of communication in the nervous system, and deeper insight into synaptic mechanisms is critical for our understanding of normal brain function as well as dysfunction in developmental and degenerative diseases. While diverse brain cell types can now be generated from human pluripotent stem cells in vitro, knowledge of human synaptogenesis remains incomplete. We therefore developed a novel and scalable assay for automated quantification of synaptic development in vitro using human neurons and astrocytes. In brief, we utilize automated liquid handling to plate precise numbers of human neurons and human astrocytes in 96-well format and immunostain for synaptic markers followed by automated high-content imaging and a custom pipeline to measure and analyze the density and size of synaptic puncta. We are now employing this assay to (i) study cell-type specific contributions to synaptic dysfunction in a model of monogenic neurodevelopmental disease, (ii) uncover modulators of human synaptogenesis utilizing a small molecule screening strategy, and (iii) optimizing CRISPRa/CRISPRi tools to facilitate genetic perturbations in both human neurons and astrocytes. Collectively, these approaches are designed to accelerate basic discovery and identify potential opportunities for therapeutic development.

Keywords: Assay, Synapse, Human

MDD318

CHROMOSOME 16P11.2 DUPLICATION IMPACTS GENE EXPRESSION, ASTROCYTE DIFFERENTIATION, NEURONAL DEVELOPMENT, AND SYNAPSE FORMATION IN CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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A rare, 650 kb duplication on chromosome 16p11.2 (dup16p11.2) is associated with neurodevelopmental disorders, schizophrenia, and bipolar disorder. This project aims to explore the use of induced pluripotent stem cell (iPSC) technology to study the biological impact of dup16p11.2 in neural cells and screen for therapeutic agents. Samples of 6 carriers and 6 noncarriers were obtained from an extended family ascertained through a proband with schizoaffective bipolar disorder or from the Rutgers Repository, reprogrammed with Sendai virus, then differentiated into neural progenitor cells (NPCs), neurons, or astrocytes. Cells were characterized with immunostaining, confocal microscopy, and bulk RNA sequencing, and NPC differentiation, neuron formation and maturation were live-imaged weekly for 20 weeks. Comparisons between carriers and sex-matched non-carriers revealed major differences in cellular differentiation and growth. Carriers generated 50% fewer neurons after 3 weeks of culture ($p<0.01$). Carrier neurons also showed fewer post-synaptic structures based on PSD95 staining. While non-carrier NPCs easily differentiated into astroctyes using standard protocols, carrier NPCs did not differentiate into mature astrocytes. RNAseq analysis found that expression of most genes within the duplication was increased in carriers. In neurons, ALDOA, KCDT13, KIF22, MAPK3, QPRT, and TMEM219 showed the greatest increases (1.5- to 2-fold). Dup16p11.2 also perturbed expression of distal genes. Functional annotation of differentially-expressed genes indicated downregulation of neuronal development ($p<0.0001$), neurite outgrowth ($p<0.0003$), and neuronal migration ($p<0.0005$), among others, consistent with our morphological imaging. As in previous studies, network analyses identified ERK1/2 as a central hub among genes dysregulated by dup16p 11.2. Experiments are now underway to rescue deficits in neuronal and synapse development in dup16p11.2 carriers. We conclude that dup16p11.2 has a widespread impact on gene expression, astrocyte differentiation, neuronal development, and synapse formation in vitro. Patient-specific iPSCs are a promising approach to the neurobiology of rare neuropsychiatric copy number variants and may provide a platform for screening novel therapeutics.

Funding source: Funded by the NIMH Intramural Research Program, grant#1ZIAMH002843 and protocol 80-M-0082.

Keywords: 16p11.2 duplication, RNAseq, neuron

MDD323

DRUG SCREENING FOR OTOPROTECTANTS USING AN IN VITRO MODEL OF INNER EAR SENSORY HAIR CELLS DERIVED FROM DIRECT CELLULAR REPROGRAMMING

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Deafness affects 360 million people worldwide, the leading cause of which is loss of sensory hair cells in the cochlea. The inner ear sensory hair cells of the organ of Corti are selectively vulnerable to numerous genetic and environmental insults. In mammals, hair cells lack regenerative capacity, and loss of these cells leads to permanent hearing loss and vestibular dysfunction. Studies for otoprotective and regenerative strategies have been limited by the paucity and inaccessibility of primary hair cells. Growing hair cells in vitro would provide a route to overcome this experimental bottleneck. We report a combination of four transcription factors (Six1, Atoh1, Pou4f3, and Gfi1) that can convert mouse embryonic fibroblasts, adult tail-tip fibroblasts and postnatal supporting cells into induced hair cell-like cells (iHCs). iHCs exhibit hair cell-like morphology, transcriptomic and epigenetic profiles, electrophysiological properties, mechanosensory channel expression, and vulnerability to ototoxins in a high-content phenotypic screening system. We have performed a non-biased screen to identify compounds that can protect iHCs against a known ototoxin, cisplatin. Cisplatin based chemotherapy can cause hair cell degeneration and permanent hearing loss in as many as 80% of patients receiving treatment. The use of iHCs to identify novel otoprotectants in a high-throughput manner has led to the identification of several protective hits in vitro. Our top five otoprotective hits have been validated in vitro using dose response curves and in organ of Corti explant cultures. We are pursuing these identified hits to test for protective effects in vivo in mice with cisplatin induced hearing loss. Our in vivo testing consists of measuring auditory thresholds as well as quantification of hair cell survival post treatment. Taken together, direct reprogramming to iHCs provides a high throughput platform to identify causes and treatments for hair cell loss, and may help identify future approaches for protecting and/or restoring hearing.

Keywords: hearing loss, induced sensory hair cells, otoprotectant drug screening

MDD333

CHARACTERIZATION OF DARK KINASES IMPLICATED IN ALS USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Although kinases are highly desirable targets with an unparalleled success in cancer therapeutics research their potential has not yet been realized in neuroscience. The intrinsic complexity linked to central neural system (CNS) drug development and a lack of validated targets has hindered progress in developing kinase inhibitors for CNS disorders. Rather than focusing on the widely studied kinases (around which most of the data, reagents, and small molecules have been generated) we concentrated on elucidating the biology, and enabling the characterization, of lesser studied kinases also called dark kinases from the Illuminating the Druggable Genome (IDG) program. Through data mining, we have identified genetic links between kinases on the IDG list and Amyotrophic Lateral Sclerosis (ALS). Following expression analysis in human spinal cord motor neurons, neuron progenitor and motor neurons derived from stem cells, prioritized kinases were selected for CRISPR-mediated genetic knockout in pluripotent stem cells to better understand their role in propagating ALS biology. Seven IDG kinases, including NEK1, DYRK2, CSNK1G3, PXK, SCYL3, STK36, and TTBR2, were knockout in both hES and iPS cells, and later differentiated into neural progenitor cells (NPC) and motor neurons (MN). Analysis of kinase KO phenotypes include cell morphology, viability, activity, response to stimuli, and protein aggregation in NPC and MN. Observed traits supported a small molecule campaign targeting the kinases of interest. We demonstrate that the identification and characterization of new kinases as potential drug targets for ALS create opportunities for the development of CNS drugs.

Keywords: ASL, Dark Kinases, Motor Neuron

MDD344

DEVELOPMENT OF HUMAN CORTICAL ORGANOIDS WITH CELLS OF MESODERMAL LINEAGE

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Human cortical organoids (hCOs), derived from human embryonic stem cells (hESCs), provide a platform to study human brain development and neurological diseases in complex 3D tissue. In either guided or unguided methods, hCOs are derivatives of neuroectoderm commitment of hESCs. Thus, most hCOs, or brain organoids, lack cell types originated from non-neuroectodermal differentiation, including endothelial cells or microglia. We previously ectopically expressrd human ETS variant 2 (ETV2) in developing hCOs to incorporate vascular-like structure in hCOs, forming vascularized hCOs (vhCOs). vhCOs displayed vascular function in vitro and in vivo, and importantly vhCOs improved the quality of cells inner most region of organoids. Using a similar approach, we introduced the myeloid-lineage cells in hCOs, producing mhCOs (microglia containing hCOs). The microglia-like cells in mhCOs demonstrated the essential function of primary microglia, such as phagocytosis. Surprisingly, the mhCOs were protected from cellular damage mediated by Abeta treatment. Using CRISPRi (CRISPR interference), we delineated the function

of AD (Alzheimer disease)-associated genes in mhCOs. Overall, we have succeeded in introducing mesodermal lineage cells into hCOs, constructing the functionally near-to-complete brain organoids.

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Keywords: Brain organoids, Microglia, endothelial cells

MDD345

AUTOMATED, HIGH-THROUGHPUT NEURONAL DIFFERENTIATIONS OF LARGE SCALE POPULATIONS USING IPSC MODELS

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While large numbers of iPSC lines are now widely available, differentiating multiple lines in parallel faces a number of technical challenges. To overcome these issues, NYSCF has developed high-throughput automation workflows capable of differentiating hundreds of lines per month into various cell types. Here we present the generation of a fully automated pipeline for the generation of excitatory NGN2-induced neurons. This workflow automates thawing of iPSCs, their subsequent culture, passaging, and seeding of targeted cell numbers across a variety of plate types. This enables reproducible viral transduction, immunocytochemistry, and lysates for RNAseq analysis. To validate our workflow, we have extensively compared differentiation protocols and manual vs. automated workflows. We show that, using lentiviral delivery of NGN2, we can create populations of neurons that express limited cell-to-cell line variation, particularly compared to classically used methods such as dual-SMAD based induction protocols. To our knowledge, this pipeline affords for the first time the high-throughput differentiation of hundreds of samples opening up the potential for population-scale, high throughput biology.

Keywords: Automation, neuronal, ngn2

MDD356

ENGINEERING HUMAN NEURAL ORGANOIDS TO EXPLORE IMPAIRED NEUROGENESIS INDUCED BY ARSENIC

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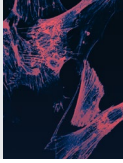
Modeling the development and function of the human brain is challenging due to the vast complexity of the organ. Recent advances in the derivation of brain-like organoids from human pluripotent stem cells (PSCs) have provided new tools to study the biology of the human brain. These 3D in vitro models have the potential to enhance our understanding of the mechanisms of developmental neurotoxicity (DNT) during the early stages of neurogenesis and offer a cost-effective approach for assessing chemical safety. Here, we have used PSC-derived embryoid bodies from 384-well plates to develop neural organoids for chemicals DNT testing. There is overwhelming evidence that environmental factors play a role in the development and progression of a host of central nervous system disorders. Arsenic (As) is widespread environmental contaminant. Human exposure to As occurs mainly through ingestion of contaminated food or water. Exposure to this inorganic is associated with developmental neural diseases. However, the mechanisms of As on DNT are not well-defined. We used 3D embryoid bodies to recapitulate events involved in early embryogenesis and neurogenesis. We found that a 7-day exposure to a human-relevant, non-cytotoxic dose (0.5 µM; 35 ppb) of As increased ectoderm differentiation within the EBs through upregulated expression of genes PAX3, PAX6, SOX1, COL2A1 and the Notch signaling pathway, which play critical roles in early embryonic development. Histological staining of As-treated EBs showed early neural rosette structure disruption. Pathological assessment validated the presence of neuroepithelial tissues, like neural rosettes and neuropil structures in 40 days neuron organoid. Immunohistochemistry showed vimentin+ astryocytes and nestin+ neural stem cells in the organoid structure. Expression of markers for other germ layers was negative. During neural organoid induction, As increased expression of neural progenitor cell marker genes NESTIN and PAX6 in early stage (day 7) of neural induction. The neural rosette structure was disrupted in day 40 neural organoid with As exposure. Using the neural organoid 3D model described here can provide valuable insights into the cellular events and molecular mechanisms to address the adverse outcome pathways associated with As-induced developmental neurotoxicity.



Keywords: Organoid, Developmental Neurotoxicity, Neurogenesis

MDD365

COMBINING TARGETED EXOME SCREENING WITH IPSC MODELING TO IDENTIFY GENETIC DRIVERS IN ALPHA-SYNUCLEINOPATHIES

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Alpha-synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies and multiple system atrophy, are all linked to the misfolding and aggregation of the alpha-synuclein (a-syn) protein. Genetic drivers, including genes encoding proteins related to the function and toxicity of a-syn, may be shared among these diseases. Our lab has previously utilized unbiased screening methodologies to uncover a network of genes that impact a-syn toxicity in cellular systems. These studies identified a number of genes enriched in Mendelian risk factors for PD. Here, we show the results of high-depth exome-capture of human orthologs of these hits in 500 patients with synucleinopathies. We identified rare (MAF<1%) variants enriched in patients versus publicly available genomes as controls, utiliing joint calling and adjusting for inflated variant capture where possible. Beyond identifying pathogenic variants in the known PD genetic risk factors GBA and LRRK2, we identified novel rare (MAF<1%) variants in genes that may modulate vulnerability to a-syn. Some of these variants were specifically enriched in GBA or LRRK2 mutation carriers. We describe a functional genomics strategy through which genes identified in this study can be assayed for biological effects in a suite of tractable human stem-cell models. In human iPSC, we use CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) systems in conjunction with transgenic overexpression of a-syn. We show that expression of different mutant a-syn transgenes result in rapid development of distinct a-syn aggregation. These neuronal pathologies reminisce of those found in postmortem patient brain. Moreover, single-cell longitudinal imaging enables robust assessment of viability. Our generalizable approach thus promises to integrate genomic and stem cell-based tools to shed light on shared mechanisms among diseases linked by a common proteinopathy, and on the basis of missing heritability and variable penetrance in genetically complex neurodegenerative diseases.

Keywords: Alpha-synuclein, Functional genomics, Human iPSC

MDD366

GENERATION OF HUMAN PLURIPOTENT STEM CELL MODELS FOR STUDYING MOLECULAR MECHANISMS UNDERLYING FRAGILE X SYNDROME

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Silencing of the FMR1 gene causes fragile X syndrome in humans. Transcriptional silencing of FMR1 is observed in human cells but not in animal models. Therefore, pluripotent stem cells (hPSCs) and derived cells serve as unique platforms for studying FMR1 silencing and re-activation as well as insights into FMRP functions in human brain. Our knowledge of the functions of the FMR1-encoded protein, FMRP, are based primarily on studies in animal models. However, failed clinical trials based on the animal studies suggest human-animal model discrepancy and a need for studies in human cells. Here, we employed CRISPR-Cas9 to generate tools to interrogate functions of FMRP in human cells. First, we generated a reporter hiPSC line by knocking in a Nano-luciferase in the FMR1 gene and screened small molecule libraries for FMR1 reactivation. Moreover, we generated FMRP-FLAG and FMRP-KO hPSCs to explored gene regulation by FMRP. We demonstrate common and distinct gene regulation by FMRP in various types of human neural cells. Our results provide new insights into functions of FMRP in human neurodevelopment.

Keywords: Fragile X syndrome, human pluripotent stem cells, CRISPR-Cas9 genome editing

MDD383

CAPTURING DOWN SYNDROME-RELATED WHITE MATTER ABNORMALITIES IN HUMAN OLIGO-SPHEROIDS

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Down Syndrome (DS), also known as trisomy 21, is characterized by the triplication of human chromosome 21 (HSA21), leading to abnormal fetal brain development, neuronal disbalance and aberrant cortical organization as well as reduced neuronal connectivity and intellectual disability. Multiple genes in the HSA21 region play crucial roles in the developing brain, including the transcription factors Olig1/2 that prime the oligodendrocyte (OL) lineage. Previous work on humans with DS and DS-mouse models have shown altered expression of myelin -related genes, as well as delayed OL maturation and disruptions in myelin production, structure and density. These white matter deficits affect neuronal connectivity, contributing, at least in part, to the intellectual deficits associated with DS. For obvious reasons, studying myelination as an ongoing process in pre and early postnatal life in humans is challenging and iPS cells allow to study biological processes in humans, while preserving the individual differences. Using an iPS cell-derived 2D OL model, we have observed deficit in the generation of neuronal stem cells from trisomic iPS cells (as compared to their euploid counterparts) and in further progression towards pre-oligodendrocyte progenitor cells (OPCs). However, 2D oligodendrocytes cultures present several limitations including the lack of interactions with CNS cells that OL encounter in vivo and the lack of ability to study the final myelination process. Recent advancements enabling the generation of 3D iPS-derived cultures promise to expand our knowledge of normal and disease-altered human brain development. Newly described 3-D organoid methodologies (by the Pasca group) enable the generation of so called oligo-spheroids that mimic the development of the OL lineage in the presence of astrocytes and neurons, making this model extremely relevant for studying myelination-related processes. Our main goal is to examine the DS-associated white matter abnormalities in human oligo-spheroids. This 3D model allows us to investigate in depth the transition of OL-lineage cells through developmental stages, as well as their migration, cytoarchitecture, myelination capacity and their interactions with other glial and neuronal cells upon which their development relies.

Keywords: Down Syndrome, Myelination, Organoids

MDD385

DECIPHERING HYPERACTIVITY OF SYMPATHETIC NEURONS IN FAMILIAL DYSAUTNOMIA USING HUMAN PLURIPOTENT STEM CELLS

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It is widely accepted that the neural circuits of the brain respond to severe stressors and pathological changes in its cells are associated with stress disorders. However, pathological changes in sympathetic neurons, one of the peripheral stress centers, are poorly defined. Familial dysautonomia (FD) is a genetic disorder that impacts both development, function and survival of sympathetic neurons (symNs), while no critical brain symptoms have been reported. For FD patients, stress can induce dysautonomic crisis, where their sympathetic nervous system becomes dysregulated evident by tachycardia, blood pressure spike and severe vomiting. We employ this disorder to first ask what the pathologies are in FD symNs and second how these pathologies lead to particular vulnerability to stress. We recently described a well characterized and efficient symN differentiation strategy. We then employed this strategy to investigate induced pluripotent stem cell (iPSC)-derived synMs in FD. Similar to previouse reports, we first showed that neural crest cells derived from FD iPSCs are not generated as efficiently compared to healthy controls. However, we found that symNs could be made at a similar rate from neural crest cells as compared to control. Interestingly, ensuing careful comparison of FD and control symNs elucidated spontaneous electrical hyperactivity of FD symNs. Higher calcium influx was also detected in FD symN by calcium imaging. We thus hypothesize that the regulation of the main neurotransmitter in symN, norepinephrine (NE), is faulty. Indeed, we found altered expression of key enzymes in NE synthesis, i.e. tyrosine hydroxylase (TH) upregulated in FD symNs. NE synthesis as well as NE metabolites are further assessed via HPLC. Additionally, we found the expression and activity of NE recycling transporter NET downregulated, suggesting a faulty auto-regulation of NE in FD symNs. Further characterization of mechanisms behind these phenotypes with or without stress stimulation need to be validated. The long-term goal of our study is the identification of specific pathologies in symNs in FD patients that have the potential to be targeted by future drugs.

Keywords: sympathetic neurons, Familial Dysautnomia, neural hyperactivity

MDD388

ATOH1 INDUCED NEURONS DERIVED FROM PARKINSONS DONORS PROVIDE A DOPAMINERGIC LIKE CULTURE SYSTEM TO ASSESS REDUCTION OF PATHOLOGIC ALPHA SYNUCLEIN

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Parkinson's disease (PD) is a progressive neurodegenerative disease hallmarked by aberrant production and aggregation of α-synuclein (encoded by the SNCA gene). Pathologic accumulation of aggregated α-synuclein is observed in the brain of both idiopathic and genetically defined cases of PD, including those caused by mutations within the SNCA locus itself, which constitutes a risk loci for disease severity and age of onset. At the cellular level, aggregated α-synuclein causes neuronal and glial dysfunction and death, with dopaminergic neurons located in the substantia nigra being highly susceptible to abnormal α-synuclein levels. α-synuclein is believed to spread extracellularly to induce pathology in adjacent cells and brain regions via a "prion-like" mechanism, serving as a template that sequesters additional α-synuclein, exacerbating aggregation and disease progression. Taken together these findings suggest a detrimental role of α-synuclein in PD. Therapeutic strategies that reduce the expression of α-synuclein might be beneficial to disease progression. We utilized a published protocol to generate dopaminergic-like neurons via Atoh1 overexpression in six iPSC lines: two healthy control donors, two donors with a triplication of the SNCA gene locus, and an isogenic pair of lines where the donor's SNCA A53T mutation was corrected by genome editing. Atoh1-induced neurons (iNeurons) were characterized by immunocytochemistry for neuronal and dopaminergic markers, and their functionality was validated by multielectrode array recordings. We assessed the lineage of Atoh1-induced cells by comparing midbrain markers in Atoh1 iNeurons to donor-matched excitatory NGN2 iNeurons and iCell Dopa Neurons from Cellular Dynamics International (CDI), and found that Atoh1 induction yields neurons with higher expression of some key midbrain markers compared to NGN2 induction. However, the expression of these markers was much lower compared to the iCell Dopa Neurons. Finally, 14-day treatment with an antisense oligonucleotide or a positive control small molecule FTX-A reduced SNCA mRNA and α-synuclein protein in patient-derived Atoh1 iNeurons. Our results provide evidence that Atoh1 iNeurons can be used as a platform for drug discovery efforts aimed at reducing α-synuclein in disease-relevant cell types.

Keywords: iNeuron, Atoh1, synuclein

MDD438

EXPRESSION PROFILING OF REST AND RCOR GENES IN NEUROGENESIS USING 2D AND 3D HUMAN PLURIPOTENT STEM CELL MODELS

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Repressor element-1 silencing transcription factor (REST) is a transcriptional repressor of neuronal genes that forms a complex with the corepressor of REST1 (CoREST1), CoREST2 or CoREST3 (encoded by RCOR1, RCOR2 and RCOR3, respectively). Emerging evidence suggests the CoREST family have the ability to target unique genes, in a REST-independent manner, in various neural and glial cell types at different stages of development. Research on REST and RCOR genes has been largely based on animal models and established cell lines. Accordingly, human stem cells offer an effective in vitro cell-based model towards defining the expression profile of REST and RCOR genes in neurogenesis and thus provide insight into their function during neurodevelopment. This study used 2D and 3D stem cell models to interrogate REST and RCOR gene expression levels during neural differentiation using RT-qPCR and Nanostring. Human pluripotent stem cells (hPSCs) were differentiated into glutamatergic cortical and GABAergic ventral forebrain neurons using a dual SMAD-inhibition driven approach, mature functional induced neurons (iNs) via neurogenin-2 (NGN2) overexpression and 3D cerebral organoids matured for 9 months. In line with previously published findings, REST and RCOR2 mRNA levels were significantly decreased with neuronal differentiation and organoid maturation. However, there was no significant change in RCOR1 expression levels in all three models. In addition, RCOR3 expression was significantly increased approximately 5-fold in both cortical ($p = 0.047$) and ventral ($p = 0.0013$) forebrain neurons and had a 4-fold increase in NGN2 iNs ($p = 0.0016$) when compared to hPSCs. In summary, this study has defined a novel expression pattern for REST and RCOR genes in human embryonic neurogenesis using 2D and 3D hPSC models.

Keywords: Neurodevelopment, Gene expression, Transcription factor

MDD451

DECIPHERING THE ROLES OF EYA1 IN HUMAN PROGRESSIVE HEARING LOSS

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Age-related hearing loss is mainly related to degeneration of hair cells and supporting cells in the inner ear, affecting 10% of all ages and 60% of those over 65 years old. At present, only a few deafness genes have been discovered, and much effort is focused onto identifying novel disease-related genes. Haploinsufficiency for Eya1 causes branchio-oto-renal syndrome (BOR) which has been associated with Progressive hearing loss. Inactivation of Eya1 in mice results in early arrest of inner ear development at the otic vesicle stage. However, the roles of Eya1 in hair cell development at later stages are still unknown. The inner ear is a complex organ requiring the coordination of multiple signaling molecules and pathways in proliferation, specification and differentiation. It has been shown to play critical roles in controlling the sensory progenitor proliferation and hair cell differentiation in the cochlea. We will investigate the role of Eya1 in hair bundle development and provide the first comprehensive description of the cellular effects of underlying progressive hearing loss in Branchio-oto-renal (BOR) syndrome. In this project, we will conditionally knockout Eya1 in hair cells at E15.5 when the cilium starts to migrate. We will test the migration of the kinocilium and hair bundle orientation and shape in cochlear hair cells in Eya1 conditional knockout mutants. We will test the function of Eya1 in cilium migration and polymerization for hair bundle formation. This study will provide new understanding of Eya1 function in hair bundle development as underlying mechanisms for hearing treatment and understand the pathogenesis of progressive hearing loss in patients with BOR syndrome.

Funding source: The fundings from Stan Perron Charitable Foundation & The Garnett Passe and Rodney Williams Memorial Foundation Australia.

Keywords: Inner ear, Hair cells, Cochlea

MDD452

INTERROGATING THE MECHANISM OF COGNITIVE IMPAIRMENTS AS A RESULT OF DIABETES IN EARLY HUMAN NEURONAL DEVELOPMENT

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The association between the dysglycaemia of diabetes and disordered human neural ontogeny resulting in functional deficits has been established for some time. Children who develop type 1 diabetes are observed to have developmental abnormalities in cognition, mental health, brain morphology and brain biochemistry. Exposure to abnormal levels of glucose and insulin conditions during pregnancy, may also have ongoing impacts on the cognition of the developing infant, including lower IQ, increase incidence of ASD and ADHD. In this study, we used cerebral-cortical organoids derived from human embryonic stem cells (hESC), as a cellular model system to examine possible mechanisms underlying the long-term detrimental consequences of dysglycaemia and abnormal insulin conditions, in the developing brain. We established a new protocol with physiological concentrations of glucose and insulin, that support hESC neural induction and cerebral-cortical organoid formation. We then transiently exposed the cortical organoids to different combinations of glucose and insulin concentrations to mimic diabetic-like conditions. Gene expression and MS proteomics, followed by IPA software analysis were used to analyse the various treatment groups and identify candidate proteins and pathways that are potentially dysregulated in neurons when exposed to altered glucose and/or insulin concentrations. Preliminary findings highlight observed changes in canonical pathways, including axonal guidance signalling, gap junction signalling and protein ubiquitination, which are critical for normal brain development. To further validate the proteomic data, reverse phase protein array has been applied. This tool allows a large number of western blots to run in a highly sensitive and high throughput manner. These findings are significant for revealing initial key events underlying cognitive impairments associated with dysglycaemia and abnormal insulin conditions during early brain development.

Funding source: University of Melbourne Graduate Research Scholarship

Keywords: Neurodevelopment, Diabetes, Cerebral-cortical organoids

MDD479

IN VITRO MODELLING OF EARLY AND LATE ONSET SCN2A DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHIES USING HUMAN PLURIPOTENT STEM CELLS

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SCN2A, encoding a brain sodium channel responsible for regulation of excitability, has emerged as a major single gene implicated in neurogenetic disorders including early- and late-onset developmental and epileptic encephalopathies, a severe epilepsy often combined with cognitive and behavioural impairments. Analysis of SCN2A variants suggests a correlation between the clinical presentation, the pharmacosensitivity and the functional impact measured in different disease models. Given the high phenotypic heterogeneity, the mechanisms underlying the SCN2A pathophysiology have not been fully unravelled and a valuable model has not been established yet. In this study, we interrogated two of the most recurrent SCN2A variants detected in early and late onset developmental and epileptic encephalopathies, respectively: R1882Q and R853Q. Patient-derived iPSC lines and the corresponding isogenic controls were differentiated using the NGN2 overexpression protocol to obtain cortical glutamatergic neurons. Electrophysiological properties and expression profile were assessed to identify any functional abnormality. R1882Q neurons showed an increased activity at cellular level early on and subsequently in networking. Instead, according to the late onset in patient, R853Q neurons showed an abnormal electrophysiological activity with an increased excitability later during differentiation. This correlated with an increase expression of SCN1A and SCN3A in R1882Q and R853Q neurons, respectively. Our data showed the validity of iPSC-derived in vitro tissue as a platform to model crucial features of DEE phenotype, therefore to investigate mechanisms underlying SCN2A genetic epilepsies and subsequently to test/screen innovative therapies.

Keywords: in vitro modelling, neural differentiation, genetic epilepsy

NEW TECHNOLOGIES

MDD389

DEFINING THE TERATOMA AS A MODEL FOR MULTI-LINEAGE HUMAN DEVELOPMENT

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Studying early human developmental processes requires access to a model system that is genetically tractable and recapitulates key features of organogenesis. We conjectured that teratoma formation, a recognized standard for validating pluripotency in stem cell lines, could be a promising platform for modeling human development. Teratomas differentiate to all germ layers, contain internal vasculature, have regions of complex tissue-like organization; and are simple and accessible, especially in comparison to studying human fetal tissue. Teratomas, however, have not been characterized rigorously to determine their relevance and tractability to human developmental studies. We thus systematically analyzed, perturbed, and engineered human pluripotent stem cell (PSC)-derived teratomas. Using histology, RNA in situ hybridization, and single cell RNA-seq (scRNA-seq) of 96,204 cells across 16 teratomas and 4 cell lines, we observed that teratomas reproducibly comprise of at least 20 cell types across all three germ layers. We found that injected PSCs engraft robustly, with a degree of heterogeneity comparable to that found in organoid systems. Additionally, we mapped teratomas to human fetal tissue and showed that the teratoma gut and brain cell types correspond well to fetal gut and brain datasets. We then performed a CRISPR-Cas9 knockout screen in PSCs targeting key genes that are embryonically lethal upon knockout and assayed their phenotypic effects in the teratoma via scRNA-seq readout, thus systematically assaying perturbations across all human lineages. Our hits included CDX2 and TWIST1, with their knockouts resulting respectively in the depletion of midgut/hindgut and mesenchyme, validating that the teratoma can serve as a platform to assay biologically accurate developmental phenotypes. Finally, we provided a demonstration of molecular sculpting, by engineering a novel miRNA-regulated suicide gene circuit that selectively depletes cells that do not express a tissue-specific endogenous miRNA. Specifically, utilizing miR-124, we were able to manipulate teratoma formation to enrich for the neural lineage. Taken together, we believe the teratoma is a promising platform for multi-lineage human development, functional genetic screening, and cellular engineering.

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Keywords: Human Development, Teratoma, Single Cell RNA Sequencing

MDD393

ENHANCING WELL-TO-WELL REPRODUCIBILITY OF COLON ORGANOIDS FOR SCREENING APPLICATIONS

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In recent years, 3D organoids have emerged as a new tool for disease modeling and for pre-clinical toxicity screening applications. Organoids are self-organizing three-dimensional structures derived from either pluripotent stem cells or from primary tissues with the ability to recapitulate some of the spatial architecture and in vivo function of organs. However, a key hurdle for widespread use of organoids in screening applications is the difficulty in controlling the numbers, sizes and shapes of the organoids. Here we describe optimization of experimental parameters that allowed us to generate more consistent numbers of similarly sized organoids in a multi-well plate format. In response to forskolin, both hiPSC derived and patient-derived colon organoids swelled in size, indicating that the cystic fibrosis transmembrane conductance regulator (CFTR) was functional. Total RNA sequencing of both hiPSC-derived and patient-derived colon organoids is being performed to show that hiPSC-derived and patient-derived colon organoids express the relevant cell types and share relatively similar transcriptional profiles. The increased reproducibility of organoids between wells should facilitate screening for agents that exert effects on the intestinal epithelium.

Keywords: Organoids, Intestinal, Gut

MDD395

BETTER ORGANOIDS THROUGH HOMOGENEITY

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While great progress has been made recently in establishing protocols to yield organoid systems starting from single or small collections of cells, the efficiency of these protocols is often poor and there is large heterogeneity in the mature organoids. This low efficiency and high heterogeneity make it difficult to perform genetic and biochemical perturbations to quantitatively understand organoid development. Here, we leverage recent advances in droplet microfluidics to specify the initial conditions of human embryonic cyst development. We encapsulated wild-type and reporter human embryonic stem cells (hESCs) in aqueous droplets in an oil carrier fluid, and screen each droplet for the number and type of the hESCs. We keep only the droplets whose contents meet the desired conditions, and deposit the encapsulated hESCs from each of the droplets in a periodic array. This approach yields hundreds to thousands of identical hESC clusters that we grow into embryonic cysts. We present our approach and data characterizing the cysts for homogeneity.

Keywords: microfluidics, human embryonic stem cells, embryonic cysts

MDD396

GENOME-WIDE CRISPR-BASED SCREENS IN IPSC-BASED MODELS OF BRAIN DISEASE

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Human genes associated with brain-related diseases are being discovered at an accelerating pace. A major challenge is the identification of the mechanisms through which these genes act, and of potential therapeutic strategies. To elucidate such mechanisms in human cells, we established a CRISPR-based platform for genome-wide screens in human iPSC-derived neurons, glia, and multi-lineage assembloids. Our approach relies on CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), in which a catalytically dead version of the bacterial Cas9 protein recruits transcriptional repressors or activators, respectively, to endogenous genes to control their expression, as directed by a small guide RNA (sgRNA). Complex libraries of sgRNAs enable us to conduct genome-wide or focused loss-of-function and gain-of-function screens. Such screens uncover molecular players for phenotypes based on survival, stress resistance, fluorescent phenotypes, high-content imaging and single-cell RNA-Seq. To uncover disease mechanisms and therapeutic targets, we are conducting genetic modifier screens for disease-relevant cellular phenotypes in patient-derived neurons and glia with familial mutations and isogenic controls. CRISPRi/a can also be used to model and functionally evaluate disease-associated changes in gene expression, such as those caused by eQTLs, haploinsufficiency, or disease states of brain cells.

Keywords: CRISPR, Functional Genomics, Disease model

MDD398

BIOLOGICALLY RELEVANT 3D SKIN DISEASE MODELS INCORPORATING PRIMARY STEM CELLS FOR DRUG DEVELOPMENT

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A major hurdle in drug screening is establishing tissue models that accurately recapitulate disease phenotypes seen in vivo. We have developed an in vitro skin model that incorporates epithelial stem cells and fibroblast progenitors that mimic many aspects of healthy and diseased skin. This Self Assembled Skin Equivalent (SASE) model utilizes disease-specific fibroblasts to secrete and organize a human 3D extracellular matrix (ECM). This disease specific ECM can support the differentiation of epithelial stem cells to create a fully differentiated skin tissue. We have also incorporated immune cells, such as peripheral blood monocytes, into 3D skin tissues to evaluate cytokine secretion, inflammation, and pyroptosis as it would occur in vivo. We have utilized this model to mimic skin diseases including diabetic foot ulcers (DFU) and scleroderma (SSC). To better understand the process of impaired healing in diabetic foot ulcers we developed a wound healing assay in the SASE model to follow the healing of epithelium on a newly wounded dermis. Immunohistochemical (IHC) staining of wounds indicated that re-epithelization occurred in SASE tissues containing both diabetic foot ulcer and healthy fibroblasts. IHC staining for K10 and Ki67 confirmed differentiation and proliferation of keratinocyte stem cells. For scleroderma, patient specific SASE models were created using matched patient fibroblasts, peripheral monocytes, and plasma, combined with neonatal human keratinocyte stem cells. ECM rigidity and stiffness was measured by atomic force microscopy. SASE tissues also showed altered ECM composition and organization, cytokine production, collagen fiber density, and crosslinking. These quantitative measures showed that the model mimics key features of clinical SSC. The SASE model is a novel tool that allows us to study mechanisms of fibrosis and wound healing, and it can be applied in drug discovery in the development of new therapies.

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Keywords: 3D Tissue, Wound Healing, Scleroderma

MDD409

DEVELOPING A HUMAN EMBRYONIC STEM CELL-BASED HIGH-THROUGHPUT PLATFORM TO SCREEN FOR DEVELOPMENTAL TOXICANTS

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Every year, millions of infants worldwide are born with a serious birth defect, which raises the risk for lifelong disabilities to those who survive and increases the economic burden to their families and society. Besides genetic or hereditary factors, many of these defects can be caused by environmental chemical exposure, such as alcohol, smoking, and drugs. While there are over 80,000 chemicals registered for use in the United States, many of them have undergone little safety testing. Therefore, a rapid and accurate method for predicting developmental toxicants in the environment to humans is strongly desired. Pluripotent human embryonic stem cells (hESCs) possess the capacity to differentiate into any cell type which makes them an ideal in vitro model to investigate developmental toxicity. In this study, we aim to develop a transcriptomic-based high-throughput platform using hESCs to screen for environmental teratogens. Three-dimensional embryoid bodies (EBs) formed from hESCs were used as the model since its formation recapitulates many early embryogenic processes. 108 selected chemicals were first administered to EBs for 7 days to evaluate their toxicities. As the result, 64 impaired EB cell viability in a dose-dependent manner, 7 increased cell viability at high concentrations, while the remaining 37 showed no effect on EB formation within the tested concentration range. Next, the impacts of 23 chemicals on key embryogenesis signaling pathways and germ layer formation were investigated by measuring the expression of 37 hallmark genes of these processes. Hierarchical cluster analysis based on the transcriptional response change showed the ability to group chemicals with similar toxicity. For a more accurate categorization, a machine learning based prediction model was built. 10 chemicals with confirmed teratogenicity from TERIS (Teratogen Information System) were selected as the training controls. With feature selection, our model showed a high accuracy (mean: 0.82) and reliability (mean: 0.70) and the prediction results for the other 13 tested chemicals were similar with the findings from existing studies, with few discrepancies. Together, these results indicate that our screening platform could be successfully applied for identifying developmental toxicants and understanding their etiology.

Keywords: Human Embryonic Stem Cell, Toxicology, Machine learning

MDD417

THE ALLEN CELL COLLECTION: HIGH QUALITY, FLUORESCENTLY-TAGGED HUMAN IPS CELL LINES TO ILLUMINATE CELL ORGANIZATION AND MODEL DIFFERENTIATION, DISEASE, AND REGENERATION

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The Allen Institute for Cell Science has created a collection of fluorescently tagged hiPS cell lines to illuminate cell organization. To date, the Allen Cell Collection consists of >40 single- or dual-edited lines that have undergone extensive quality control testing to ensure genomic, cell biological, and stem cell integrity. We have tagged all the major cellular organelles, a few signaling molecules, membrane-less structures, phase transition markers, transcription factors, and cardiomyocyte-specific structural markers. Our most recently released lines focus on nuclear structures such as nuclear speckles (SON-mEGFP) and the nuclear envelope (LMNB1-mTagRFP-T). Our first triple-edited cell line (FBL-mEGFP/NPM1-mTagRFP-T/dCas9-KRAB-TagBFP) enables CRISPRi-mediated knock-down of target genes and visualization of two major nucleolar compartments. Here, we present our gene-editing and quality control workflows for mono- and biallelic editing of expressed or silent genes that are expressed during differentiation. We also highlight a newly adopted procedure for testing dCas9-KRAB function in hiPSCs and cardiomyocytes. Furthermore, we will discuss two of our applications of the Allen Cell Collection. We use high-replicate, high-resolution, 3D live images of our cell lines as well as quantitative analysis and modeling approaches to create integrative cell models such as the Integrated Mitotic Stem Cell and the integrated nucleus. The Integrated Mitotic Stem Cell captures a holistic view of the human cell division using 15 key cellular structures. The integrated nucleus will conjoin key nuclear organizational landmarks with chromatin architecture in collaboration with the 4D Nucleome Project. Our cell lines, the plasmids used to generate them, thousands of segmented single cell 3D images of our lines, analysis and visualization tools, integrated cell models and biological findings are available to the research community (www.allencell.org).

Keywords: gene editing, fluorescent tagging, iPSCs

MDD430

NOVEL METHODS FOR ASSESSING ELECTROPHYSIOLOGICAL FUNCTION OF CARDIAC AND NEURAL THREE-DIMENSIONAL CELL CULTURES WITH MEA TECHNOLOGY

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Three-dimensional induced pluripotent stem cell (iPSC)-derived in vitro models, commonly referred to as spheroids, organoids, or “mini-brains”, more accurately recreate the multicellular organization and structure of in vivo tissues when compared to traditional monolayer stem cell cultures. However, to effectively characterize 3D iPSC cell cultures, or to extract meaningful and predictive information from these models, new technology is required for evaluating functional cellular and network responses. For electro-active cells, like cardiomyocytes or neurons, measurements of electrophysiological activity across a networked population of cells provide a comprehensive view of function. Microelectrode array (MEA) technology offers such a platform by directly connecting key biological variables, such as gene expression or ion channel distributions, to measures of cellular and network function. However, placing 3D culture models on traditional multiwell MEA plates can be time-consuming and labor-intensive. Here, we characterize a novel insert for 6-well MEA plates that facilitates the placement, attachment, and long-term culture of 3D cell models for electrophysiological analysis. Human iPSC-derived cardiomyocytes and neural spheroids were cultured on 6-well MEA plates and monitored throughout maturation of their network connections. Cardiac electrophysiological activity, including spike amplitude and field potential duration, were recorded in response to compounds to provide information on the depolarization and repolarization of the cardiomyocyte action potential. For neural cultures, functional endpoints, such as network bursting and synchrony, were measured to define cellular activity across neural spheroids within a network. These results support the continued development and use of human iPSC-derived cardiomyocyte and neural spheroid assays on multiwell MEA technology for high throughput drug toxicity and safety assessment, evaluation of phenotypic disease-in-a dish models, and cell development.

Keywords: Organoid, Electrophysiology, Disease-in-a-Dish Model

MDD431

COMPARISON OF ELECTROPHYSIOLOGICAL TOOLS FOR BRAIN ORGANOIDS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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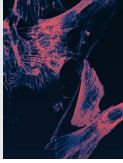
Human induced pluripotent stem cell (hiPSC)-derived brain organoids provide a 3D culture condition to study brain development, characterize disease phenotypes, and to test drug toxicity/efficacy, in vitro. Compared to monolayer cell cultures, brain organoids have demonstrated formation of cortical layers,



which can potentially model in vivo-like neuronal network activity. Although the anatomy of organoids has already been shown through imaging techniques, functional analysis is still at an early stage. To advance the functional analysis of complex brain organoids, there is call for a reliable and comprehensive recording methodology to simultaneously capture both network and single cell activities. Traditional MEA and calcium imaging are useful tools to measure electrophysiological features of brain organoids, each with their limitations. High-density microelectrode array (HD-MEA) technology offers a label-free solution to examine neuronal spiking activity throughout the development of organoids at high spatiotemporal resolution. In this study, we utilized MaxOne HD-MEA (MaxWell Biosystems, featuring 26,400 microelectrodes within a sensing area of 3.85 × 2.10 mm2) to detect spontaneous electrical activity from intact hiPSC-derived brain organoids. These brain organoids are differentiated for more than 60 days and cultured on HD-MEA for 2-3 weeks. We recorded neuronal action potentials from the organoids and analyzed electrical activity at network and single cell level. We detected network bursts, indicating the development of synaptic connectivity. Pharmacological manipulation of network bursts was performed using NMDA and MK801. We compared the readouts of HD-MEA with that of traditional MEA and calcium imaging. With HD-MEA, we were able to electrically visualize the extracellular action potential spatial distribution of single cells and identify sub-cellular features, such as axonal action potential propagation. In conclusion, the approach for electrophysiological characterization of hiPSC-derived brain organoids using HD-MEAs may enable long-term investigation of brain organoid function throughout development and upon drug application at different physiological scales.

Funding source: This work was supported by the European Community through the Marie Skłodowska-Curie Individual Fellowships Grant 798836 'MAPSYNE'.

Keywords: Brain organoids, Functional phenotype, MEA

MDD434

DEVELOPMENT OF A HIGH THROUGHPUT IMAGING AND SORTING APPROACH FOR 3D CELL CULTURE

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One of the challenges to the adoption of 3D cell culture models for high-throughput screening is a lack of control over 3D cell culture formation and a reliable non-invasive readout for these 3D cell culture models. Here, we were able to analyze, select, and sort organoids and spheroids using a large object imaging flow cytometer, the COPAS VISION. The COPAS VISION is a large object flow cytometer capable of imaging, analyzing, and sorting cell clusters, spheroids, and organoids from 10 µm to 700 µm. In contrast to traditional methods, where organoids are manually sorted, our approach offers a high-throughput manner to assess quality and to sort uniform organoids/spheroids to be used for testing, transplantation, and disease modeling. These were analyzed and sorted on the basis of size, levels of fluorescence, the localization of fluorescence, and the brightfield images of the sample objects. We successfully sorted 7-day iPSC-derived organoids expressing Thy1-GFP (a marker for Retinal Ganglion Cells (RGCs)) and 24-day Rx-DsRed (early retinal field marker), marking the areas of retinal tissue development. Cell clusters varied in size, shape, and expression levels of markers. Cell clusters were dispensed individually into wells of 96-well and 384-well plates and microscope observations showed that no damage occurred to the dispensed cell clusters despite their differences in appearance. Organoids selected by the COPAS VISION to have few differentiated neurons were confirmed by microscopy to have this phenotype. Using GFP as a fluorescent marker and the size of the spheroids as our sorting criteria, a population of well-formed, singular spheroids was sorted into 96 and 384 well plates and verified by microscopy. Sterility of the sorted spheroids was also confirmed 24 hours post sorting by microscopy. We demonstrated organoids and other 3D cell culture models can be analyzed and sorted up to 30 events/sec. The data presented supports that the COPAS VISION can automate the handling of large numbers of organoids and spheroids in an unbiased fashion, dispensing them into multi-well plates as well as characterizing the various sub-populations within each type of cell cluster. Analyzing and sorting organoids provides an approach for large scale quality control and high throughput screening assays.

Keywords: Large object flow cytometry, High throughput imaging & sorting, Spheroids, organoids, 3D cell culture

MDD504

EVALUATION OF THE HPSC SCORECARD ASSAY IN A HUMAN EMBRYONIC STEM CELL TEST FOR DEVELOPMENTAL TOXICITY SCREENING

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The US Environmental Protection Agency (EPA) Center for Computational Toxicology and Exposure employs high-throughput screening approaches to identify environmental chemicals that can pose a risk to human health. Key provisions in the Frank R. Lautenberg Chemical Safety for the 21st Century Act promotes the use of non-animal, new approach methods to identify chemical risks to susceptible populations including pregnant women. Most of the current assays within the US EPA's ToxCast and Tox21 portfolio are not designed to evaluate cellular processes associated with human development, therefore cell-based models that recapitulate signaling pathways for defined endpoints in early embryonic patterning are needed to identify potential hazards during pregnancy. The objective of this study was to adapt human induced pluripotent stem cells (hiPSCs) to a 96-well embryoid body (EB) stem cell test to identify chemicals that perturb early germ layer differentiation. EB formation, maintenance, and treatment was optimized for seeding density, plate type, and media changes using automated liquid handling. The commercial TaqMan hPSC Scorecard Assay gene-signature array was used to conduct temporal analysis of spontaneous differentiation. Pronounced EB differentiation was noted as early as day six, with maximum germ layer expression plateauing at day ten. Solvent tolerance and concentration-range finding experiments were used to define a reference set of eight chemicals ranging the spectrum of FDA pregnancy risk categories (A-X) at known teratogenic and non-teratogenic

doses. Disruption of germ lineage commitment and progression was determined after six days of chemical exposure by calculating the difference in weighted Z-test scores (ΔZ-test) relative to pre-aggregated cells. No consistent differences in EB differentiation were observed at day six when compared to mean ΔZ-test values for ectoderm (15.6 ± 2.7), mesoderm (5.1 ± 1.6) and endoderm (12.6 ± 0.9) in solvent treated controls. Further investigation is warranted to determine if exposure frequency, duration, and endpoint analysis are suitable for evaluating hiPSC EB differentiation using the TaqMan hPSC Scorecard Assay gene panel. This abstract does not necessarily reflect EPA policy. Mention of trade names is not an endorsement or recommendation for use.

Funding source: United States Environmental Protection Agency

Keywords: Embryonic Stem Cell Test, hPSC Scorecard Assay, Developmental Toxicity Screening

Theme: Tissue Stem Cells and Regeneration

ADIPOSE AND CONNECTIVE TISSUE

TSC425

INDUCED REJUVENATION OF MESENCHYMAL STEM CELLS DERIVED FROM AGED HUMAN BONE MARROW USING SMALL MOLECULE PROGRAMIN 2

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Human mesenchymal stem cells (MSCs) are multipotent connective tissue cells that are one of the most attractive stem cell source for tissue engineering and cell therapy. MSCs possess a potential to self-renew and differentiate into various types of cells such as osteoclasts, adipocytes, chondrocytes and neurons. Compared to the pluripotent stem cells these cells are also easier to harvest and free from ethical complications. MSCs have also good immunosuppressive capacity and they do not form teratomas in vivo. MSCs proliferate well in vitro but old patients' MSCs enter senescence a lot faster compared to the young patients' MSCs. This limits their use in tissue engineering and therapy since old patients are usually in higher demand of treatments. Therefore, it is crucial to enhance the proliferation capacity of these cells. In this project we tested an ability of the small molecule Programin 2 to reverse the senescence of human bone marrow derived mesenchymal stem cells (hBM-MSCs). Level of various proliferation markers such as phosphorylated histone H3 (PH3) and senescence associated b-galactosidase staining were observed. In addition, the protein or mRNA level of proliferation associated markers such as Ki67 and SIRT1 and senescence associated markers such as p21 and p16 were investigated after the Programin 2 treatment. Also the morphological changes were detected after the treatment. Furthermore, the expression of b-catenin and its downstream signalling genes were significantly elevated after Programin 2 treatment. These results of this project have shown evidences that small molecule Programin 2 has potential to re-activate the proliferation in senescent hBM-MSCs via Wnt/b-catenin signalling pathway.

Keywords: Mesenchymal Stem Cells, Small Molecule, Rejuvenation

CARDIAC

TSC405

BIOMECHANICAL ENHANCEMENT OF CARDIAC PHENOTYPE IN HUMAN ADIPOSE-DERIVED STEM CELLS

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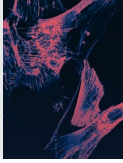
Cardiovascular disease continues to be the leading cause of mortality worldwide. Extensive research has been done to study cell-based therapy for regeneration of myocardium damaged as a result of myocardial infarction. Adipose-derived stem cells (ADSC) are prime candidates for revascularization therapies as these cells can be obtained easily from patients using less invasive methods, expanded in culture, and differentiated into cardiovascular cell types. Various factors have been identified that are able to induce ADSC cardiac differentiation, these include biochemical cues, biomechanical forces, and substrate topography. Despite their therapeutic potential stem-cell based transplantation therapy is limited by inconsistent clinical significance and long-term function in vivo. In this study we examined a broad range of biochemical factors, pharmacological inhibitors, and biomechanical forces in order to enhance ADSC cardiac differentiation and regenerative therapeutic potential. Cells were cultured on flexible substrates and exposed to cardiac differentiation treatments and mechanical load which included a 10% cardiac strain waveform, mimicking physiological loading in the left ventricle, and a 10% sinusoidal strain waveform at frequency of 1 Hz. We demonstrate that physiological loading in combination with 5- Aza treatment for 24 hours synergistically increased protein expression of cardiac markers including cardiac transcription factors GATA4 and Nkx2.5. Next generation sequencing showed that cyclic strain activated differential expression of genes associated with cardiac cell types. We also investigated the synergistic effect of cell alignment and mechanical load on ADSC cardiac differentiation. We were able to demonstrate that mechanically loading ADSCs seeded onto micropatterned PDMS substrates enhanced the expression of cardiac markers. Most notably we observed that stretching ADSCs for 7 days with 10% cardiac strain waveform induced significant increased expression of mature cardiac marker cardiac troponin T on larger microgroove patterns. Our study



supports that biomechanical regulation in combination with either topographical or biochemical cues work synergistically to enhance ADSC cardiac differentiation.

Funding source: The author acknowledge material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1610403.

Keywords: cardiac differentiation, myocardial infarction, adipose-derived stem cell

EARLY EMBRYO

TSC128

CELL COMPETITION CONSTITUTES A BARRIER FOR INTERSPECIES CHIMERISM

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Shortage of organs for transplantation is one of the largest unmet medical needs. Pluripotent stem cells (PSCs) offer a potentially unlimited source of donor organs. Despite decades of research, however, it remains infeasible to generate organs from PSCs in vitro. To overcome this barrier an in vivo approach known as interspecies blastocyst complementation has been developed to enable the formation of an organ from one species inside another, which raises an intriguing possibility to produce human organs in animals. One of the keys to success for interspecies blastocyst complementation is the ability of donor PSCs to contribute to chimera formation in the host species. Rat and mouse PSCs can extensively contribute to chimera formation in mouse and rat, respectively. To date, however, robust chimerism between distantly related species has not been achieved. A multitude of factors can differ significantly between species of distant evolutionary origin, which preclude extensive chimerism. Cell competition, the process of eliminating viable but “less fit” neighbor cells, has been proposed as a surveillance mechanism to ensure normal development and maintain tissue homeostasis. During interspecies chimera formation, cells from the donor species may be treated as unfit or aberrant cells targeted for elimination. Our central hypothesis is that cell competition constitutes a major component of the xenogeneic barrier and overcome this competition improves chimerism between evolutionarily distant species. To date, however, cell competition has not been examined in an interspecies context during early development due to lack of an in vitro model. We developed an interspecies PSC co-culture strategy and uncovered a previously unrecognized mode of cell competition between species. Interspecies PSC competition occurs during primed but not naïve pluripotency, and between evolutionarily distant but not closely related species. Inhibition of apoptosis could effectively overcome interspecies PSC competition. Suppressing interspecies PSC competition significantly improved the survival of donor human cells in early mouse embryos. Thus, strategies to overcome interspecies PSC competition may help improve the degree of chimerism between evolutionary distant species.

Keywords: Cell competition, Pluripotent stem cell, Interspecies chimerism

TSC130

PODOCALYXIN-LIKE PROTEIN 1 REGULATES HUMAN PLURIPOTENT STEM CELL SELF-RENEWAL THROUGH THE CHOLESTEROL BIOSYNTHESIS PATHWAY

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To understand the early stages of embryonic development helps people know how life begins and find clues to the diseases. Regularly cultured hESCs can not contribute to the inner cell mass in the mouse embryo. Therefore, regularly cultured hESCs are considered as ‘primed’ state of stem cells. Inhibitions of some signaling pathways by a chemical compound approach are reported recently to convert hESCs into less differentiated naïve human pluripotent stem cells (hPSCs) that can contribute to mouse embryo with low efficiency, but do not develop into placenta. Recently, a study reported the extended pluripotent stem cells (EPSCs) with their biological uniqueness of a high chimerism rate and the developmental abilities into both the inner cell mass and trophoblasts (placenta) while compared with hESCs in primed and naïve stages. However, the signaling mechanisms of extended pluripotency and the metabolism in hPSCs have rarely been investigated. Here, we demonstrated Podocalyxin-like protein 1 (PODXL) is abundantly expressed in primed hPSCs/hEPSCs and functions as a transmembrane protein. Downregulation of PODXL in undifferentiated hPSCs

significantly blocks self-renewal, leads to decreased protein expression of c-MYC and telomerase, and inhibits the formation of induced pluripotent stem cell (iPSC) and extended EPSC colonies. Accordingly, overexpression of PODXL promotes hPSC self-renewal, c-MYC and telomerase expression, and iPSC/EPSC formation. In a mechanism level, PODXL also regulates HMGCR and SREBP1 and SREBP2 expression to control intracellular cholesterol levels. Importantly, hPSCs are more sensitive to cholesterol depletion than fibroblasts, resulting in pluripotency loss. Of note, cholesterol can fully restore PODXL knockdown-mediated pluripotency loss. Cholesterol is known for serving as an important role in cell membrane integrity and lipid raft formation. Moreover, PODXL regulates lipid raft formation and dynamics. Finally, loss of ITGA2 blocks hESC renewal. Our data highlight the important roles of PODXL in controlling cholesterol metabolism to achieve hPSC self-renewal. This study may imply that PODXL/cholesterol functions importantly in early human embryogenesis and shed light on potential cancer treatment.

Keywords: Cholesterol, Pluripotency, Lipid raft

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

TSC135

EFFICIENT DIFFERENTIATION OF HOMOGENEOUS FUNCTIONAL HEPATOCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (iPSCs) hold tremendous promise for regenerative medicine, disease modeling, drug screening, and toxicology studies. Directed differentiation of iPSCs into hepatocytes could afford unlimited supply of liver cells for various liver-based applications. A variety of protocols have been established during the past decade for the differentiation of iPSCs into hepatocytes through recapitulating major signaling pathways involved in the different stages of embryonic hepatogenesis, using either growth factors or small molecules. Although successful derivation of hepatocyte-like cells (HLCs) has been achieved through those protocols, the differentiation efficiency is mostly not ideal, and the cells at the end of the differentiation always showed a heterogeneous population. Herein, we tested and compared four previously published protocols, and based on the results established an improved protocol that enables highly efficient, homogeneous, and reproducible differentiation of iPSCs into HLCs. The protocol started with single-cell culture (instead of colonies) of iPSCs, and employed both growth factors and small molecules for the differentiation. Compared to the existing protocols, the new protocol yielded a differentiated HLC population that was more homogeneous and with a morphology more closely resembling that of primary human hepatocytes (PHHs). The final population of cells not only expressed specific hepatic markers at both the transcriptional and the protein levels, but also possessed key hepatic functions, including serum protein (albumin, fibronectin, and alpha-1-antitrypsin) secretion, urea synthesis, glycogen storage, and more importantly for toxicology applications, cytochrome P450 activity. Therefore, our method would be a valuable tool for highly efficient generation of homogeneous functional hepatocytes from human iPSCs, and the resultant HLCs could be potentially useful as an in vitro model for predictive toxicology.

Keywords: hepatocytes or hepatocyte-like cells (HLCs), stem cell differentiation, induced pluripotent stem cells (iPSCs)

TSC138

GENERATION AND CHARACTERIZATION OF STEM CELL-DERIVED PANCREATIC DELTA CELLS

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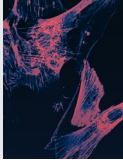
Recent progress in generating a limitless supply of pancreatic cells through the directed differentiation of human embryonic stem cells holds promise as a cell replacement therapy for type 1 diabetes. While the field of diabetes stem cell research has focused on generating insulin producing beta cells, a growing body of evidence suggests that the glucagon producing alpha cells and the somatostatin producing delta cells are important for precise coordination of hormone secretion and glucose homeostasis. In this study, we developed a chemically defined protocol for the generation of pancreatic delta cells through direct differentiation of human embryonic stem cells. Building on our previously described differentiation protocol that generates pancreatic progenitor cells, we performed a combinatorial screen of 20 compounds and found that omitting factors favored stem cell-derived delta cell commitment, resulting in a ~15-20% mono-hormonal somatostatin expressing population as characterized by FACS. In order to assess stem cell-derived delta cell function and overcome the limitations of current antibody-dependent somatostatin quantification assays, we engineered a novel luminescent reporter of somatostatin secretion by targeting the HiBiT reporter system at the endogenous somatostatin locus in human embryonic stem cells. Using this highly sensitive system in static and dynamic perfusion assays, we showed that stem cell-derived delta cells secrete somatostatin in response to glucose stimulation. Further, the somatostatin secretion from high glucose challenges improved upon addition of the beta cell hormone urocortin 3, which has reportedly been shown to



stimulate delta cells. Altogether, we have described for the first time the in vitro generation of functional stem cell-derived delta cells which brings us one step closer to assembling the three major endocrine cells (beta, alpha and delta) into designer islet organoids for type 1 diabetes disease modeling and islet-cell replacement therapy.

Keywords: Delta cells, Somatostatin, Pancreas

TSC150

DONOR AGE ALTERS THE FUNCTION AND REGENERATIVE CAPACITY OF HUMAN LIVER EXTRACELLULAR MATRIX.

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Liver diseases pose a big threat as 20 million individuals are estimated to have cirrhosis and/or liver cancer globally. Although orthotopic liver transplantation leads to high survival rates, there is an increasing global demand for donor livers which results in 8 patients dying awaiting transplantation daily. Despite the organ shortage many livers are still not being considered eligible for transplantation leading to an estimated 250,000 donor livers being discarded. Our group established methods to decellularize whole livers for expanding the donor pool. The future use of such ECM scaffolds relies on the success of decellularization and their successful recellularization with patient-specific iPSC-derived cells. However, donated human livers found not suitable for transplantation vary widely in age and quality. This raises the question for their suitability in supporting and inducing the regenerative capacity of the resulting liver substitute. To answer this question, we decellularized human liver sections from 19-year-old (young), 46-year-old (mature), and 60-year-old (aged) donors and examined the changes in their composition as well as their potential role in human iPSC differentiation to hepatocytes. Our results showed that human decellularized liver matrices (hDLM) go through a dynamic change through aging process: total collagen and glycosaminoglycan content peaks at mature stage followed by a decrease with age. Importantly, although differentiation on hDLM of all age groups induced higher expression of hepatocyte specific markers compared to the matrigel controls, the age-dependent changes in liver ECM affected the differentiation significantly. We observed that important markers including HNF4a, CYP1A2, CYP2C9, and CYP3A4 were expressed significantly higher on mature liver ECM compared to both young and aged. These findings show that the composition and functionality of liver ECM is affected by donor age. Through further investigation on the effect of donor age on the regenerative capacity of hDLMs we will obtain the recipe for developing organ grafts with high functionality, minimal immunogenicity, and high regeneration capacity.

Keywords: Liver, extracellular matrix, organ engineering

TSC154

ENGINEERING A NOVEL LUMINESCENT REPORTER OF INSULIN SECRETION IN HUMAN STEM CELL-DERIVED BETA CELLS

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The long-standing “gold-standard” for quantification of insulin secretion is ELISA. However, ELISA-based quantification of insulin secretion is not compatible with high-throughput applications. Consequently, several groups have attempted to circumvent ELISA by engineering fluorescent or luminescent reporters of insulin secretion. Yet, whilst these reporters are useful for visualizing insulin secretion, they only provide relative insulin quantification. Thus, to address these limitations, we developed a novel luminescent platform of insulin quantification that has the potential for high-throughput adaptation. Specifically, we targeted the 11 amino acid HiBiT tag and an additional proconvertase 2 (PC2) site between the genomic region encoding for C-peptide and the alpha chain of the INS gene in human embryonic and induced pluripotent stem cells. When combined with the detection reagent that consists of the LgBiT subunit, the resulting high-affinity complex of HiBiT:LgBiT reconstitutes active nano luciferase, which emits bioluminescence that is sensitive enough to measure insulin secretion in minutes without the need for antibodies or multiple liquid-handling steps. Our luminescent reporter demonstrated 3 orders of magnitude greater dynamic range, increasing sensitivity at low and high insulin concentration by ~2 and ~1.5 orders of magnitude respectively versus ELISA. In addition, the luminescent reporter is 8-fold more time and cost-effective than the traditional ELISA. Glucose-stimulated insulin secretion (GSIS) of differentiated SC-beta cells demonstrated overlapping GSIS profiles by luminescence and ELISA. To demonstrate the potential of the novel reporter for screening of compounds that improve insulin secretion, SC-beta cells were assessed for insulin secretion with a panel of insulin secretagogues. In conclusion, we have developed a novel luminescent platform of insulin secretion with the potential for high-throughput screening of compounds that enhance insulin secretion and improve SC-beta cell function.

Keywords: Beta cells, Insulin secretion, Luminescent reporter

TSC424

IN VITRO 3D LIVER MODEL UTILIZING HUMAN IPSC INDUCED HEPATOCYTES FOR DRUG TESTING

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Liver is responsible for the detoxification in our bodies, and it is exposed to different threats in everyday life. Despite of the strong regenerating property of the organ, when the exposure of toxic substances is prolonged and beyond its limits, the damage could be irreversible, as the extracellular matrix proteins which are supposed to heal wound over accumulates, forming extensive scar tissues in the liver. Indeed, chronic liver diseases including liver cirrhosis have been one of the highest leading causes of death in Hong Kong and in other developed countries for these decades, yet no cures are found as the drug discovery process relies on the in vitro cell model growing on petri dishes, which is lack of representativeness to their in vivo counterparts, on the other hand animal models are found not predictive for the human responses to drug. Our study aims at creating a novel 3D in vitro functional liver model by co-culturing human induced pluripotent stem cells (iPSCs) induced hepatocytes with non-parenchymal liver cells including hepatic stellate cells, endothelial cells as well as mesenchymal stem cells. We successfully generated vascularized liver organoids with these four cells types in which cell-cell/cell-ECM interactions and liver functions are regained, the human liver microenvironment is hence better mimicked for the more accurate drug screening. We believe that this study using iPSC induced hepatocytes gives novel insight in development of personalized medicine for liver diseases, increasing the effectiveness of pre-clinical studies and reducing the number of experimental animals needed in drug discovery process.

Keywords: Liver Organoids, Liver Disease Model, Tissue Engineering

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

TSC395

IMPAIRED PRO-ANGIOGENIC FUNCTION IN MESENCHYMAL STEM CELLS OF WERNER SYNDROME

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WRN mutation causes a premature aging disease called Werner syndrome (WS). However, the mechanism by which WRN loss leads to progeroid features evident with impaired tissue repair and regeneration remains unclear. To determine this mechanism, we performed gene editing in reprogrammed induced pluripotent stem cells (iPSCs) derived from WS fibroblasts. Gene correction restored the expression of WRN. WRN+/+ mesenchymal stem cells (MSCs) exhibited improved pro-angiogenesis. An analysis of paracrine factors revealed that hepatocyte growth factor (HGF) was downregulated in WRN-/- MSCs. HGF insufficiency resulted in poor angiogenesis and cutaneous wound healing. Furthermore, HGF was partially regulated by PI3K/AKT signaling, which was desensitized in WRN-/- MSCs. Consistently, the inhibition of the PI3K/AKT pathway in WRN+/+ MSC resulted in reduced angiogenesis and poor wound healing. Our findings indicate that the impairment in the pro-angiogenic function of WS-MSCs is due to HGF insufficiency and PI3K/AKT dysregulation, suggesting trophic disruption between stromal and epithelial cells as a mechanism for WS pathogenesis.

Funding source: Hong Kong RGC General Research Fund Project No. 14121618
Hong Kong RGC General Research Fund Project No. 14169717

Keywords: Werner syndrome, Mesenchymal stem cells, Angiogenesis

EPITHELIAL

TSC165

CELL ATLASES TO FUNCTIONS: HOW INFLAMMATION SHAPES HUMAN EPITHELIAL AND STROMAL CELLS

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For a barrier tissue to effectively learn from previous immunological experiences, it can sense, adapt, and store this information in essential or resident cell types. The foundational architecture of barriers such as the skin, airway, and intestine include essential stromal and tissue stem-cell derived epithelial cell subsets that collectively perform key tissue functions. This design is critical for homeostatic regeneration and responding to

induced demands, together with the support of resident immune cells. However, this system becomes dysregulated in chronic disease states driven by immune effector cytokines such as inflammatory bowel disease (IBD). Identifying the essential cell types, states and circuits of communication which predict and sustain disease in humans is vital to effectively treating these conditions. We hypothesized that cytokines may act directly on, and be remembered by, discrete essential stem and niche cell subsets to influence differentiation and function in disease. To address this challenge, our lab has pioneered the application of tissue-scale single-cell RNA-sequencing to resolve cell states and their interactions in healthy and diseased human intestine. Insights from healthy individuals and advanced IBD patients highlighted a novel TNF-induced inflammatory fibroblast cell state (IL11+IL13RA2+) associated with clinical resistance to therapy, and significant alterations to the circuitry between epithelial stem and immune cell subsets in the intestine (366,650 cells; 30 people), which we validated histologically. Furthermore, our unpublished findings from a treatment-naïve longitudinal cohort of IBD patients and age-matched controls (438,585 cells; 27 people) identify discrete essential and immune cell subsets at diagnosis that can predict disease severity and response to therapy. We present functional experiments testing whether and how immune effector cytokines lead to tissue stem cell adaptation, with our results demonstrating that epithelial stem cells can "store" inflammation. Collectively, we propose that inflammatory adaptation and memory may be distributed across diverse cell types, providing a tissue-scale basis for persistent human disease, and suggesting therapeutic tissue reprogramming strategies.

Funding source: Richard and Susan Smith Family Foundation (to J.O.-M.) NIH U19AI095219 NIH 5U24AI118672

Keywords: Stem Cells and Their Niche, single-cell RNA-sequencing, Inflammatory Memory

TSC179

CHARACTERIZING THE ROLE OF LACTATE DEHYDROGENASE (LDH) AND MITOCHONDRIAL PYRUVATE CARRIER 1 (MPC1) IN MELANOCYTE STEM CELL (MESC) HOMEOSTASIS

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We are studying melanocyte stem cell (MeSC) metabolism and its effect on melanocyte homeostasis. Transgenic mouse models were generated using TyrCreERT to create melanocyte-specific lactate dehydrogenase A (Ldha) deletion in MeSCs. Ldha is an enzyme that catalyzes the NADH-dependent reduction of pyruvate to lactate in glycolysis. Inactivation of Ldha in mice MeSCs resulted in depigmentation of the skin. Taking advantage of a genetic reporter (LSL-tdTomato), we show that MeSCs are still viable after Ldha deletion as tdTomato-positive MeSCs were detected in the epidermis. This phenotype is being characterized in detail using immunostaining technics to quantify tdTomato-positive cells both in wildtype (WT) and knockout (KO) skin. In parallel, differentiation markers (Dct, Gp100, Tyrosinase) are being evaluated to determine if the phenotype is due to failure in melanocyte differentiation or MeSC activation. Additionally, transgenic TyrCreERT mice with a melanocyte- specific mitochondrial pyruvate carrier 1 (MPC1) deletion were generated. MPC1 is a mitochondrial transporter that facilitates entry of pyruvate to the mitochondria for oxidative phosphorylation; its inactivation redirects glycolytic flux towards lactate. Currently, MPC1 KO mice display similar pigmentation as MPC1 WT mice. Immunostaining strategies are being employed to quantify and assess MeSCs and melanocytes in MPC1 WT and KO skin. Taken together, we expect to reveal whether targeting key metabolic nodes in MeSCs affects their homeostasis.

Funding source: California Institute of Regenerative Medicine

Keywords: Melanocyte Stem Cell Homeostasis, Melanocyte Stem Cell Metabolism, Skin and Hair Pigmentation

TSC185

FRIZZLED5 MEDIATED WNT SIGNALING MAINTAINS MOUSE INTESTINAL HOMEOSTASIS

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The role of noncanonical Wnt signaling in regulating intestinal stem cell, epithelial homeostasis, and regeneration is poorly understood. Previously, it was reported that loss of Fzd5 led to migration of Paneth cells from crypt bottom to the villus region. We found that Frizzled5 (Fzd5) is predominantly expressed in intestinal crypt, including intestinal stem cells (ISCs), compared to other members of the Fzd family. Furthermore, we observed that deletion of Fzd5 from either +4 position (CK19-Cre) or from CBC (Lgr5-Cre) did not lead to crypt loss, but instead, that loss of crypt occurred only with Fzd5 deletion from both +4 and CBC positions (Villin-Cre). Given that Fzd5 may mediate either canonical or noncanonical Wnt signaling, the finding that deletion of Fzd5 resulted in reduced active form of Cdc42 suggests that Fzd5 may also play a role in controlling the planar cell polarity (PCP) via Cdc42. Reduced CDC42 activity led to disorganized cytoskeleton structure. Fzd5 knockout significantly inhibited the in vitro growth of organoids derived from Villin-CreERT-Fzd5f/f mouse. On the other hand, Fzd5 knockout also inhibited the activation of β-catenin, affected the expression of c-Myc and Axin2, and caused loss of stem cells, eventually promoting cell differentiation into enterocytes in intestine, especially in jejunum. We also examined intestinal biopsies from patients with inflammatory bowel disease (IBD) and found Fzd5 highly expressed in the regenerating epithelial cells, revealing that aberrant Fzd5 expression might be linked to IBD.

Keywords: Intestinal stem cells, Fzd5, Wnt signaling

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

TSC189

CHALLENGES OBSERVED IN ESTABLISHING AN IPSC RESOURCE: OBSERVATIONS FROM THE PERSPECTIVE OF A GOLD STANDARD BIOBANK.

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Induced pluripotent stem cells (iPSCs) provide an invaluable tool for studying human disease at the cellular and molecular level. Human iPSCs can be derived from multiple cell types, using multiple reprogramming methods and they have enabled disease-in-a dish modeling and drug discovery. Coriell Institute for Medical Research recognized the value of human iPSCs and over the past decade, has created a diverse, publicly available collection from apparently healthy donors and individuals with complex disorders and inherited genetic diseases. Coriell's iPSC bank started by obtaining pre-established iPSCs from the scientific community, however, priorities shifted to establishing lines internally due to a number of challenges observed. The incidence of quality failures observed in external lines, including submissions from reputable national laboratories, was substantial (approximately 65% failure rate) and necessitated the need to define best practices in iPSC establishment, maintenance and storage. Here we will present results from our findings and also report other challenges observed, such as the lack of ethnic diversity, problems with donor consent language, and submissions from minors, which raised ethical considerations. In an effort to ensure the quality and long-term sustainability of its iPSC bank, Coriell established standard operating procedures for the collection, processing, storage and distribution of iPSCs. Coriell's dedicated ISO9001:2015 certified Stem Cell Laboratory routinely performs screening for sterility (including mycoplasma), bloodborne pathogens, cell identity, chromosomal integrity, pluripotency, and differentiation potential. Coriell's robust iPSC pipeline has resulted in a 8% failure rate over the past three years. Additional molecular characterizations are also recommended for disease confirmation and/or gene engineered cell lines. With the advent of targeted differentiation approaches, 3D organoid models, and gene engineering technology, the utility of human iPSCs in advancing biomedical research and curing disease continues to grow. Quality control testing of these derivative products is equally critically required, however, leaders in the field still need to define what these parameters are for these new product types.

Keywords: IPSC, CHARACTERIZATION, QUALITY

EYE AND RETINA

TSC200

CYTOSKELETON AND NUCLEAR LAMINA REMODELING IN DIFFERENTIATING RETINAL PIGMENT EPITHELIUM DERIVED FROM GFP TAGGED IPSC FOR TUBULIN1B AND LAMINB

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The inherent capacity of Pluripotent Stem Cells to differentiate into any cell type coupled with the ability to endogenously GFP-tag proteins linked to the cytoskeleton, nuclear lamina or any other organelle in Induced Pluripotent Stem Cells (iPSCs) allows visualization of cellular architecture remodeling during differentiation. We used our developmentally guided iPSC to Retinal Pigment Epithelium (RPE) differentiation protocol, to generate fully functional mature and pure RPE cells. The RPE is a monolayer of post-mitotic, polarized, pigmented, and hexagonal cells in the back of the eye. Embryonically, the RPE is derived from the neuroectoderm. We tweaked the dual SMAD inhibition to generate the RPE primed optic neuroectoderm, then directed the differentiation of the neuroectodermal cells to RPE progenitors by inhibiting the FGF and TGF pathways, and later used Activin-A to induce the fate of the RPE progenitors to reach the RPE committed stage. Specific arrangements of the ACTIN and MICROTUBULIN filaments are necessary for RPE cells in a monolayer to attain complete polarization, hexagonal morphology, and full functionality. Here, we used GFP tagged TUBA1B and LAMINB iPSC reporter lines generated by the Allen Institute to study the changes in the arrangement of the cytoskeleton (tubulin filaments) and nuclear lamina as the pluripotent cells differentiated into mature RPE. The iPSCs were seeded on iBidi chamber slides for differentiation and fixed after the end of the early neuro-ectoderm, progenitor middle, or final RPE committed phase. Following fixation, the cells were stained for early and late RPE specific markers and imaged under an LSM800 microscope. The expression levels of RPE specific transcription factors, PAX6 and MITF, determined the differentiation of optic neurectoderm to RPE progenitor cells; while the presence of adult RPE markers, RPE65 or BEST1, confirmed the maturity of the RPE cells. The structural analysis of the mature cells was assessed using our custom ReShape software, which utilizes artificial intelligence and machine learning to provide a morphometric analysis of the RPE cells. In conclusion, our live high content imaging allowed us to

capture cytoskeleton and nuclear lamina changes during RPE differentiation and provides deeper insight into the mechanism of RPE polarization.

Keywords: iPSC to RPE differentiation, GFP tagged iPSCs, Cellular architecture development

HEMATOPOIETIC SYSTEM

TSC231

CASPASE-8 IS SELECTIVELY REQUIRED FOR PREVENTING RIPK3-MEDIATED NECROPTOSIS IN C-KIT+ HEMATOPOIETIC STEM AND PROGENITOR CELLS BUT NOT CKIT-HEMATOPOEITIC CELLS

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Myelodysplastic syndromes (MDS) are a group of heterogeneous pre-leukemic diseases. Despite the normal or hyper-cellular appearance of the bone marrow (BM), peripheral cytopenia is a common feature among majority of MDS patients, due to ineffective hematopoiesis. Increased apoptosis of hematopoietic stem/progenitor cells (HSPCs) was believed to be the cause of ineffective hematopoiesis. However, our recent study has demonstrated a significant increase in cellular necroptosis probably due to the reduced caspase 8 expression in most MDS BM samples, suggesting a critical role for necroptosis in the pathogenesis of MDS. Increased concentrations of TNF-α, Fas ligand and TRAIL are commonly detected in MDS patients. Such pro-inflammatory cytokines stimulate both Caspase 8-mediated apoptosis and RipK1-RipK3 mediated necroptosis. Normally, RipK1-RipK3 mediated necroptotic signaling is repressed by Caspase 8 in most tissue cells; thus inactivation of Caspase 8 would promote inflammatory cytokine-stimulated necroptosis in these tissue cells. To study the role of necroptosis in the pathogenesis of MDS, we generated polyI:C-inducible conditional Caspase 8-knockout mice. We found that all mice died within 10 days of poly I:C injection. Flow cytometry analysis suggested that Caspase 8 deletion caused a near-complete loss of c-Kit+ HSPCs when examined on day 6 post-poly I:C injection. However, the c-Kit- mature lymphoid and myeloid cell subsets in the BM as well as in peripheral blood were not significantly affected. Consequently, mice with Caspase 8 deletion showed BM hypo-cellularity. We also determined that loss of c-Kit+ HSPCs in the Caspase 8-knockout setting results from enhanced necroptosis of HSPCs in a RipK3 dependent manner, because it can be prevented by inactivation of RipK3. Our preliminary results hence suggest that Caspase 8 is required for the maintenance of c-Kit+ HSPCs in mice by preventing RipK3 mediated necroptosis. We are determining whether the loss of HSPCs in Caspase 8-knockout mice is due to the loss of Caspase 8 only or due to increased sensitivity of Caspase 8-knockout HSPCs to inflammatory stress. Given that approximately 30% MDS patients progress to Acute Myeloid Leukemia, we are also exploring the role of Caspase 8 in regulating the clonogenic expansion of HSPCs for leukemic transformation.

Keywords: Caspase 8, cKit+ Hematopoietic Stem and Progentor Cells, RipK3 mediated Necroptosis

TSC379

HIGH PLOIDY LARGE CYTOPLASMIC MEGAKARYOCYTES: KEY NEGATIVE REGULATORS OF HEMATOPOIETIC STEM CELLS AND CRITICAL FOR PLATELET PRODUCTION

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Hematopoietic stem cells (HSC) are regulated by their bone marrow (BM) microenvironment. High ploidy megakaryocytes (MK), not only generate platelets, but have been recently shown to regulate HSC. We report MK of individual ploidies can be further sub-fractionated into large cytoplasmic MK (LCM) and small cytoplasmic MK (SCM), and LCM not only negatively regulate HSC, but when prospectively isolated from mouse BM and transplanted, more readily form platelets in vivo than SCM. In a mouse model with normal MK numbers, but essentially devoid of LCM (Pf4-Cre-Srsf3fl/fl, MKΔ/Δ), we

found a pronounced increase in BM HSC concurrent with both endogenous mobilization and extramedullary hematopoiesis. We provide evidence that this is through previously reported mechanisms such as thrombopoietin and thrombin-cleaved osteopontin. Thrombocytopenia was also observed in animals with diminished LCM despite no change in MK ploidy distribution, thus, uncoupling endoreduplication and platelet production. The absence of LCM, an increased BM HSC pool and thrombocytopenia were recapitulated when HSC isolated from MKΔ/Δ mice were used to reconstitute hematopoiesis in irradiated mice. In contrast, following a competitive transplant using minimal numbers of MKWT/WT HSC with HSC from MKΔ/Δ mice, MKWT/WT HSC recapitulated the LCM population, preventing the HSC defect as well as thrombocytopenia. We have further characterized LCM via flow cytometry, TEM and performed mitochondrial network analysis using confocal microscopy. Importantly, we provide evidence that LCM are conserved in humans and hypothesize they have similar functions. Ploidy alone is not sufficient for accurately predicting platelet generation potential, and therefore, therapeutic efforts to increase platelet production need to specifically increase LCM; however, careful consideration needs to be placed on the possible consequences of increasing LCM in vivo since LCM are an important component of the BM microenvironment, providing regulator signals to HSC.

Keywords: HSC, Megakaryocytes, Microenvironment / niche

IMMUNE SYSTEM

TSC427

FUNCTIONAL AND GENETIC ANALYSIS OF THYMI AND T CELLS IN THYMUS-COMPLEMENTED MICE GENERATED BY BLASTOCYST COMPLEMENTATION

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Blastocyst complementation is a promising technology that potentially allow the generation of humanized animals for use in regenerative medicine and drug discovery. To realize the purpose, it is important to demonstrate physiological functions of complemented organs/cells in the chimeras. Although thymus-complemented animals have been successfully produced by blastocyst complementation, physiological functions of complemented thymi and T cells are not fully understood. Thus, we characterized thymi and T cells in thymus-complemented mice at molecular and functional levels. The chimeras were generated by injection of EGFP-positive C57BL/6 (B6) ESCs into blastocysts of KSN/Slc (Foxn1nu/nu) mice. (They varied in coat-color chimerism.) Flow cytometry analysis showed that their thymic epithelial cells were derived from donors (EGFP+) and they produced T cells derived from both donors (EGFP+) and hosts (EGFP-) in peripheral blood. The scRNA-seq analysis of thymi showed similar tSNE clusters between B6 and chimeric mice, suggesting that normal development of T cells occurred in complemented thymi at gene expression levels. For functional analysis, splenic T cells isolated from the chimeras were stimulated with anti-CD3/CD28 antibodies to evaluate proliferation potential of T cells by priming through costimulatory signals. As a result, splenic T cells of the chimeras were proliferated normally upon stimulation with anti-CD3/CD28 antibodies in the same manner as those isolated from B6. In addition, EGFP+ and EGFP- splenic T cells showed an equivalent proliferation rate in an individual chimera. Further functional analysis is ongoing. These data showed that thymi can be successfully complemented by blastocyst complementation and they function physiologically in terms of maturation and proliferation potential of T cells. Our basic studies would be useful for realizing the generation of humanized animals by blastocyst complementation.

Keywords: Blastocyst complementation, Thymi and T cells, Tissue regeneration

MUSCULOSKELETAL

TSC248

FUNCTIONALLY HETEROGENEOUS HUMAN SATELLITE CELLS IDENTIFIED BY SINGLE CELL RNA SEQUENCING

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Although the existence of heterogeneity is recognized within the satellite cell pool, a comprehensive understanding of distinct subpopulations and their functional relevance is lacking. We used a combination of single cell RNA sequencing and flow cytometry to identify, distinguish, and physically separate novel subpopulations of human satellite cells from multiple, diverse uninjured muscles. We found that, although relatively homogeneous compared to activated satellite cells and committed progenitors, the human satellite cell pool contains clusters of transcriptionally distinct cells. Analysis revealed new surface marker combinations enriched in transcriptional subclusters, including a subpopulation of human satellite cells marked by CXCR4/CD29/CD56/CAV1 and expressing a transcriptional signature consistent with a more quiescent state. In vitro, CAV1+ satellite cells are characterized by a longer time to first division and lower MyoD expression compared to CAV1- satellite cells. In vivo, CAV1+ satellite cells demonstrated increased engraftment potential after transplantation in mice. Our findings provide a comprehensive transcriptional view of normal human satellite cells describing new heterogeneity, and enabling enrichment of a functionally distinct subpopulation from the human satellite cell pool.

Keywords: Muscle Stem Cells, Quiescence Satellite Cells, Transcriptome

TSC249

CRITICAL ROLE OF CRY2 FOR CIRCADIAN REGULATION OF MYOGENIC DIFFERENTIATION

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Circadian rhythms regulate cell proliferation and differentiation; however, little is known about their roles in myogenic differentiation. Our synchronized differentiation studies demonstrate that myoblast proliferation and subsequent myotube formation by cell fusion occur in circadian manners. We found that one of the core regulators of circadian rhythms Cry2, but not Cry1, is critical for the circadian patterns of these two critical steps in myogenic differentiation. This is achieved through the specific interaction between Cry2 and Bclaf1, which stabilizes mRNAs encoding cyclin D1, a G1/S phase transition regulator, and Tmem176b, a transmembrane regulator for myogenic cell fusion. Myoblasts lacking Cry2 display premature cell cycle exit and form short myotubes due to inefficient cell fusion. Consistently, muscle regeneration is impaired in Cry2-/- mice. Bclaf1 knockdown recapitulated the phenotypes of Cry2 knockdown: early cell cycle exit and inefficient cell fusion. This study uncovers a post-transcriptional regulation of myogenic differentiation by circadian rhythms.

Keywords: circadian rhythm, myogenesis, muscle regeneration

TSC260

EXPANSION OF BONE PRECURSORS THROUGH JUN AS A NOVEL TREATMENT FOR OSTEOPOROSIS-ASSOCIATED FRACTURES

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Osteoporosis and osteoporotic fractures lead to decreased life quality and high health care costs. Current treatments prevent losses in bone mass and fractures to some extent but have side effects. Therefore, better therapies are needed. This study investigated whether the transcription factor JUN has a specific pro-osteogenic potency and whether modulating JUN could serve as a novel treatment for osteoporosis-associated fractures. We demonstrate that ectopically transplanted whole bones and distinct osteoprogenitors increase bone formation. Perinatal JUN induction disturbs growth plate architecture, causing a striking phenotype with shortened and thickened bones. Molecularly, JUN induces hedgehog signaling in skeletal stem cells. Therapeutically, JUN accelerates bone growth and healing in a drilling defect model. Altogether, these results demonstrate that JUN drives bone formation by expanding osteoprogenitor populations and forcing them into the bone fate, providing a rationale for future clinical applications.

Funding source: T.L. received a research fellowship from the German Research Foundation (DFG)

Keywords: Bone disease, Osteoprogenitor, Fracture

TSC262

BIOACTIVE BIOMATERIAL SUBSTRATE FOR TISSUE ENGINEERING

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Scaffold design for tissue engineering, with properties and functions that mimic native tissues, has attracted significant attention in recent years. There have been new developments in this field, including the design of new biomaterials and their composites, the development of implantable scaffolds, as well as the understanding of cell–biomaterial scaffolds interaction. To fully realize the potential of biomaterials, it is crucial to have a consistent biomaterial design approach to create novel scaffolds and other material systems suitable for tissue repair and regeneration. Acellular substrates were manufactured from human cadaveric tissues, which were segmented by dissociation of the tissues in a crystalloid solution to facilitate stimulation and release of cellular and extracellular proteins. The solution was filtered to remove any large tissue fragments and the remaining material was precipitated out of solution to create a stable 3-dimensional matrix dressing that can be stored for long-periods. The resultant product was preserved or solidified using lyophilization. The structural and chemical properties of the substrates were examined and revealed 3D-geometry, non-toxic biodegradation products, tunable degradation kinetics, mechanical properties and solubility resembling native ECM. The protein release from these substrates could be sustained for up to a month with retained bioactivity over time. The substrates were tested in vitro with dermal fibroblasts, C2C12 myoblasts and MG-63 cells. The substrates, which were made from musculoskeletal tissues, were found to have low immunogenicity, great cytocompatibility (high cell adhesion and proliferation) and potential for osteoinductivity. Further in vivo testing is underway to demonstrate that application of these bioactive substrates enhances tissue regeneration.

Keywords: biomaterial, tissue engineering, bioactive scaffold

TSC266

CRITICALLY SIZED VOLUMETRIC MUSCLE LOSS LEADS TO ABERRANT FIBRO-ADIPOGENIC PROGENITOR POPULATION IN THE MOUSE QUADRICEPS

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Skeletal muscle's remarkable regenerative capacity relies on the temporal activation and regulation of immune cells, fibro-adipogenic progenitors (FAPs), and muscle stem cells (MuSCs). However, after a vast loss of skeletal muscle due to traumatic injury or surgery, known as volumetric muscle loss (VML), this coordinated regenerative response is diminished, resulting in fibrosis, inflammation, and chronic functional deficits. The goal of this study was to compare the temporal response and phenotype of key cell populations, including macrophages, FAPs, and MuSCs following critical and sub-critical VML injuries in the mouse quadriceps. Unilateral VML injuries were used to quantify cell type distribution via flow cytometry at day 1, 3, and 7 post-VML (pVML, n=4 per injury size and time point). At day 7 pVML, anti-inflammatory M2 macrophages, FAPs, and MuSCs were all present in significantly elevated numbers per tissue volume in critical (3-mm) injuries compared to sub-critical (2-mm) injuries ($p<0.05$). Using multi-dimensional flow cytometry analysis, a distinct sub-population of FAPs was identified in critically sized VML injuries which had larger cell content and highly expressed β1-integrin cell-surface marker. FAPs from quadriceps at day 7 pVML (VML-FAPs) were purified using fluorescence-activated cell sorting and cultured in vitro. VML-FAPs exhibited increased proliferation, increased cell area, and showed elevated levels of β1-integrin expression. In co-culture experiments, VML-FAPs were also shown to significantly decrease the myogenic capacity of primary MuSCs by quantification of myotube formation ($p<0.05$). When cultured with the anti-inflammatory cytokine TGF-β1, VML-FAPs more readily responded differentiating down a fibroblastic lineage. Taken together, these results indicate there is a fundamental shift in the FAPs cell population following VML, potentially driven by the accumulation of M2 macrophages, towards a lineage which is persistent, pro-fibrotic, and anti-myogenic. The identification of this shift in VML-FAPs will be key to development of pro-regenerative therapeutics for VML, as success of these interventions will rely on the ability to downregulate the fibrotic lineage of FAPs while maintaining their traditional pro-myogenic qualities during regeneration.

Keywords: fibro-adipogenic progentiors, volumetric muscle loss, muscle stem cells

TSC275

CANINE MESENCHYMAL STROMAL CELL MEDIATED BONE REGENERATION IS ENHANCED IN THE PRESENCE OF SUB-THERAPEUTIC CONCENTRATIONS OF BMP-2 IN A MURINE CALVARIAL DEFECT

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The dog is a strong translational model for cell-based bone repair strategies. While human MSCs (hMSCs) undergo osteogenesis in response to established protocols, canine MSCs (cMSCs) exhibit little alkaline phosphatase activity or monolayer mineralization without addition of bone morphogenic protein-2 (BMP-2) or other agonists. When compared directly to hMSCs, cMSCs also perform poorly in vivo in the murine calvarial defect model. These findings suggest that from both mechanistic and clinical perspectives, cMSC and hMSC-mediated osteogenesis may differ. We hypothesized that, in vivo, cMSC-mediated bone healing would be improved with co-administration of a sub-therapeutic concentration of BMP-2 and that cMSCs would not express the BMP-2 transcript. Calvarial defects (4mm) were created in 60-day Nu/J mice (n= 5 mice/group). Defects were treated with either 2x106 cMSCs, sub-therapeutic recombinant human (rh) BMP-2 (6 µg/ml), or the combination of 2x106 cMSCs and sub-therapeutic rhBMP-2 (C+BMP-2 L) in 20 µL murine plasma and thromboplastin C to initiate fibrin clot. A positive control group received therapeutic rhBMP-2 (100 µg/ml) in murine plasma on a gelatin sponge. A negative control group (n=4) received the plasma/thromboplastin alone. At day 10, calvarial RNA was extracted from groups that received cMSCs for species-specific qPCR to quantify cMSCs within the defect. At 4 weeks, mice were terminated and assessed for healing using micro-CT and histology. Quantitative data were reported as mean ± SD and analyzed using one-way ANOVA with Dunnetts multiple comparisons post-test. For calvarial defects that received cMSCs, only 2-20 total cMSCs were detected in defects at day 10, demonstrating that cMSCs within a fibrin clot are rapidly cleared during early wound healing. While there were differences in several groups, the largest difference in bone volume (0.205 vs. 0.696 mm3; p=0.001) and surface area (5.06 vs. 10.29 mm2; p<0.0001) was between the negative controls and C+BMP-2 L group, respectively. In vitro, canine BMP-2 transcription was absent in control and osteogenic cMSCs, even with addition of BMP-2. This is the first in vivo study in support of previous in vitro findings regarding cMSC osteogenesis, namely that cMSCs require additional agonists to initiate robust osteogenesis.

Funding source: CSTR Institute, Texas A&M University; Bone & Joint Fund, Texas A&M Foundation

Keywords: canine MSCs, bone regeneration, calvarial defect

NEURAL

TSC279

CRISPR-CAS9 REPROGRAMMING OF HUMAN STEM CELLS INTO MOTOR NEURONS

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Neuromuscular junctions (NMJs) are formed when motor neuron axons terminate on skeletal muscle myocytes, translating nerve impulses into physical muscle contractions. Human models of NMJs are challenging to create because motor neurons are terminally differentiated, preventing further growth or replication. One solution is to differentiate stem cells into motor neurons and while this can be accomplished using small molecules and growth factors, it is laborious and unreliable. An alternative approach is to genetically manipulate stem cells to express key transcription factors required for motor neuron differentiation. Cell markers of mature motor neurons include Isl1 and Lhx3, transcription factors which promote motor neuron specification. The CRISPR-Cas9 gene editing technique can be applied to human stem cells to precisely target and modify specific DNA sequences. Using this approach, we overexpressed specific transcription factors required to differentiate stem cells into motor neurons. Two strategies for lineage-directed stem cell reprograming were applied: 1) CRISPR-Cas9 insertion of transcription factors required for motor neuron differentiation, or 2) direct expression of endogenous motor neuron transcription factors using deactivated Cas9 linked to a transcription activator (dCas9-VPR). Ideal culture conditions for the CRISPR-Cas9 edited cells included small molecules and growth factors to reliably produce mature, functional motor neurons. Ultimately, mature motor neurons will be co-cultured with myocytes to establish NMJs and functionality will be measured by electrophysiological assays. Reliable human NMJs will be a valuable tool to investigate neural injury, neurodegenerative disease, and provide reproducible and robust assays for high throughput drug discovery.

Funding source: Los Alamos National Laboratory Directed Research and Development - A CRISPR Future

Keywords: CRISPR-Cas9, iPSCs, Neurogenesis

TSC280

EXPLORING HUMAN STEM CELL-DERIVED NEURONS TO EVALUATE RISK OF DEVELOPMENTAL NEUROTOXICITY FROM PSYCHOTROPIC DRUGS

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The developing human nervous system is highly susceptible to damage from environmental agents including heavy metals, organophosphate pesticides, drugs-of-abuse and pharmaceutical agents including psychotropic drugs (1). Determining risk of neurotoxicity in humans using animal models is unreliable. Our lab has been pioneering the study of human stem cells as a model of neurogenesis to address development neurotoxicity in vitro. We have previously reported on the impact of the antiepileptic drugs phenobarbital, valproic acid, carbamazepine and lamotrigine on human stem cell viability and their ability to differentiate into neurons in vitro (2). We have now extended this work by investigating the effects of gabapentin (Neurontin) and topiramate (Topamax) on stem cell viability, proliferation and their potential to differentiate into neurons in vitro. Non-differentiating human TERA2.cl.SP12 stem cells were grown in normal culture media or media plus either gabapentin (10-1000mM) or topiramate (10-1000µM) for 3 days. Stem cells differentiating to a neural phenotype were also cultured in the presence of topiramate (3-300µM) or gabapentin (3-300µM) for 30 days. Neither Gabapentin nor topiramate affected stem cell viability or cell death indicated by the Reliablue" Cell Viability and lactate dehydrogenase assays. Topiramate decreased stem cell proliferation after 3 days but gabapentin did not. The proportion of human stem cells differentiating to a neural phenotype, as indicated by ^{2}-III tubulin and MAP2 immunocytochemistry, increased over 3 months from approximately 5% at day 10 to over 35% at day 80 in vitro. Inclusion of topiramate in the culture media reduced the number of b-III tubulin+ cells in a time and concentration-dependent manner. However, there was no significant effect on number of b-III tubulin+ cells after exposure to gabapentin. These new experiments indicate that chronic exposure to topiramate (a widely used anticonvulsant/antimigraine agent) may have detrimental actions on the early developing nervous system. Notably, epidemiological data is also consistent with increased risk of developmental neurotoxicity with topiramate. Further studies are currently underway to address the mechanisms underlying impaired neurogenesis.

Keywords: Stem cell, Psychotropic Drugs, Neurotoxicity

TSC285

ENGINEERING A THREE-DIMENSIONAL STEM CELL NICHE IN THE INNER EAR BY APPLYING A NANOFIBRILLAR CELLULOSE HYDROGEL WITH A SUSTAINED-RELEASE NEUROTROPHIC FACTOR DELIVERY SYSTEM

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Although stem cell replacement therapies involving the application of human embryonic stem cells (hESCs) to treat sensorineural hearing loss show promise, they are hindered by low cell survival rate post-transplantation within the inner ear. Here, we aim to enhance the in vitro and in vivo cell survival rate of otic neuronal progenitors (ONPs) and promote their neuronal differentiation by generating an artificial stem cell niche consisting of three-dimensional hESC-derived ONP spheroids with a nanofibrillar cellulose hydrogel and a sustained-release brain-derivative neurotrophic factor delivery system. Our results demonstrate that transplanted hESC-derived ONP spheroids survive and differentiate towards an otic neuronal lineage in vitro and in vivo. In vivo, we show that these spheroids extend neurites toward the bony wall of the cochlea. Our in vitro and in vivo data presented here provide sufficient evidence that we have established a robust, reproducible protocol for in vivo transplantation of hESC-derived ONPs to the inner ear. Using our protocol to create an artificial stem cell niche in the inner ear, it is now possible to work on integrating transplanted hESC-derived ONPs further, with a future goal of achieving fully functional auditory neurons generated from hESCs. Our findings suggest that the provision of an artificial stem cell niche is a fruitful approach to stem cell replacement therapy for inner ear regeneration.

Funding source: NIH K08 Clinician Scientist Award K08DC13829-02, Office of the Assistant Secretary of Defense of Health Affairs through the Hearing Restoration Research Program (Award #: RH170013:WU81XWUH-18-0712)

Keywords: stem cell niche, stem cell replacement therapy, inner ear

Keywords: iPSC derived from Bipolar patients, Astrocytes from Bipolar patient IPSC, Exosomes from astrocyte culture medium

TSC292

EXPRESSION AND FUNCTION OF EXCITATORY AMINO ACID TRANSPORTERS IN ADULT MOUSE HIPPOCAMPAL NEURAL STEM CELLS

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Adult neurogenesis supports hippocampal learning and memory and responds dynamically to physiological or cognitive experience. Neurotransmitter signaling is one mechanism by which experience can modify multiple stages of the neurogenic cascade. Glutamate, the major excitatory neurotransmitter in the adult dentate gyrus (DG) niche where neurogenesis occurs, stimulates radial glia-like neural stem cells (NSCs) to exit quiescence and supports survival and proper synaptic development of maturing adult-born neurons. Regulation of the glutamatergic milieu proximate to NSCs depends both on glutamate release by local neurons such as hilar mossy cells and subsequent glutamate clearance. Clearance functions in most brain regions are attributed to astrocyte- or neuron-expressed excitatory amino acid transporters (EAATs), a class of membrane-integral proteins that mediate glutamate removal from the extracellular space. However, within the DG neurogenic niche, rodent adult NSCs also express EAATs. This raises the possibility that NSCs shape their own and/or their neighboring progeny's glutamatergic environment, potentially modulating stem cell quiescence/activation and adult-born neuron integration/elimination. However, few detailed studies of NSC EAAT expression and function exist, and whether NSC-expressed EAATs are functionally relevant to hippocampal physiology is unknown. Here we use multiple methods of RNA and protein analysis to demonstrate that mouse hippocampal NSCs express 2 of the 5 EAAT proteins, EAAT1/GLAST and EAAT2/GLT-1, at physiologically relevant levels. Furthermore, in vitro functional analysis demonstrates that NSCs substantially clear extracellular glutamate in an EAAT-dependent manner. We are currently testing whether NSC-expressed EAATs are necessary for normal dynamics of neurogenesis in cell culture and rodent models using pharmacological and gene silencing methods to perturb EAAT function. These studies will advance our understanding of brain signaling mechanisms regulating adult neurogenesis and may elucidate new targets for restoring altered neurogenesis associated with aging or disease.

Keywords: Neurogenesis, Neural stem cell, Excitatory amino acid transporter

TSC298

EVALUATION OF THE DIFFERENTIATION STATUS OF NEURAL STEM CELLS DERIVED FROM HUMAN IPSCS

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Neural Stem Cells (NSCs) can be derived from induced Pluripotent Stem Cells (iPSCs) and then used to make cell-based therapies for the treatment of neurodegenerative diseases. The goal of this project is to identify methods that can be used to evaluate the differentiation status of NSCs expanded in culture as neurospheres or as adherent cells. When iPSC derived NSCs are grown as neurospheres and then analyzed by flow cytometry, the cells appear to have two distinct types of morphology. One subset of cells has low Side Scatter (SSC) and high Forward Scatter (FSC), the other has high SSC and low FSC. The low SSC and high FSC cells express Notch and Sox2, two markers of undifferentiated NSCs, and can form new neurospheres. Cells from outside this region do not express Notch and Sox2 and cannot form new neurospheres. In contrast, when iPSC derived NSCs are grown as adherent cells and then analyzed by flow cytometry, only one cell population based on SSC and FSC is observed. Interestingly, NSCs grown as adherent cells express Notch2 in early passages but not in later passages and as the level of Notch2 expression decreases, the expression of beta-Tubulin III increases. Early passage Notch2(+) NSCs can also more efficiently form tyrosine hydroxylase expressing cells than Notch2(-) cells. It appears that Notch expression can be used as a marker for undifferentiated NSCs regardless of whether they are grown as neurospheres or adherent cells.

Keywords: Induced Pluripotent Stem Cells, human Neural Stem Cell, Notch

TSC318

CONTROLLED ASTROGLIOGENESIS IN CHEMICALLY DEFINED (SERUM-FREE) CONDITIONS, BYPASSES NEUROGENESIS AND ENABLES AUTOMATED, HIGH-THROUGHPUT GENERATION OF ASTROCYTES FROM HUMAN PLURIPOTENT STEM CELLS

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Astrocytes play important roles in normal brain development, function, and various pathological conditions. Derivation of human astrocytes from induced pluripotent stem cells (iPSCs) is an attractive approach for disease modeling and drug discovery; however, present protocols are variable, inefficient, and last up to 6 months. Here, we developed a highly efficient, chemically defined and controlled astrocyte differentiation protocol that requires neither serum nor genetic manipulation. By identifying and simultaneously manipulating several critical pathways, we obtained astrocytes from iPSCs with over 90% efficiency in less than 30 days. These cells displayed astrocyte morphologies and expressed typical markers, whereas genes indicative of other cell types (e.g. neurons, oligodendrocytes) were absent. Compared to the popular dual-SMAD inhibition (dSMADi) approach for neural induction, our method resulted in more efficient generation of symmetrically dividing BLBP+ radial glial cells within 7 days. By day 14, BLBP+ cells differentiated into S100B+ astroglia while TUJ1+ neuroblasts were absent, followed by NFIA expression (day 21). At day 30, strong induction of CD44 and glutamate transporter SLC1A2 was observed. Cell maturation over two passages then robustly induced expression of GFAP and HEPACAM (day 50). iPSC-derived astrocytes were capable of taking up the neurotransmitter glutamate, displayed calcium transients, stored glycogen, promoted neuronal survival, neurite outgrowth and synaptic activity when co-cultured with neurons. We also utilized iPSC-astrocytes for disease modeling (e.g. Alexander disease, Zika virus infection), high-throughput drug screening and cell grafting experiments in mice. Lastly, the protocol was automated using a robotic cell culture system, which now enables the standardized production of billions of well-characterized human astrocytes.

Keywords: ASTROCYTES, RADIAL GLIA, DRUG SCREENING

TSC385

IMPAIRED NEUROGENESIS IN THE DENTATE GYRUS OF ADULT PARKINSON'S DISEASE-ASSOCIATED MUTANT VPS35 MOUSE THROUGH STIMULATING AMYLOID PRECURSOR PROTEIN

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Vacuolar protein sorting protein 35 (VPS35) is a core component of the retromer complex involves in regulating protein trafficking and retrieval. Recently, using exome sequencing, a missense mutation, Asp620Asn (D620N), in VPS35 (PARK17) has been identified to be a pathogenic mutation for late-onset autosomal dominant Parkinson's disease (PD). Although PD is characterized by a range of motor symptoms associated with loss of dopaminergic (DA) neurons in the substantial nigra, non-motor symptoms such as impaired hippocampal neurogenesis was observed in both PD patient and PD animal models caused by multiple PD-linked genes mutation such as alpha-synuclein and LRRK2. However, the role of VPS35 D620N mutation in the adult hippocampal neurogenesis remains unknown. In this study, we revealed that VPS35 D620N mutation strikingly impaired hippocampal neurogenesis in adult transgenic mice expressing VPS35 D620N mutation gene. Specifically, we showed a reduction in neural stem cells pool, neural stem cells proliferation and differentiation, retarded migration, and impaired neurite outgrowth in the 3-month-old VPS35 D620N mutant mice. Moreover, we found that VPS35 D620N mutation induces the hyperphosphorylation of amyloid precursor protein (APP) at Thr668, stimulates the luciferase activity of APP intercellular domain (AICD) and its accumulation. Notably, by crossing VPS35 D620N mutant with APP knockout (KO) mice, we showed that loss-of function in APP rescues VPS35 D620N mutation-inhibited neurogenesis, migration and neurite outgrowth. Our study provides an important evidence for APP being the functional target of VPS35 D620N mutation in regulating adult neurogenesis, which shed the light on the pathogenic mechanisms in PD.

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Keywords: Vacuolar Protein Sorting-35 (VPS35), D620N mutation, adult neurogenesis

TSC394

FUNCTIONAL CHARACTERIZATION OF DORSAL ROOT GANGLIA SENSORY NEURONS FROM HUMAN PLURIPOTENT STEM CELLS USING EXOGENOUS EXPRESSION OF NEUROGENENIN-2

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Emerging strategies to study and characterize peripheral neuropathies in vitro involve the use of human pluripotent stem cells (hPSCs), which can be differentiated to generate populations of dorsal root ganglion (DRG) sensory neurons. A more recent and promising approach for directing hPSC differentiation towards functionally mature neurons is using exogenous expression of Neurogenin-2 (NGN2) during the differentiation. Here we report generation of DRG sensory neurons using induced expression of NGN2 in hPSC-derived neural crest progenitors. Neural crest cells were derived from hPSCs via a small molecule approach and the migrating neural crest cells (66% SOX10+ cells) were transduced with an inducible lentiviral expression vector to transiently express NGN2 and were then matured for a further 3 weeks. The induced neural crest sensory neurons (iSNs) expressed sensory neuron markers such as BR3NA (82% BRN3A+ cells), ISLET1 (91% ISLET1+cells), TRKA, TRKB and TRKC, as confirmed through ICC and PCR. Functionally, action potential firing is supported largely by the activity of TTX sensitive sodium channels as well as high and low voltage activated calcium currents. In the presence of TEA, substantial impairment of action potential repolarization is observed, in congruence with the presence of robust delayed rectifier voltage dependent potassium currents. These results are significant for using hPSC-derived sensory neurons for modelling human DRG development and DRG peripheral neuropathies.

Keywords: Sensory neuron, Differentiation, Electrophysiology

TSC413

GENERATION OF INDUCED NEURAL STEM CELLS BY ARTIFICIALLY ENHANCED TRANSCRIPTION FACTORS

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Direct lineage reprogramming which switches cell types from one to another using defined transcription factors (TFs) has re-shaped traditional understandings of the epigenetic stability of specialized cells. With rapidly increasing cell types produced by direct reprogramming recently, this technology has become a promising approach to study disease processes, test newly developed drugs on human patient cells, and generate new functional cells and tissues for regenerative medicine. Although the technology is developing fast recently, low efficiency, slow rate, and irreproducibility in the process of cell fate conversions restrict its application. Native TFs are not ideal for the artificial lineage reprogramming process. We have previously shown that single amino acid substitutions can convert lineage reprogramming factors into potent inducers of pluripotency. Therefore, these TFs are amenable to be functionally enhanced by protein engineering. A method termed directed evolution of reprogramming factors by cell selection and sequencing (DERBY-seq) has been developed and used to identify artificially evolved and enhanced TFs (eTFs) with high-performance in pluripotency reprogramming. Several TF cocktails were reported to trans-differentiate mouse and human somatic cells into induced neural stem cells (iNSCs). The goal of this research is to apply DERBY-seq to identify new eTFs based on the reprogramming incompetent factor SOX17 that can improve the trans-differentiation of somatic cells to iNSCs. SOX17 variants outperformed the wild-type reprogramming factor SOX2 in iNSC reprogramming cocktails. The application of eTFs to generate iNSCs could be regarded as a new approach to provide adequate cell sources for research and the therapy of neurodegenerative diseases.

Funding source: Health and Medical Research Fund, Grant No. 260870977.096828.22600.440.01

Keywords: induced neural stem cells, direct reprogramming, artificially enhanced transcription factors

NEW TECHNOLOGIES

TSC345

EFFECTS OF DEFINED AND UNDEFINED EXTRACELLULAR MATRICES ON HUMAN IPSC MORPHOLOGY, EXPANSION AND DIFFERENTIATION

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Human induced pluripotent stem cells (iPSC) hold great potential for generating 2D and 3D organoid models that facilitate the treatment of disease. In addition to molecular factors, the extracellular matrix (ECM) is critical for maintaining iPSC survival, pluripotency, and differentiation potential. In this work, the effects of defined (vitronectin and fibronectin) and undefined substrates (basement membrane extract (BME)) on the morphology of human iPSC colonies and their differentiation potential were assessed. Colony formation was observed for cells cultured on all defined and undefined matrices, with colonies exhibiting tight cell packing, smooth and clearly defined edges, and no signs of spontaneous differentiation. Although slight differences in cell proliferation were observed, flow cytometry assessment did not identify significant differences in Oct-3/4 (>95%) or SSEA-1 (<1%) expression between the matrix substrates. However, the

morphology of the iPSC colonies cultured on vitronectin differed significantly from the colonies grown on fibronectin and Cultrex® BME. Despite the cells being from the same line, the vitronectin-cultured iPSC colonies were round/oval shaped whereas the iPSC colonies grown on fibronectin and Cultrex® BME were more irregular with many more colonies being polygonal shaped. To investigate these differences in colony morphology, especially the cells at the periphery of the colonies, integrin signaling and focal adhesion kinase were assessed on different defined/undefined substrates. The impact of defined and undefined substrates on germ layer and terminal differentiation potential of iPSCs were also investigated. These data stress the importance of understanding substrate engagement with integrin signaling pathways which may possibly lead to subtle differences in iPSC morphology or differentiation potential. In addition, identifying and characterizing chemically-defined surfaces for culturing iPSCs will be beneficial for establishing consistent stem cell-based disease models and producing clinical-grade therapeutics.

Keywords: iPSC, Extracellular Matrix, focal adhesion

TSC346

DISSECTING STEM CELL FUNCTION IN VIVO THROUGH SIMULTANEOUS READOUT OF LINEAGE HISTORIES AND GENE EXPRESSION IN SINGLE CELLS

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Tracing the lineage history of cells is key to answering diverse and fundamental questions in biology. To date, lineage tracing research has been hindered by the technically difficult and time-consuming task of labeling and tracking individual cell populations. Here, we present a new mouse model that can be used to simultaneously interrogate the lineage and gene expression information of single cells in vivo. This mouse model, named CARLIN (CRISPR Array Repair LINeage tracing), exploits CRISPR-Cas9 technology to generate inducible, transcribed barcodes that can be detected in an unbiased and global manner in single cells. We demonstrate that CARLIN mice can be used to generate up to 44,000 unique barcodes marking individual cells of the mouse, and that these barcodes can be detected using single-cell droplet sequencing. We also find that multiple pulses of labeling can be used to enhance our understanding of tissue phylogeny during embryogenesis. Finally, we have applied our tools to investigate the clonal dynamics of hematopoiesis in development and in adulthood following perturbation. First, by labeling the earliest emerging embryonic hematopoietic stem cells and tracing them to adulthood, we observed a bias in the spread of HSC clones across different adult bones, pointing toward heterogeneity in the behavior of developmental blood progenitors. Second, we analyzed clonal dynamics of hematopoiesis following chemotherapy-induced ablation and observed that replenishment of the blood is largely driven by a small number of highly active HSC clones, with the majority of HSCs remaining quiescent. We used the gene expression data to obtain a signature marking the active HSC clones, demonstrating that CARLIN can be used to uncover molecular drivers of functional heterogeneity in cellular populations. Our work represents a new resource in lineage tracing research and sheds light on the dynamics of stem cell differentiation in development and in the adult following stress.

Keywords: Lineage tracing, Hematopoiesis, Functional heterogeneity

TSC357

DEVELOPING CELL ADHESION ENGINEERING TECHNOLOGY TO IMPROVE STEM CELL DELIVERY TO THE SITE OF INJURY

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Stem cell therapy has advanced many fields of research in recent years. At the forefront, an increasing number of studies show the potential of stem cells in reversing heart remodeling and improving the loss of heart function in cardiovascular diseases. However, targeting therapy noninvasively has proven to be challenging as stem cells naturally lack the ability to home at the site of injury. In this work, we are presenting a method for engineering stem cells to improve their homing at the site of injury that is inflamed in nature. For this purpose, we genetically modified cardiosphere-derived stem (CDCs) to mimic leukocytes in their initial step of rolling and engraftment at the site of inflammation. These modified CDCs stably express specific glycoproteins that are essential in binding to selectin molecules on activated neutrophils and inflamed endothelium. First, in a series of in-vitro assays, we demonstrate that modified CDCs act in a similar manner as neutrophils in binding to stimulated endothelial cells under hydrodynamic conditions. Additionally, this modification enables CDCs to form aggregates with activated neutrophils, increasing the chance of their engraftment at the site of inflammation. Further, we translate this work in-vivo using a mouse model of ear inflammation. In this model, modified and normal CDCs are intravenously injected into an animal with one ear subjected to local inflammation while the contralateral ear serves as a control. Tracking the cells one hour after injection

shows the localization of modified CDCs in the inflamed ear. These primary results indicate that Glycoengineered CDCs can be directed more efficiently to the inflamed site of injury. Consequently, this may decrease the number of cells required for the stem cell therapy and increase the chance of beneficial effect at the site of injury, thus enhancing the success rate of current and future clinical trials.

Funding source: NYSTEM, American Heart Association (AHA), Translational Imaging Shared Resource (TISR) of the Roswell Park comprehensive cancer center grant S10 OD016450

Keywords: Targeting, Inflammation, Adhesion Molecules

TSC359

ECM MIMETIC CRYOELECTROSPUN SCAFFOLD AS AN MSC DELIVERY VEHICLE FOR THE REMEDIATION OF MOUSE SALIVARY GLAND FIBROSIS

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Fibrosis is characterized by aberrant deposition of extracellular matrix (ECM) and is a contributor to about 45% of deaths worldwide. Fibrosis in the salivary gland results in salivary hypofunction characterized by reduced saliva output or changes in its composition, leading to poor oral and digestive health. Current therapies for salivary hypofunction are palliative and inefficient. Hence, regenerative strategies that would remediate fibrosis and restore salivary gland function are an appealing alternative. Exogenous application of mesenchymal stem cells (MSCs) can limit fibrosis; however therapeutic effects are transient. Scaffolds may improve therapeutic responses by localizing MSCs near the tissue, promoting cell survival and/or engraftment. Further, soft scaffolds (low kPa stiffness) impede the fibrotic nature of myofibroblasts, one of the key mediators of fibrosis and promote tissue regeneration. Ideally, scaffold-based regenerative strategies should emulate native ECM of the region of interest in its developmental stage and be conducive to cell function and differentiation. Current ECM-mimicking scaffolds, such as nanofiber mats, sponges, hydrogels and nanofiber-hydrogel composites show promise, however, fail to concurrently mimic the 3D topography, stiffness, or porosity of healthy soft-tissue ECM. To address the limitations of current scaffolding technologies, we explored an emerging technique – cryoelectrospinning, and scaffold chemistry to fabricate scaffolds with minimal fibrous backbone, porous morphology, and physiological stiffness. We demonstrated 3D stromal and epithelial growth on our cryoelectrospun scaffolds and showed that their coculture facilitated cell-cell interactions resembling normal tissue structure. We further tested the feasibility of maintaining MSC-like primary embryonic mesenchyme on cryoelectrospun scaffolds. Our work lays the foundation to explore the use of cryoelectrospun scaffolds for the delivery of MSCs, to remediate fibrosis and restore salivary epithelial cell function.

Funding source: NIH - NIDCR 1R01DE027953-01A1

Keywords: Cryoelectrospinning - Scaffolds, Extracellular Matrix, Fibrosis

PLACENTA AND UMBILICAL CORD DERIVED CELLS

TSC421

INTERPLAY OF NF-KB/P21 IN REGULATION OF ADHESION AND MIGRATION OF MSCS UNDER MICRO-ENVIRONMENTAL STRESS CONDITIONS.

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Mesenchymal stem cells (MSCs) play a promising role in cell-based therapies due to their immunomodulatory properties and migration abilities. Post transplantation, exposure to micro-environmental stress conditions created by inflammation, serum deprivation, hypoxia, etc, leads to poor survival, low retention and reduced engraftment of MSCs, thereby reducing the efficiency of treatment. Thus, it becomes critical to understand the effect of micro-environmental stress conditions such as febrile temperature, which is a hallmark of inflammation, and serum starvation, on migration and adhesion of MSCs. In our study, we showed that MSCs on exposure to febrile temperature stress had significant increase in cell adhesion and spread area along with reduction in migration speed and suppressed directionality ratio, as evident from the cell trajectory patterns. Adhesion and migration studies were done via cell-matrix de-adhesion kinetics study and time-lapse live cell imaging, respectively. Extracellular matrix genes like collagens and vitronectin were up-regulated, while matrix metalloproteinases like MMP-1 showed a significant down-regulation under febrile temperature stress. siRNA mediated knockdown of NF-κβ pathway, showed a reversal effect on gene expression pattern, as well as on cell spreading, adhesion and migration observed under febrile temperature stress. siRNA mediated knockdown of NF-κβ or p53 independently suggested an inverse relationship between p53/p21 and MMP-1 expression both at mRNA and protein levels under febrile temperature stress while inhibition of JNK pathway led to further down-regulation of MMP-1 gene expression. Surprisingly, we observed that serum starvation of MSCs showed a similar pattern of changes in gene expression, morphology and migration as those under febrile temperature stress and NF-κβ knockdown resulted to a similar rescue

phenomenon. Thus, our study showed that p65/p53/p21, with a possible involvement of JNK pathway, caused repression of MMP-1 expression under febrile temperature stress. Also, NF-κβ could be playing an important role in regulating migration of MSCs under both febrile temperature and serum starvation stress conditions.

Funding source: DST-SERB and UGC

Keywords: Mesenchymal Stem Cells (MSCs), matrix metalloproteinase 1 (MMP-1), cell migration

FRIDAY, JUNE 26, 2020

POSTER SESSION III
05:00 – 07:00

Theme: Cellular Identity

CARDIAC

CI294

PROFILING HAND1 AND HAND2 EXPRESSIONS DURING CARDIOGENESIS USING HIPSCS

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The heart is developed from heat tube to four chambers without stopping heart beats which is achieved by spatial, temporal expression of genes. HAND1 and HAND2 which were thought to be expressed in left and right ventricle during development, respectively. Recent papers with single cell RNA sequencing revealed HAND2 is not specific for RV, but expressed ubiquitously. On the other hand, HAND1 is expressed in LV, and its function and regulatory mechanism are still unclear. To analyze HAND1 and HAND2 expression in vitro and clarify their role in cardiomyocytes (CMs), we established HAND1, HAND2 and MYH6 triple reporter human induced pluripotent stem cells (hiPSCs) and observed the expression dynamics of HAND1 and HAND2 during cardiomyocyte differentiation.

EARLY EMBRYO

CI117

HOW TO RIGIDLY EVALUATE DIFFERENTIATION POTENTIALS AND MAKE THE RIGHT IPS CELL CLONE?

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During development, transcription factors of adjacent cell populations mutually inhibit their expression, a phenomenon known as cross-inhibition. This operation establishes a sharp boundary between the cell populations and lays the foundation of multi-cellularity. Pluripotent stem cells, including iPS cells (iPSCs), are expected to faithfully follow a certain cell fate when directed by a developmental rationale which in turn dictates the expression profiles of the due master regulators. Although haploinsufficiency of transcription factors is known to cause developmental aberrations, we pay little attention to the expression levels of transcription factors. Using a ventral midbrain neuronal precursor (DANP) differentiation model of human iPSCs, we sought to tackle this problem through the inspection of this cross-inhibition. During ventral midbrain development, LMX1A-positive DANPs emerge juxtaposed by more dorsal cells which are NKX6.1- or PAX6-positive. Our in-house made human iPSC clones exhibit relatively high differentiation efficiencies to LMX1A-DANPs, ranging between 70-90%. Importantly, one of them, 1T47 cell line, differentiates into 90% LMX1A-, 9.5% NKX6.1- or PAX6-positive leaving only 0.5% of the cells undefined. We interpret this finding as an indication of the adequate functionality of the transcription factors analyzed and, therefore, a faithful execution of developmental rationale. We failed in observing such quasi-total differentiation from any other iPSC clones. 1T47 clone is unique in that we performed iPSC reprogramming by adding TET1 to the Yamanaka factors. We will discuss the effect of TET1 on reprogramming this “new-type” iPSC by focusing on enhancer DNA methylation.

Funding source: This work was supported by AMED (grant no. 19bk0104090h0001).

Keywords: Pluripotency, Differentiation potentials, TET1

CI118

SELF-ORGANIZATION OF THE IN VITRO ATTACHED NON-HUMAN PRIMATE EMBRYO

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Blastocyst implantation is a landmark event in mammalian embryonic development. After implantation, mammalian embryos develop inside the uterus making it difficult to observe the developmental process and perform experimental analysis. Therefore, molecular mechanisms of mammalian early development are poorly understood. Recently established in vitro implantation platforms in the mouse, human, and cynomolgus monkey have expanded our knowledge of post-implantation development. However, human embryos cannot be extensively used because of ethical reasons, and these studies show that the mechanism of early development in primates differs from that of the mouse. Common marmoset (Callithrix jacchus, marmoset) is an excellent model for studying primate embryogenesis. Common marmoset is the only model which allows access to both in vitro fertilized embryos and naturally conceived embryos. In this study, we established in vitro post implantation culture in the marmoset embryos. Our results show that all embryos successfully attached to our in vitro implantation platform. Sixteen of 33 (48.5%) embryos successfully developed and expressed Oct4 in the epiblast and Gata6 in the hypoblast. Although the development of these marmoset cultured embryos was slower than in human and cynomolgus monkey, they recapitulated the structure observed in other primates. Immuno staining and qPCR results show that the Burachury gene, a primitive streak marker expressed during the developmental stage, was clearly expressed in marmoset cultured embryos. We have observed primitive streak cells merged with GATA6 positive cells and are currently analyzing the details of differentiation of the primitive endoderm. In the future, this in vitro marmoset post implantation embryo culture system would be invaluable for improving our understanding of the mechanisms of early development.

Keywords: in vitro culture, non-human primate, implantation

CI122

MUCIN-TYPE O-GLYCOSYLATION REGULATES PLURIPOTENCY IN MOUSE EMBRYONIC STEM CELLS VIA WNT RECEPTOR INTERNALIZATION

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Embryonic stem cells (ESC) are a promising tool for regenerative medicine and to dissect the mechanisms underlying mammalian embryonic development. In the past decades, ESC have been intensively studied to unravel the complex mechanisms underlying pluripotency. Nonetheless, glycosylation role within the pluripotency network remains largely unexplored, hampering ESC exploitation. To address this, we performed a preliminary evaluation by screening approximately 100 glycosyltransferases using gene silencing in mouse ESC (mESC), followed by the alkaline phosphatase activity assay as an indicator of the pluripotent state. As a result, the mucin-type O-glycosylation pathway was identified as a putative candidate. Mucin-type O-glycosylation is characterized by the initial addition of N-acetylgalactosamine (GalNAc) to serine or threonine residues and, together with N-glycosylation, is the most abundant form of glycosylation present on membrane-anchored and secreted proteins. Previously, our and other groups reported that mucin-type O-glycosylation is crucial during development across different species. However, the function of mucin-type O-glycosylation and its relation with signaling in mESC still remains undefined. In the present study, we identified the mucin-type O-glycosylation elongation pathway via C1GalT1, which synthesize T antigen, as the most prominent in mESC. Manipulation in the expression of C1GalT1 resulted in the loss of mESC pluripotency. Furthermore, we observed that T antigen on Wnt receptor Frizzled regulates its internalization finely modulating Wnt signaling outcome, unveiling a novel Wnt signaling regulatory mechanism. Our results provide the first demonstration that mucin-type O-glycosylation regulates mESC pluripotency by directly modulating Wnt receptor Frizzled internalization. Our findings advance understanding of ESC pluripotency regulation accelerating the exploitation of ESC in both regenerative medicine and developmental biology research.

Funding source: This work was supported by JSPS KAKENHI Grant Number JP18K06139 and JST-Mirai Program Grant Number JPMJMI18GB.

Keywords: Glycosylation, T antigen, Wnt signaling pathway

CI127

UNVEILING THE EPIGENETIC MECHANISMS UNDERLYING MOUSE GERM LAYER FORMATION

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Primary germ layers possess the potential to form all tissues in the mature organism. The formation of primary germ layers during gastrulation requires precise epigenetic modulation of regulatory elements. However, the underlying epigenetic mechanisms remain largely unexplored. Here, we profile the spatiotemporal landscape of the epigenome and transcriptome

of the mouse gastrula. We reveal the asynchronous dynamics of proximal chromatin states during germ layer formation as well as unique gastrula-specific epigenomic features of regulatory elements, which have strong usage turnover dynamics and clear germ layer-specific signatures. By using the transgenic mice and genome editing system, we demonstrate that an ectoderm-specific enhancer-Ect2, which executes important function during mouse neural differentiation. Through constructing Ect2 activity-based lineage tracing mouse, we identify the potential for direct ectoderm lineage tracing and explore the molecular mechanisms underlying the ectoderm-neuronal developmental trajectory. Taken together, our study provides the comprehensive epigenetic information for embryonic patterning during mouse gastrulation, demonstrates the importance of germ layer-specific enhancers in regulating the correct development of the mouse embryo, and broadens the current understanding of mammalian embryonic development and related diseases.

Keywords: Mouse germ layer formation, Epigenetic regulation, Enhancer usage turnover

CI148

AGE-DEPENDENT CHARACTERISTICS OF TRANSCRIPTION AND METABOLISM IN PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) offer an unlimited source for elucidating early embryo development as well as developing autologous cell replacement therapies. Recent findings revealed that mitochondrial genome (mtDNA) mutations and genomic instability of iPSCs generated from aged donor are accumulated compared to embryonic stem cell (ESC) conterparts, which can lead to rejection of autologous iPSC-derived cells, tumorigenesis and respiratory defects. Two stable pluripotent states have been derived in the mouse; pre-implantation naive and post-implantation primed states. The naive-to-primed transition accompanies a pronounced metabolic switch from a bivalent to a highly glycolytic state. During reprogramming from primed to naive states of pluripotency, it is reported that LIF/Stat3 promotes mitochondrial transcription and facilitates metabolic resetting. Thus, establishing naive pluripotent cells from the primed state might be the key to revert the mtDNA sequence. To elucidate the molecular pathways involved in age-dependent deterioration of stem cell function, we have isolated parthenote-derived ESCs and skin-tissue-derived iPSCs from mice of different ages: young (6 to 8 weeks), middle-aged (6 months) and aged (12 to 14 months). Immunohistochemistry of pluripotent stem cells derived from all ages uniformly proved self-renewal capacity and pluripotency. RNA-sequencing analysis revealed that gene expression level responsible for defense and cytokine response in iPSCs derived from aged mice increased compared to ESCs derived from young mice (y-ESCs). The functional analysis of mitochondria displayed that stem cells in primed state show poor glycolytic rate, whereas cells in naive state show improved mitochondrial oxidative capacity. In addition, it projected the transition of ATP production rate with age; alongside no significant age-associated changes in respiratory function, proton leak, and coupling efficiency. In the current study, we have demonstrated age-dependent characteristics of transcription and metabolism in pluripotent stem cells. Our data potentially leads to develop a path toward iPSCs without undesirable genetic abnormalities, which is more desirable for cell replacement therapies.

Keywords: pluripotent stem cells, mitochondria, aging

CI149

HIGHLY SULFATED HYALURONIC ACID MAINTAINS UNDIFFERENTIATED STATE OF HUMAN IPS CELLS

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Glycosaminoglycans (GAGs), such as heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratan sulfate, regulate signaling pathway and cell fate decisions in pluripotent stem cells. Hyaluronic acid (HA), one of glycosaminoglycans (GAGs), is composed of repeating disaccharide units of glucuronic acid (GlcA) and N-acetylglucosamine (GlcNAc). Unlike other GAGs, HA is not sulfated naturally. We chemically synthesized low sulfated HA (HA-LS) and highly sulfated HA (HA-HS). The effect of sulfated HA addition to human induced pluripotent stem (iPS) cell culture medium was analyzed, and non-sulfated HA (HA-NS) was added to the culture medium as a control. When HA-HS was added to the culture medium, human iPS cells maintained undifferentiated state even without feeder and the addition of basic fibroblast

growth factor (bFGF), an essential signaling factor to maintain undifferentiated state of human iPS cells. The increase of bFGF/Extracellular Signal-regulated Kinase (ERK) signal activity was observed as a result of HA-HS addition to culture medium. It was speculated that bFGF alone which human iPS cells themselves secreted was sufficient to maintain undifferentiated state. On the other hand, the undifferentiated state of the human iPS cells was not maintained when HA-NS and HA-LS were added to culture medium, indicating that the effect to maintain undifferentiated state was a HA-HS-specific effect. Furthermore, human iPS cells that had maintained undifferentiated state by the HA-HS addition could differentiate into three germ layers and maintain pluripotency. In a competitive ELISA analysis, the binding ability of HA-HS to bFGF was the highest among GAGs including heparin which is known to bind to bFGF. This is the first report to clarify the effects of sulfated HA on the culture of mammalian pluripotent stem cells and provide a novel culture method that can maintain human iPS cells under feeder-free and bFGF-free conditions.

Keywords: Sulfated hyaluronic acid, FGF signaling, Human induced pluripotent stem cell

CI151

ROLE OF H4K20ME1 AND H3K27ME3 DURING X-CHROMOSOME INACTIVATION IN MOUSE ESC

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During stem cell differentiation, transcriptional and chromatin modification changes co-occur but the order and causality of events often remain unclear. We explore the interrelationship of these processes by using X-chromosome inactivation (XCI) as a paradigm of facultative heterochromatin formation. To this end we have developed probes for tracking the accumulation of two repressive histone modifications: H3K27me3 and H4K20me1. We combine these reporters with live imaging of Xist non-coding RNA in female mouse embryonic stem cells. We reveal that both histone modifications simultaneously accumulate within hours of Xist RNA accumulation. Allele-specific profiling of both marks allowed for the identification of regions most susceptible to H4K20me1 and H3K27me3 accumulation. Strikingly, we report that H4K20me1 marks actively transcribed genes prior to XCI. However, upon Xist upregulation H4K20me1 accumulates mainly at intergenic regions. While H4K20me1 and H3K27me2 accumulation pattern is distinct they both favor regions rapidly coated by Xist. Together, striking correlations in the timing of accumulation of both marks and their relative distribution indicated a common molecular pathway for their targeting. Indeed, we show that Xist B+C repeat mutant unable to recruit Polycomb complexes not only fails to enrich H3K27me3 at the inactive X but also does not accumulate H4K20me1. This region of Xist is dispensable for the initiation of gene silencing and so is H4K20me1. All in all, we provide tools for tracking chromatin changes in living cells and uncover the functional relevance of specific histone modifications during early steps of facultative heterochromatin formation.

Funding source: Novo Nordisk Foundation, ERC

Keywords: X chromosome inactivation, embryonic stem cells, chromatin

CI152

NON-CANONICAL BAF COMPLEX MEMBER BRD9 IS A BARRIER FOR HUMAN SOMATIC CELL REPROGRAMMING

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ATP-dependent chromatin-remodeling complexes have important functions in the regulation of gene expression, and some have significant effects on reprogramming of somatic cells to pluripotency. However, until now, the role of the recently identified ncBAF (non-canonical BRG1-or BRM-associated factors) complexes in reprogramming were unknown. Here, we show that BRD9, a member of ncBAF complex, is a strong barrier to cell fate transitions. Through an epigenetics-focused chemical screen, we identified an inhibitor BRD9, LP99, as an enhancer of human somatic cell reprogramming. Two structurally distinct

BRD9 bromodomain inhibitors, I-BRD9 and BI-7273, and a PROteolysis TArgeting Chimera (PROTAC) degrader, dBRD9, phenocopied this effect. In addition, genetic suppression of BRD9, by shRNAs and CRISPR-mediated knockout validated the inhibitory effect of BRD9 on reprogramming. Importantly, treating fibroblasts with a degrader or a bromodomain inhibitor of BRD9 enabled reprogramming with only two-factors, OCT4 and SOX2. In contrast, loss of bromodomain containing 7 (BRD7), a member of the PBAF complex, did not enhance reprogramming, suggesting a specific role for the ncBAF complex. RNA-seq and ATAC-seq analyses demonstrated that inhibition of BRD9 results in decreased somatic cell type-specific gene expression and chromatin accessibility at associated regulatory regions. Together these results indicate that ncBAF complex functions to maintain somatic cell identity, and its inhibition, lowers the epigenetic barriers to reprogramming. Our findings highlight the role of a newly characterized chromatin remodeling complex in cell fate transitions and show that small molecule-based targeting of this complex can be employed to derive human iPSCs with high efficiency and fewer transgenes.

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Keywords: Mechanism of reprogramming, Epigenetics and chromatin, Induced pluripotency stem cell (iPSC)

CI160

THE FUNCTION OF LNCRNA IN EXPANDING MOUSE EMBRYONIC STEM CELLS DEVELOPMENTAL POTENCY

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Mouse embryonic stem cells (mESC) derived from the inner cell mass can give rise to ectodermal, mesodermal, endodermal lineages but not the extra-embryonic lineages. Many efforts have been made to establish embryonic stem cells with extra-embryonic developmental potency through complex culture conditions adjustment. However, whether the expanded potency could be established through simple alternative paths remains unknown. Here, we successfully acquired mESC with expanded extra-embryonic potency, which can be cultured under conventional conditions, by genomic deletion of lncRNA GM26793 (GKO-mESC). We found that GKO-mESC exhibits both embryonic and extra-embryonic lineage differentiation capability, which were demonstrated by in vitro differentiation and in vivo blastocyst chimeras. Mechanistic analyses revealed that the deletion of GM26793 can reactivate Fgf-Erk signaling, which further boost the expanded potency of the mouse embryonic stem cells. Further analyses demonstrated that GM26793 could inhibit Fgf signaling through cis- but not trans-regulation. Collectively, we provide a novel way for the establishment of expanded pluripotent stem cell, and demonstrate the direct link between expanded potency and specific genomic element, which will broaden the current understanding of stem cell pluripotency.

Keywords: extra-embryonic lineages, GKO-mESC, Fgf-Erk signaling

CI170

MECHANISTIC INSIGHT TO GROW SELF-ORGANIZED EMBRYO FROM EMBRYONIC STEM CELLS

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Early mammalian development comprises of two major processes: development of epiblast, and development of extraembryonic layers like trophoblast and hypoblast for providing support to the epiblast. I am particularly curious to understand how these very early cells of embryo decide to become foetus or supporting cells, using mouse as a model system. Developmental potential of Embryonic stem cell (ESCs) known to restrict to embryo proper and hypoblast but not trophoblast. However recent reports in the past one year have shown that ESCs cultured in extended potential media can form extraembryonic layers including trophoblast. However, mechanism of such process is still unknown. In this study we have identified one of the major signalling pathways and the molecular players essential for attainment of the extended pluripotent and differentiation to trophoblast lineage. We have also identified a complex interaction between three transcription factors regulated by small molecules leading to trophoblast differentiation of pluripotent cells. In addition, we have derived trophoblast cell lines and also show that the cells can contribute to trophoblast lineage when injected into 8-cell morula. With these mechanistic insights and few other perturbations, we were able to self-organise preimplantation embryo like structure solely from mouse embryonic stem cells in-vitro. We also show that these embryo-like structures can implant and develop till dpc 7.5.

Funding source: The work has been funded by the Council of Scientific & Industrial Research (CSIR), India and University Grants Commission (UGC), India.

Keywords: Trophoblast, Self-Organization, Extended Pluripotency

CI171

NANOG AUTO REPRESSION OPERATES THROUGH AN EXTENDED SIGNALLING PATHWAY

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Embryonic stem cells are isolated from the inner cell mass of a blastocyst. They are pluripotent and can differentiate to the three primary germ layers. Pluripotency in ES cell is maintained by a network of core pluripotency factors viz Nanog, Oct4 and Sox2. Among the core factors, Nanog shows heterogeneous expression. The mosaic expression of Nanog is observed in the epiblast of blastocyst from which ES cells are isolated. Heterogeneous gene expression is known to have a functional role in cell fate decisions, which might allow a window of opportunity to direct differentiation into a particular lineage. However, it is not known how the heterogeneous expression of Nanog is induced and maintained. The ectopic overexpression of Nanog in pluripotent cells resist their differentiation, thereby embryos fail to undergo normal embryogenesis. Hence, maintaining the expression of Nanog in ES cells within threshold limits is essential for retention of differentiation potential along with self-renewal. This level is maintained in ES cell by an auto feedback repression loop. In addition, it is known that this loop is also operational in serum-free '2i LIF' conditions. However, mechanistic details operating for maintaining Nanog levels in this loop are poorly understood. Nanog shows monoallelic expression when ES cells are cultured in Serum/LIF condition. Whereas when cultured in '2i LIF' it shifts to biallelic expression. Here we have identified an elaborate FGF signalling based autocrine/paracrine signalling loop which regulates Nanog levels. The Fgf signalling cascade activates downstream molecules like MEK1/2 and ERK1/2, which repress the activity of Nanog locus. We further show that the same Fgf signalling is essential for inducing heterogeneous expression of Nanog in ES cells. We also show that MEK1/2 is essential for monoallelic expression of Nanog. We suggest that MEK1/2 acts as a molecular fulcrum integrate 3 regulating paradigms of Nanog expression in pluripotent stem cells.

Funding source: This work is funded by Council of Scientific and Industrial Research (CSIR) and Indian Council of Medical Research (ICMR)

Keywords: Autorepression, Heterogeneous expression, Biallelic expression

CI173

RNA-SEQUENCING ANALYSIS OF TURNER SYNDROME PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS REVEALS CIRCULAR RNA-MEDIATED DYSREGULATION OF DEVELOPMENTAL GENES VIA COMPETING ENDOGENOUS RNA NETWORK

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Turner syndrome (TS), also known as monosomy X, has occupied 19% chromosomal abnormalities of human embryos. Circular RNA (circRNA) is one class of competing endogenous RNA (ceRNA) that regulates mRNA expression by acting as an microRNA sponge. It is abundant in human embryos and has a pivotal role in regulating the embryonic development process. However, its role in TS embryonic development remains unclear. We have established 3 TS patient-specific induced pluripotent stem cell (iPSC) lines as the TS group and 3 normal iPSC lines as the control group. Total RNA-seq, microRNA-seq and circRNA-seq were performed to obtained the expression profiles of mRNA, microRNA and circRNA in these cell lines. Differentially expressed genes (DEGs) were identified between the TS and control groups. CeRNA network was built with the DEGs by seed match analysis based on the "circRNA-microRNA-mRNA" regulatory module. The TS-iPSC lines have exhibited a significantly lower efficiency in embryoid body formation compared to the normal iPSC lines, though they still maintain the potentials to differentiate into cell types of the three germ layers. From the RNA-seq data, we have discovered 829 differentially expressed circRNAs in the TS group, named "TS-circRNAs". Gene annotation enrichment analysis of the TS-circRNA parental

genes showed that the top enriched GO terms are insulin receptor binding, dystroglycan binding and Ran GTPase binding, which are highly relevant with embryonic development. CeRNA network analysis has built a gene regulatory network that includes a number of developmental genes. The top ranking circRNA regulatory modules include “circSHROOM2-miR3663-DIAPH2”, “circRPS6KA6-miR4435-GLIS3” and “circFIRRE-miR139-PRKX”. DIAPH2, GLIS3 and PRKX are key regulators of ovary, thymus and kidney development, respectively. These findings implicate the important roles of circRNAs in mediating ovary, endocrine system and kidney malfunction during TS embryonic development. In this study, we have discovered a novel panel of TS-circRNAs associated with embryonic development, and provided valuable insights into the underlying mechanisms of circRNA-mediated dysregulation of ovary, endocrine system and kidney development in TS embryos.

Funding source: This work was supported by grants from the Guangzhou City and Liwan District Science, Technology and Innovation Commission (201804010340, 201804013), and The Third Affiliated Hospital of GMU Fund Project (110217103, 110217110)

Keywords: Turner syndrome, Induced pluripotent stem cell, Circular RNA

CI307

THE BIPHASIC ROLE FOR BCATENIN SIGNALING IN NAÏVE AND PRIMED PLURIPOTENT STEM CELL

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The βcatenin is expressed in a variety of cells and is a molecule involved in many cell kinetics. Similarly, it profoundly involved in cellular properties in pluripotent stem cells(PSC), but its function is known to be different in naïve and primed pluripotency. However, the detailed molecular mechanism is still unclear.The present study aimed to elucidate the relationship between the state of PSC and βcatenin, and attempted to elucidate a novel pluripotency related molecular mechanism. In the study, mouse embryonic stem cell(ESC) and mouse epiblast stem cell(EpiSC) were used as models for naïve and primed PSC. Activation of βcatenin by small molecule compounds or overexpression plasmid by using amino acid substitutions was performed. We used microarray, in silico ChIP and Co-IP analysis were performed to evaluate the function of βcatenin in ESC and EpiSC. When the activation state of βcatenin was observed, the amount of nuclear βcatenin was unchanged in both. However, activation of βcatenin promotes maintenance of undifferentiated in the naïve state, while primed state shows a tendency to differentiate. Therefore, we speculated about the molecules that bind to the upstream region of the TSS of βcatenin upregulated genes in EpiSCs using in silico ChIP, and several pluripotency-related genes were identified. More interestingly, Co-IP showed that these molecules interacted with βcatenin in EpiSCs, but not in ESCs. These results suggest that βcatenin alters partner molecules in a cell-state-dependent manner and has a strong effect on pluripotency. We consider that the action of βcatenin shown in this study is a function of regulate the pluripotency state and differentiation determination. Identifying the key factors involved in the stability and conversion of pluripotent states is essential for a understanding of pluripotency and could lead to a more ideal model system for studying early human development.

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Keywords: βcatenin, pluripotent stem cell, naive and primed pluripotent state

CI313

CDK8/19 INHIBITION TRIGGERS GLOBAL HYPERACTIVATION OF ENHANCERS AND STABILIZES HUMAN AND MOUSE NAIVE PLURIPOTENCY

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

Funding source: AGAUR

Keywords: plasticity, Mediator-complex, CDK8

CI316

DOT1L INTERACTING PROTEIN AF10 IS A BARRIER TO SOMATIC CELL REPROGRAMMING

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Epigenetic regulators have important roles during embryonic development as well as somatic cell reprogramming. We previously showed that inhibition of DOT1L, the histone H3 lysine

79 methyltransferase, increases the efficiency of reprogramming via regulation of lineage specific genes. However, the role of DOT1L-interacting proteins in reprogramming remains unknown. In this study, DOT1L interactors were identified using the BioID method in which a promiscuous BirA ligase (BirA*) was employed to biotinylate DOT1L-proximal proteins. The resulting interaction candidates were investigated for their effects on reprogramming. Candidate genes were knocked-down in human fibroblasts via shRNAs followed by reprogramming. Our results indicated that knock-down of AF10 (MLLT10), significantly increased the iPSC generation efficiency, suggesting that it acts as a barrier to reprogramming similar to DOT1L. This finding was verified by CRISPR/Cas9 mediated knockout of AF10. Overexpression of AF10 reversed the effect of AF10 knockout and decreased reprogramming efficiency. To determine how AF10 silencing changes the gene expression, RNA-sequencing was performed on human fibroblasts undergoing reprogramming. AF10 suppression resulted in downregulation of fibroblast-specific genes and accelerated the activation of pluripotency-related genes. Our analysis also demonstrated that silencing of AF10 results in gene expression changes similar to DOT1L inhibition during reprogramming. Taken together, this study uncovered AF10 as a novel barrier to reprogramming and contributed to our understanding of epigenetic mechanisms that maintain cell identity.

Funding source: TÜBİTAK
KUTTAM

Keywords: AF10, reprogramming, DOT1L

CI334

EFFECT OF INTERLEUKIN-7(IL-7) ON IN VITRO MATURATION OF PORCINE OOCYTES

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Interleukin-7 (IL-7), a cytokine secreted mostly by stromal cells in bone marrow and thymus, stimulates the survival, differentiation, proliferation and development of lymphocytes. It is also a oocyte secretion factor (OSF) that interacts with cumulus cells and surrounding somatic cells. The purpose of this study was to investigate the effects of IL-7 on porcine oocytes in vitro maturation. Each concentration (0, 0.1, 1 and 10ng/ml) of IL-7 was supplemented to maturation media (TCM199-PVA) during in vitro maturation. Porcine cumulus oocytes complexes (COCs) were treated for 40-42 hours with and without IL-7 treatment. Subsequently, we investigated nuclear maturation, intracellular glutathione(GSH), reactive oxygen species (ROS) levels. As a result, there was a significant difference in the nuclear maturation between control group and 1ng/ml IL-7 supplementation group. The rate of oocytes in the stage of metaphase II was observed on nuclear maturation from the different concentration (0, 0.1, 1, and 10 ng/ml) of IL-7 supplementation groups (91.6, 92.2, 97.3, and 92.1%, respectively) compared with the control ($P < 0.05$). The 1ng/ml IL-7 treated groups showed a significant ($P < 0.05$) increase intracellular GSH levels compared with other group. The 1- and 10-ng/ml IL-7 treatment groups showed significantly ($P < 0.05$) lower intracellular ROS levels compared with control group. Based on these results, IL-7 has a good effect on nuclear maturation and cytoplasmic maturation on porcine oocytes. As a further experiment, parthenogenetic activation (PA) experiments may be able to demonstrate that IL-7 aids embryo development.

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Keywords: Porcine oocyte, Interleukin-7, In vitro maturation

CI336

ECTOPIC EXPRESSION OF LIMA1 IN PRIMED PLURIPOTENT STEM CELLS ENABLES EFFICIENT GENERATION OF MOUSE-MOUSE AND MOUSE-HUMAN EMBRYOS

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Pluripotency defines the ability of a cell to differentiate into all somatic tissues and the germline. Known and described for almost a century, pluripotency as a developmental state has just recently been found to comprise different sub-states - namely naïve, formative and primed pluripotency, which consequently transform into each other as embryogenesis advances. Mouse embryonic stem cells (mESCs) reside in a naïve pluripotent state, exhibiting full developmental potential, whereas mouse epiblast stem cells (mEpiSCs) and conventional human induced pluripotent stem cells (hIPSCs) capture primed pluripotency features. Characteristically and in contrast to naïve pluripotent cells, primed pluripotent cells only integrate with low efficiency into pre-implantation mouse embryos, showing that a poorly understood developmental barrier sets apart the naïve and primed states. Here, we identified an effector molecule, LIMA1 (LIM domain and actin-binding 1, a.k.a. EPLIN), that allows both mouse and human primed pluripotent cells to trespass the developmental barrier, without inducing changes (reprogramming) in their pluripotent state. We determined that Lima1 expression is associated with naive pluripotency, both in vitro - in mESCs, and in vivo - in blastocyst stage embryos. We found that Lima1 function is involved in the regulation of the energy metabolism in mESCs and is required for the maintenance of the inner cell mass in mouse embryos. During the transition from naïve to primed state, Lima1 expression is downregulated, following the dynamics of the bona fide naïve markers such as Nanog. Interestingly, overexpressing lima1 in both mouse and human primed pluripotent cells allowed efficient generation of chimeric embryos, enabling also interspecies chimerism. We are currently investigating the molecular mechanism of Lima1 function in order to decipher the nature of this developmental barrier. Our ongoing analyses point to a mechanism that comprises of LIMA1-mediated mechanobiological changes in the cytoskeleton that affect the metabolic and epigenetic status of the primed cells, altogether directing their potential for integration into host embryos.

Funding source: German Research Foundation (DFG)
CiM-IMPRS Joint Graduate School of University of Münster and Max Planck Society

Keywords: LIMA1/EPLIN, Chimerism, Blastocyst

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CI179

EX VIVO EVALUATION OF HUMAN HEPATOCYTE PLASTICITY

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Hepatocyte plasticity, i.e., the ability to convert into proliferation progenitor cells with bi-potential differentiation capacity to replace a lost liver mass, is essential for the homeostasis and regeneration of the liver. So far, the plasticity of human hepatocytes has not been evidenced. Here, we established a method to evaluate the plasticity of human hepatocyte ex vivo. After six days of induction, we found that human hepatocyte can be reprogrammed into a proliferation statue with hepatic progenitor characteristic. Moreover, these progenitor cells can be differentiated into cells with a typical hepatic morphology and albumin production, and also developed into a cyst structure with an up-regulated expression of cholangiocyte signatures. Thus, this study demonstrates the plasticity of human hepatocyte ex vivo and provides a useful tool for further study of liver regeneration in humans.

Keywords: Hepatocyte plasticity, Hepatic progenitor cell, Bi-potential differentiation capacity

CI190

ESTABLISHMENT OF A NOVEL CELL FATE CONVERTING MODEL TOWARDS FUNCTIONAL INSULIN-PRODUCING CELLS FROM ENDOCRINE PROGENITOR CELLS LINE TEC-3P AND SOMATIC CELLS

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The reprogramming of somatic and stem cells into pancreatic β cells is a promising approach for curing type I diabetes as a cell replenishment therapy. Pancreatic acinar, duct and alpha cells can transdifferentiate into β cells in vivo. Instead of limited these cells in vivo, however, a large pool of somatic cells such as fibroblasts is needed for the feasible therapy as a resource, and cell fate converter into direct β cell reprogramming remains to be uncovered in vitro in the context of potential future therapy for all T1D patients. We found, however, that no induction of insulin in murine embryonic fibroblasts (MEFs) by above 3 factors. Here we propose a hypothesis in which other cell fate key driver might be involved in MEFs-derived cell reprogramming. To address this, we have established a novel cell line, Tec-3p which is very likely immature endocrine progenitors. We have also developed the lineage-tracing model from endocrine progenitors to beta cells in dual-labeled transgenic mice expressing eGFP and DsRed2 driven by the Ngn3 and insulin promoters. Integrative screening analyses with our unique models has performed to identify potential key factors such as growth factors facilitating beta cell differentiation from progenitors mediated by Notch signaling. Moreover, we found that these factors accelerated the differentiation through the downregulation of Neurog3 gene expression through epigenetic control, indicating that chromatin remodeling mediated epigenetically by coordinating the integrative Notch and ErbB signaling contributes to progenitor maintenance and pancreatic endocrine cell fate determination together with potent cell fate converters we identified. Therefore, we propose herein a potential therapeutic approach towards T1D.

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Keywords: differentiation, reprogramming, endocrine cells

GERMLINE

CI315

NON-CANONICAL FUNCTIONS AND NOVEL ISOFORM OF DNA METHYLTRANSFERASE 3-LIKE IN GERM CELLS AND BEYOND

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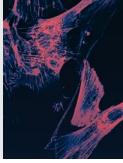
DNA methyltransferase 3-like (DNMT3L) is one of the key epigenomic modulators that, despite not having DNMT enzymatic activity by itself, is critical for setting up parental origin specific imprinting marks as well as providing proper silencing of transposable elements (TEs) after the dramatic epigenomic reprogramming event in primordial germ cells. In this proposed presentation, we will go through our decade long effort in identifying DNMT3L's molecular function beyond facilitating



de novo DNA methylation; DNMT3L's expression beyond embryonic male germ cells, female germ cells in developing follicles and embryonic stem cells; the phenotypes of Dnmt3l KO mice beyond infertility. We recently identified novel isoforms of DNMT3L in postnatal male germ cells and the implications of their interaction with piRNA pathway in modulating TEs in post-transcriptional gene silencing level. Beyond the germ lines, we demonstrate how DNMT3L legacy is important for preventing premature aging in cultured fibroblasts and multipotent mesenchymal stromal cells. Ectopic expression of DNMT3L, on the other hand, is capable of halting senescence partly through triggering DNMT3L-DNMT3A-KAP1-SETDBA-HDAC1 complex formation and silencing of TEs by H3K9me3, as well as collaborating with PRC2 complex to maintain H3K27me3 mark on aging associated derepressed genes. The importance of DNMT3L modulated homeostasis in germ cells and beyond will be discussed in detail.

Funding source: Ministry of Science and Technology, Taiwan; National Taiwan University

Keywords: Epigenetics, piRNA, DNMT3L, germ cells, MSCs, aging, senescence

HEMATOPOIETIC SYSTEM

CI218

SINGLE CELL QUANTIFICATION OF ATP CONCENTRATION IN LIVING MOUSE HEMATOPOIETIC STEM CELLS IDENTIFIES A STEM CELL-SPECIFIC PLASTICITY OF ATP PRODUCTION

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Metabolic dynamics control the fate of hematopoietic stem and progenitor cells (HSPCs). Despite its importance, the metabolic difference between hematopoietic stem cells (HSCs) and progenitors is yet unclear due to paucity of methods that can quantitatively measure metabolic profile. For instance, to what extent each of the HSPC fraction depends on glycolysis and mitochondrial oxidative phosphorylation (OXPHOS) is still an open question. Adenosine triphosphate (ATP) plays a central role in metabolism and kinetics of which represents metabolic status inside cells. We took advantage of ATP biosensor-knock-in mice to quantify ATP concentration ([ATP]) within the HSPC fraction with high temporal resolution at single cell level thereby examining how HSPCs adapt to nutrient environment, cell-cycle status and metabolic perturbation. We first examined the dependence on glycolysis and OXPHOS within the HSPC fraction by measuring [ATP] following pharmacological inhibition under a nutrient-deficient condition. Inhibition of glycolysis by 2-deoxy-D-golucose (2-DG) resulted in rapid reduction of [ATP] in Lineage-negative c-Kit+ Sca-1- myeloid progenitors (MPs) while HSCs and common lymphoid progenitors exhibited slower ATP reduction rate. Inhibition of OXPHOS by oligomycin, by contrast, led to rapid decrease in [ATP] either in HSCs or in progenitors, contrary to the previous notion that HSCs depend more on glycolysis than progenitors. Among the HSPC fraction, only HSCs restored ATP production by adding glucose upon OXPHOS inhibition, suggesting that HSCs but not progenitors plastically accelerate glycolysis to compensate for decrease in OXPHOS-dependent ATP production. We then tested whether the glycolytic acceleration could occur in a situation where ATP requirements have increased in vivo. In HSCs after 5-FU treatment, ATP levels greatly decreased by 2-DG treatment than that of the PBS-administered group, suggesting that activated HSCs accelerate glycolytic ATP production in vivo. We searched for chemical compounds that inhibit glycolytic acceleration in HSCs upon OXPHOS inhibition and identified that inhibitors for AMPK or PFKFB3 reduced [ATP] in oligomycin-treated HSCs. Collectively, HSCs plastically upregulate the glycolytic ATP production in a demand-driven manner.

Funding source: AMED, JSPS/MEXT

Keywords: stem cell metabolism, single cell analysis, hematopoietic stem cell

CI221

CD62L EXPRESSION LEVEL DICTATES THE CELL FATE OF COMMON MYELOID PROGENITORS AND GRANULOCYTE-MONOCYTE PROGENITORS BOTH IN MICE AND HUMAN

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Hematopoietic cells are hierarchically differentiated from hematopoietic stem cells through several progenitors. Recent studies showed each progenitor population has substantial heterogeneity and some of the subsets have skewed differentiation potential. However, it has not been elucidated when common myeloid progenitors (CMPs) and granulocyte-monocyte progenitors (GMPs) acquire different fates. We thoroughly explored surface markers with heterogeneous expression patterns in the GMP population by using publicly available single cell RNA-seq data and identified CD62L as a candidate to refine the differentiation potential of CMPs and GMPs. At CMP level, CD62L expression was widely distributed. When CMPs were divided into low, middle and high CD62L expression, colony forming-cell assay showed CD62L-high CMPs only produced granulocytes and macrophages in mice and human. Liquid culture assay in mice showed CD62L-low CMPs differentiated into GMPs and megakaryocyte-erythrocyte progenitors (MEPs), however CD62L-high CMPs only produced GMPs. Also, single-cell RNA-seq data of murine and human samples showed CD62L-low CMPs exclusively had gene expression profiles characteristic of CMPs. These data suggest that CD62L-high CMPs are restricted to GMP potentials. At GMP level, CD62L showed trimodal expression patterns: negative, low and high. Colony forming-cell assay in mice showed CD62L-negative GMPs partially had CMP potentials and single-cell RNA-

seq data supported this finding, suggesting that the bona fide GMPs were restricted to CD62L-low to high GMPs. Furthermore, CD62L-low GMPs produced granulocyte colony (CFU-G) 73.2 ± 6.1% and macrophage colony (CFU-M) 17.4 ± 3.3%, whereas CD62L-high GMPs produced CFU-G 46.6 ± 1.7% and CFU-M 42.9 ± 2.7%. CD69 showed similar patterns and co-staining of CD62L and CD69 further discriminated neutrophil- or monocyte-skewed subpopulations; CD62L-low/CD69-low GMPs mostly produced CFU-G (84.1 ± 1.5%), whereas CD62L-high/CD69-high GMPs mainly produced CFU-M (65.1 ± 9.1%). Moreover, in vivo transplantation assay showed murine CD62L-low GMPs produced more neutrophils (95.3 ± 2.1%) than bulk GMPs (82.0 ± 4.9%). In summary, CD62L expression refines the definition of CMPs and GMPs, which elucidates the differentiation mechanism of myeloid cells in more detail.

Keywords: Common myeloid progenitor (CMP), Granulocyte-monocyte progenitor (GMP), CD62L

MUSCULOSKELETAL

CI298

NOTCH REGULATES THE FATE OF HUMAN SKELETAL MUSCLE PROGENITORS VIA PROSTAGLANDIN EP2 RECEPTOR

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Understanding how proliferation and differentiation of skeletal muscle progenitors are regulated is essential for establishing cell transplantation therapies for Duchenne muscular dystrophy (DMD). We found that DAPT, an inhibitor of Notch signaling, promotes the fusion of human skeletal muscle progenitors in vitro and improves their engraftment into the tibialis anterior muscle of immunodeficient mice. To understand the mechanisms by which Notch regulates proliferation and differentiation of muscle progenitors, we investigated which genes were up-regulated or down-regulated in muscle progenitors by DAPT treatment. RNA-seq analysis revealed that PTGER2, which encodes prostaglandin E2 receptor 2 (EP2), was significantly down-regulated by DAPT treatment. Functional analysis of EP2 using inhibitors, overexpression, and shRNA-mediated knockdown showed that Notch signaling inhibits the differentiation of skeletal muscle progenitors via PGE2/EP2 signaling. Interestingly, inhibitor experiments suggested that another signaling pathway other than the cAMP/PKA pathway regulates the cell fate of skeletal muscle progenitors downstream of Notch/PGE2/EP2.

Keywords: skeletal muscle progenitors, differentiation and proliferation, Notch signaling

NEURAL

CI238

IN VITRO RECORDING OF PROPAGATING ACTION POTENTIAL ALONG SENSORY AXON WITH HIGH DENSITY MICROELECTRODE ARRAY

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In vitro models of neurodegenerative disorder are promising for developing novel medical treatment. A lot of model using iPS cell derived central nervous system (CNS) neurons have been developed. However, little is known about sensory neurons, and myelination and subsequent increase of conduction velocity. Here, we aim to develop a new method for evaluating salutatory conduction from myelinated sensory axons. First, sensory neurons and Schwann cells were cultured on high-density microelectrode array (HD-MEA) chips. Myelin sheath formation was induced by adding ascorbic acid in culture medium, and was confirmed with immunocytochemistry with antibodies for myelin-related proteins. Spontaneous activity was induced by capsaicin stimulation and recorded with HD-MEAs. Axonal signal was detected from averaged extracellular signal by triggering somatic activity. Instantaneous and mean conduction velocity was calculated from propagating action potentials. As a result, we observed that action potential propagated along axon more than 2 mm, which is much longer than propagation of CNS neurons. Mean conduction velocity was 0.1-0.5 m/s, suggesting normal conduction along unmyelinated fiber. As a next step, we try promoting myelin sheath formation. Our method is suitable for evaluating conduction properties of sensory axons.

Funding source: This study was partially supported by JSPS KAKENHI (19H05323, 19H04437), and Murata Science Foundation.

Keywords: Sensory neuron, High-density microelectrode array, axon

CI250

EVALUATING THE EFFECT OF HYPOXIA ON HUMAN FETAL NEURAL STEM CELLS IN VITRO

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Fetal neural stem cells (FNSCs) can differentiate into neuronal and glial lineages. “Physiological hypoxic condition” influences their growth and differentiation. We wanted to evaluate the effect of hypoxia on FNSCs. In this study, we expose Fetal Neural Stem Cells (FNSCs) to various grades of hypoxia (20%, 6%, 2% and 0.2%) and evaluate their cell fate after hypoxia. With Ethics Committee clearance and informed consent, aborted fetuses were collected from the Department of Obstetrics and Gynaecology, AIIMS, New Delhi. FNSCs were isolated from the sub-ventricular zone of the fetal brain and expanded in culture. FNSCs were exposed to various oxygen concentrations (20%, 6%, 2% and 0.2%) for 48 hours and a panel of hypoxia markers (CA9, PGK1 and VEGF) validated exposure to hypoxia by qPCR. Annexin V assay was used to evaluate cell death. Microarray was done using Agilent platform to study differentially expressed genes between normoxic and hypoxic FNSCs. GeneSpring GX and MetaCore software were used to analyze data. Nestin and Sox2 expression were detected in isolated FNSCs by immunocytochemistry and flowcytometry. The expression of CA9, VEGF and PGK1 were found to be up regulated in hypoxic FNSCs by qPCR. Hypoxia did not cause cell death in FNSCs. It was observed that genes related to neurogenesis were getting enriched in FNSCs exposed to hypoxia in the microarray analysis. The pathway analysis of these enriched genes showed that WNT signaling played a role in determining the cell fate of FNSCs. Validation of the microarray analysis showed that early neuronal markers DCX and ACSL1 were upregulated in FNSCs exposed to hypoxia whereas markers for gliogenesis were downregulated. It was also observed that canonical WNT pathway was upregulated in FNSCs exposed to hypoxia by QPCR. Thus, it was concluded that when FNSCs were exposed to hypoxia, they were getting primed for neuronal lineage and not towards glial lineage.

Funding source: Department of Biotechnology, Government of India

Keywords: Neural Stem cells, hypoxia , Neurogenesis, Wnt, Neural Stem cells, hypoxia , Neurogenesis, Wnt, Neural Stem cells, hypoxia , Neurogenesis, Wnt

NEW TECHNOLOGIES

CI283

MOGRIFY: A UNIVERSAL CELL REPROGRAMMING PLATFORM TO TRANSFORM THE DEVELOPMENT OF LIFESAVING CELL THERAPIES

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Despite major investment in the field, few cell therapies have gained regulatory approval with limited commercial success. The success of therapeutic products is defined by three factors: safety, efficacy and scalability. Achieving these for cell therapy presents new challenges; overcoming transplant rejection (and GvHD in the case of immune cells), producing and culturing functional cells and developing an off-the-shelf therapy with a scalable manufacturing process. Gene editing technologies are paving the way towards allogeneic safety with numerous developments towards “universal donor cells”. However, such progress would be almost idle without the possibility to produce and culture functional cells at scale. Underpinned by a decade of development through an international research effort, Mogrify has developed a computational approach powered by genetic, proteomic and epigenetic data to systematically control the cellular transcriptomic network underlying cellular identity. This platform can identify key factors required to culture or convert any cell type to any other cell type, a process called transdifferentiation. We have applied Mogrify to 173 human cell types and 134 tissues, defining an atlas of cellular reprogramming including both known transcription factors used in transdifferentiation and new ones, never implicated in these cellular conversions. Mogrify in silico predictions have been validated in vitro in over 20 cell conversions, including the generation of endothelial cells, astrocytes and cardiomyocytes. We present the latest advances in our prediction technology, as well as new data on our internal cell therapy programs, aiming to develop new therapies for blood, immune, eye and musculoskeletal indications.

Keywords: Transdifferentiation, cell reprogramming platform, cell identity

CI285

APPROACHES TO THE GENERATION OF FELINE INDUCED PLURIPOTENT STEM CELLS USING SENDAI VIRUS VECTORS

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Establishment of feline induced pluripotent stem cells (fiPSCs) would enable us to differentiate various cells for use in veterinary regenerative medicine. However, there are very few reports on the generation of fiPSCs. Known iPS cells have been generated using a lentivirus vector but fiPSCs without gene insertion have not been reported yet. Sendai virus vector (SeVdp (KOSM)302L), which expresses exogenous genes without genomic insertion and can be automatically erased by the microRNA-302 (miR-302) expressed in PSCs, can be used to effectively generate human iPSCs. We have successfully generated canine iPSCs using this vector. In this study we attempted to generate fiPSCs, free from exogenous and integrated DNA, from feline embryonic fibroblasts, using SeVdp (KOSM)302L. We could establish three fiPSC lines by co-culture with mouse fetal fibroblasts (MEFs). All iPSC colonies showed flat morphology similar to human iPSCs. The cell lines were maintained for several passages by dissecting colonies into a number of small clumps by mechanical slicing. They showed alkaline phosphatase activity and RT-PCR confirmed the expression of SeVdp genes in all cell lines. However, immunostaining showed a mixture of SeVdp-positive and -negative cells in the same colony. To isolate SeVdp-negative cells, the resulting fiPSC colonies were enzymatically separated into individual cells and cultured in Cellartis® DEF-CS™ 500 Culture System without MEFs (single-cell culture). As a result, a total of 27 clones were generated from fiPSC colonies. All of these clones tested positive for alkaline phosphatase activity. RT-PCR confirmed the expression of the SeVdp gene in all clones. In conclusion, using SeVdp (KOSM)302L, we were able to reprogram feline embryonic fibroblasts without genome insertion; however, Sendai virus was not completely removed. Further studies are needed to generate exogenous and integrated DNA free fiPSCs.

Funding source: This work was supported by JSPS KAKENHI Grant Numbers JP18K19273 and JP18H02349.

Keywords: induced pluripotent stem cells, feline, Sendai virus vector

CI286

EFFICIENT GENERATION OF CANINE INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS USING A SMALL MOLECULE COCKTAIL

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Induced pluripotent stem cells (iPSCs) are expected to provide new cell sources for development of regenerative medicines and genetic disease models in the veterinary field. Canine peripheral blood cells (PBMCs) are advantageous because they are noninvasive and easily obtained from blood samples of healthy donors and patients. However, it is difficult to generate canine iPSCs (c-iPSCs) from PBMCs owing to their very low reprograming efficiency. For efficient generation of c-iPSCs from PBMCs, we used a Sendai virus vector, SeVdp (KOSM)302L, which has a high gene transfer efficiency. Furthermore, we determined its reprograming efficiency in combination with small molecules. We found that c-iPSCs can be efficiently generated from PBMCs on feeder cells using Stemfit® supplemented with leukemia inhibitory factor (LIF) and a small molecule cocktail (Y-27632, PD0325901, CHIR99021, A-83-01, Forskolin, and L-Ascorbic acid). We generated five c-iPSC lines in Stemfit® supplemented with LIF. The SeVdp(KOSM)302L viral vectors were silenced automatically in four c-iPSC lines. The c-iPSC lines were positive for alkaline phosphatase activity and expressed pluripotent markers, including OCT3/4, NANOG, and SSEA1, as determined by immunocytochemistry. The c-iPSCs were able to form embryoid bodies and differentiate into all three germ layers, as indicated by expression of the endoderm marker SOX17 and mesoderm marker DESMIN and ectoderm marker TUBB3. Consequently, we achieved more efficient, noninvasive, and easy generation of canine iPSCs from PBMCs. In conclusion, our method presents several advantages to be used for the development of clinical research tools for regenerative medicine and pathological elucidation.

Funding source: This work was supported by JSPS KAKENHI Grant Numbers JP18K19273 and JP18H02349.

Keywords: canine induced pluripotent stem cells, peripheral blood cells, reprograming

CI287

SINGLE-CELL CRISPR SCREEN IDENTIFIES NEW EPIGENETIC REGULATORS FOR NAIVE PLURIPOENCY MAINTENANCE

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The transcriptional and epigenetic status restricts the self-renewal ability and lineage specificity of the naïve and primed mouse embryonic stem cells (mESC). CRISPR/Cas9-based functional screening coupled with single cell RNA-seq (CROP-seq) establishes relationships between gRNA-mediated knockout genotypes and transcriptome phenotypes, and is a novel tool to unravel gene regulatory networks. Here, we used CROP-seq approach to dissect the transcriptional and epigenetic regulation of the pluripotency network in mESCs. This highly sensitive and efficient method identified key genes for acquisition of and exit from pluripotency, and uncovered a novel role for H3K36me2 and H3K27me3 in mESC pluripotency state transition. We found that loss of Nsd1-mediated H3K36me2 led to delayed naive state exit while loss of Ezh2-mediated H3K27me3 promoted transition to primed state. Together, our study reveals an epigenetic-transcription factor regulatory network that determines mESC state transition.

Keywords: Single-cell CRISPR screen, stem cell state transition, epigenetic modification

CI289

REACTIVATION OF X CHROMOSOME STARTS FROM THE IDENTICAL REGION DURING MOUSE IPSC GENERATION

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Induced pluripotent stem cells or iPSCs are produced by forced expression of four ectopic reprogramming genes, which reset the epigenomic status of somatic cells. One of those epigenomic events is termed X chromosome reactivation (XCR). XCR erases Xi-heterochromatin marks and activates transcription from Xi allele. The reversed phenomena, X chromosome inactivation (XCI) during development/differentiation was studied well. XCI is a stepwise event, which starts from Xist RNA expression, and spreading the whole chromosome while establishing the heterochromatin marks. It was believed that XCR is in the late phase of reprogramming, considered to correlate to pluripotency. However, the detail mechanism, whether it's a stepwise process or whether it has the specific starting locus like XCI still remains unclear. Here, we investigate how XCR proceed related to these questions. As a first step, we tried to establish a quantitative method to detect transcription of any genes in X chromosome. To distinguish 2 alleles using SNPs, Mus Musculus C57/BL6J (B6) and Mus Spretus (Sp) are crossed to obtain the hybrid embryonic fibroblast (MEFhs). We designed TaqMan probe which can specifically recognize the target SNPs of B6-allele or Sp-allele on X chromosome and labelled with different color for RT-qPCR. Conclusively this system, named SNP cDNA typing, enable us to quantify X chromosome status between two alleles. Next, MEFhs were reprogrammed and iPSCs with different XCR status in a time course were isolated with confirmation by SNP cDNA typing. We applied those sets of iPSCs to allele-specific RNA-seq, to know how reactivation of Xi allele proceeds in fact. Contrary to the previous studies, we found that XCR started in relatively early phase of reprogramming and gradually proceeded. Moreover transcriptional activation starts when still Xist RNA expression is observed. Interestingly, transcriptional activation reproducibly started from one specific 0.5 Mb region and spread to the whole chromosome. The region is totally different from Xist, or X-Controlling center which are important for XCI. Those results suggest that XCR occurs in stepwise manner, starting from one identical region, and spreading to whole chromosome as XCI does. However, the regulation of XCR is distinct from XCI, XCR dynamics is not just the reversal of XCR.

Keywords: iPSCs, epigenetics, X Chromosome Reactivation

CI290

REPROGRAMMING OF CANINE EMBRYONIC FIBROBLASTS TO PRIMED AND NAIVE LIKE PLURIPOTENT STATES

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Pluripotency of cells is divided into primed and naïve states. In general, cells in naïve state such as mouse pluripotent stem cells, are comparatively undifferentiated and therefore have a better differentiation ability than primed state such as human pluripotent stem cells. Both types of pluripotency are considered useful for regenerative medicine, especially cells in naïve state may be useful in saving endangered species, as they can differentiate into germ cells. Although some studies reported the generation of primed canine induced pluripotent stem cells (ciPSCs), the reprogramming efficiency was very low. Therefore, it is necessary to improve the low reprogramming efficiency to widen the downstream applications. Furthermore, the generation of naïve ciPSCs has not been reported so far. In this study, we aimed to generate ciPSCs in primed and naïve states with high reprogramming efficiency by treatment with TGFβ inhibitor and a combination of GSK3β and MEK inhibitors (2i), respectively. Our results showed that the TGFβ inhibitor, specially A83-01, significantly improved the reprogramming

efficiency. The morphology of the primary colonies was similar to the human pluripotent stem cells in primed state. Furthermore, the cells retained their morphology and pluripotent state after several passages as shown by alkaline phosphatase (AP) staining, RT-PCR, and immunocytochemistry. To generate naïve ciPSCs, we tested various combinations of culture media (KSR vs. N2B27), growth factors (bFGF only vs. LIF only vs. bFGF+LIF), and small molecule compounds (+/- 2i). Our results showed that N2B27 medium supplemented with 2i and LIF resulted in the highest reprogramming efficiency. Moreover, the morphology of the cells reprogrammed in this media was similar to the naïve mouse pluripotent stem cells, and did not change after multiple passages. However, these cells were AP-negative. Next, we added 8 small molecular compounds simultaneously to N2B27 supplemented with 2i and LIF. Our results showed that when reprogrammed in presence of these compounds, the cells stained AP-positive. Taken together, our results showed a high efficiency reprogramming protocol to generate ciPSCs in primed and naïve like pluripotent states. Owing to their translational implications, our results warrant further validation.

Funding source: This work was supported by JSPS KAKENHI Grant Numbers JP18K19273, JP18H02349, JP19J22851.

Keywords: canine induced pluripotent stem cells, primed and naive states, small molecule compounds

CI332

SINGLE-CELL TRANSCRIPTOMIC ATLAS OF PRIMATE OVARIAN AGING

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The molecular mechanisms underlying ovarian aging as well as the major risk factor of physiological reproductive decline and female infertility remain unclear. Here, we surveyed the single-cell transcriptomic landscape of ovaries from both young and aged non-human primates (NHPs; cynomolgus monkeys). Distinct gene-expression signatures were identified for ovarian somatic cell types and oocytes at various stages of follicular development. Aging resulted in the cell type-specific downregulation of antioxidant genes, such as GPX1 and GSR in oocytes and IDH1 and NDUFB10 in granulosa cells, indicating that oxidative damage is a determinant process in the decline of NHP ovarian function with age. Similarly, in humans, increased ROS and apoptosis, as well as downregulation of IDH1 and NDUFB10 expression were observed in granulosa cells from aged versus younger women. Knockdown of IDH1 or NDUFB10 in human granulosa cells led to defects in oxidative stress responses, linking the aging phenotype with altered antioxidant gene expression. This study provides a comprehensive platform for the understanding of the cell type-specific mechanisms underlying ovarian aging in primates at single-cell resolution. The data also reveal new biomarkers for diagnosing aging-associated human ovarian disorders, provide targets for developing interventions to treat these disorders and may help develop new approaches for rejuvenating aged oocytes for assisted reproductive therapies.

Keywords: single-cell RNA sequencing, ovary, aging

CI340

CORRELATION BETWEEN CELL MORPHOLOGICAL INFORMATION OBTAINED FROM DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY (D-IHM) AND FUNCTIONAL CHARACTERISTICS OF EPITHELIAL CELLS

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We have previously shown the utility of cell-specific scores obtained from D-IHM to predict the cell function in mesenchymal stem cells. In this study, we will report on the utility of morphological scoring derived from D-IHM as a novel non-invasive method for predicting functional properties of epithelial cells. We focused on the morphological information of epithelial cells obtained from digital in-line holographic microscopic images (D-IHM) acquired by CultureScanner CS-1. Chronic inflammation or neoplastic changes cause epithelial cell damage to parenchymal cells such as intestinal epithelial cells, hepatocytes, and renal tubular epithelial cells. Epithelial cells are connected to each other by various structures, which prevents some molecules from passing through the intercellular space and can contribute to the communication between each cell. Epithelial cells also have polarity, which plays an important role in the secretion and absorption of functional substances. Accordingly, structural and functional disorders of epithelial cells are closely linked to the pathology of these diseases. The evaluation of epithelial cells is mainly based on functional analysis such as the “transmission method,” which measures the uptake and excretion of drugs and fluorescent substances, or “transepithelial electrical resistance (TEER),” which measures the impedance of the epithelial cell sheet by applying a weak flow of AC electricity. In addition, measurement of the LDH response to cytotoxic stimulation or expression of tight junction proteins has also been performed. However, these methods are affected by the heterogeneity of the cultured cell sheet, making it difficult to detect changes in individual cells, and cell invasive methods. In contrast, morphological characteristics, such as individual cell shape, cell density, and cell adhesion are obtained through phase contrast microscopy non-invasively, but it is difficult to estimate these features objectively or further predict cell function. Therefore, we focused on the morphological analysis using D-IHM, which provides information concerning optical transmittance and cell contours of cultured cells non-invasively. The morphological information is quantitatively evaluated as the epithelial cell-specific score.

Funding source: Shimadzu Corporation

Keywords: Digital in-line holographic microscopy, Epithelial cells, Morphological scoring

Theme: Clinical Applications

ADIPOSE AND CONNECTIVE TISSUE

CA102

A NEW NONTOXIC AND BIOSAFETY CRYOPROTECTIVE AGENT FOR TISSUE BULK PRESERVATION

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Long term preservation of adipose tissue is curial for clinical application. Choosing cryoprotective agent (CPA) for adipose tissue preservation should consider both efficiency and bio-safety. Glycerol is an ingredient that protect cell from freezing injury mainly by reducing intracellular ice crystal formation and osmotic damage. The purpose of this study is to evaluate the efficacy of glycerol as CPA for adipose tissue cryopreservation. Fresh human adipose tissues were obtained from ten liposuction patients and was divided to 1ml samples. Each sample was randomly mixed with 1 ml CPA: 60 to 100% glycerol, 0.25 mol/L of trehalose or DMSO+FBS and cryopreserved in -196°C liquid nitrogen for one month. After thaw and elution, tissues were immediately evaluated for activity in vitro, as well as for structural integrity. For evaluating the effect of freezed fat in transplantation, 0.2ml of each sample was transplanted subdermally to nude mouse dorsum and harvested after one month for histological examination. After cryopreservation, samples treated with DMSO/FBS, trehalose, 60% and 70% glycerol had a more integrated structure. But only tissues preserved with 70% glycerol had highest tissue activity, close to that of fresh tissues. The growing rate and differentiation capability of stem cells separated from different groups were similar. In vivo study showed that 70% glycerol treated fat had superiors in retention rate and structural integrity, with less inflammation. Our study elucidated that glycerol solutions can serve as cryoprotective agents for adipose tissue long-term cryopreservation. Among all concentrations studied, 70% glycerol solution was superior at preserving adipose tissue bioactivity, reducing the retention rate, as well as preventing tissue fibrosis and inflammation in cryopreserved adipose tissue. Furthermore, glycerol is a nontoxic and nonimmunogenic agent thus is safer and more compatible with clinical use.

Keywords: cryopreservation, adipose tissue, cryoprotective agent,

CARDIAC

CA113

AUTOLOGOUS SKELETAL MYOBLAST SHEET AMELIORATED CARDIOMYOCYTE ISCHEMIA AND RIGHT HEART DYSFUNCTION IN PRESSURE-OVERLOADED RIGHT HEART PORCINE MODEL

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Severe heart failure (HF) with congenital heart disease (CHD) have demonstrated life threatening disorder despite of remarkable progress in medical therapies. Autologous skeletal myoblast sheet (ASMS) transplantation showed clinical efficacy for left ventricular dysfunction by cytokine paracrine effects, which are expected to be sufficiently effective against right ventricular (RV) dysfunction which is often seen in end-stage of CHD patients with severe HF. So, we hypothesized that, an ASMS transplantation alleviates RV dysfunction in a pressure-overloaded right heart in a porcine model. Five-to-six-month-old Göttingen mini-pigs underwent pulmonary artery banding. Two months after banding, ASMS was placed on the epicardium of the RV free wall and followed for 2 months and sacrificed for histologic analysis. Groups were as follows: control (C, n=4), sheet implantation (S, n=4). Before sheet implantation, RV dysfunction was equal in groups; however, 2 months after sheet implantation, RV dysfunction and myocardial ischemia was significantly ameliorated in group S than group C. On CT, RV ejection fraction exacerbation were well controlled in Group S compared to Group C (S 44.9+/-2.2 vs C 31.9+/-2.1 % [p=0.0042]). UCG and Cath revealed well maintained systolic and diastolic function in Group S compared to Group C (Tei index: S 0.42+/-0.06 vs C 0.70+/-0.07 [p=0.0004], Fraction Area Change: S 45.8+/-7.8 vs C 19.5+/-1.3 % [p=0.0002], Isovolumic Relaxation Time; S 44.3+/-9.2 vs C 97.3+/-9.5 ms [p<0.0001]). On C11-Acetate PET, myocardial ischemia was more prominent in Group C compared to Group S (K mono-Rest/Stress: S 3.17+/-0.69 vs C 2.03+/-0.65 min-1 [p=0.0421], Myocardial Blood Flow-Rest/Stress: S 3.22+/-0.39 vs C 2.13+/-0.92 min-1 [p=0.0421]). In histologic analysis, Group S presented less progressed hypertrophic change in periodic acid-

Schiff stain (S 13.5+/-0.9 vs C 18.0+/-3.0 µg [p<0.0001]) and anti-fibrotic changes in picrosirius red stain (S 3.0+/-0.3 vs C 4.2+/-0.2 % [p<0.0001]) and more angiogenesis in CD31 expression (S 18.3+/-1.5 vs C 10.7+/-2.8 /104 µm2 [p=0.0099]) and less hypoxia in dihydroethidium stain (S 1741+/-83 vs C 627+/-107 units/mm2 [p=0.0304]). ASMS transplantation alleviates cardiomyocyte Ischemia and RV dysfunction in a porcine model of pressure-overloaded right heart.

Funding source: None

Keywords: Autologous skeletal myoblast sheet, Pressure-overloaded right heart failure, Porcine model

CA114

A PHARMACOLOGICAL APPROACH FOR IMMUNOSUPPRESSION REGIMEN IN PIGS TO ACCEPT HUMAN IPSC-DERIVED CELLS

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Cardiovascular disease is the leading cause of mortality worldwide. Cell-based therapies using human iPSC-derived cardiomyocytes (hiPSC-CMs) are promising interventions for the treatment of severe heart failure patients. Transplantation of hiPSC-CMs into the myocardium leads to the sustained improvement of cardiac function through the cell engraftment and synchronized contraction with host myocardium. To prove this concept in preclinical studies, large animal studies are needed because extrapolation of results from small animal studies to humans is difficult given the heart size and heart rate of these animals A calcineurin inhibitor, cyclosporin A (CyA) or tacrolimus (Tac), is generally used for immunosuppression for xenotransplantation of human cells in swine. However, it is unclear which is suitable for porcine immunosuppression in xenogeneic transplantation. The appropriateness of immunosuppressants also differs depending on the type of pig. First, we determined the IC50 of CyA and Tac in phytohemagglutinin-induced lymphocyte proliferation assay using the peripheral blood mononuclear cells from the blood of micro-miniature pigs (MMPs), resulting in the IC50 values as about 1 µg/mL and 1 µg/mL, respectively. Next, the drugs were orally administrated to MMPs at the maximum tolerable doses (50 mg/kg b.i.d. of CyA or 1.5 mg/kg b.i.d. of Tac) for one week, then the plasma drug concentration at trough was determined. Because protein-unbound (free) drug is available for pharmacological activity, we measured free drug levels in the medium or the plasma. As a result, more than 100-times higher equivalent drug concentration was observed in the blood of CyA-administered swine compared to that of Tac. These data suggest that CyA is more suitable for immunosuppression of porcine than Tac. Finally, we examined the cell engraftment of hiPSC-CMs in the heart of MMPs treated with our immunosuppression regimen (CyA, mycophenolate mofetil, and prednisolone). The main immune organs (thymus and spleen) of the MMPs were surgically removed. Robust engraftment of hiPSC-CMs was observed by immunohistochemistry analysis in the porcine heart at three weeks after transplantation This finding is useful to conduct the xenogeneic transplantation studies of cell-based therapy using a porcine model.

Keywords: xenotransplantation, immunosuppression, human iPS cell-derived cardiomyocytes

CA115

WHICH HYDROGEL MATRIX TO CHOOSE IN CARDIAC TISSUE ENGINEERING: A COMPARISON BETWEEN FIBRIN AND COLLAGEN

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Cardiovascular diseases are both the leading cause of death worldwide and a major safety concern during novel drug development. Animal models have been traditionally used in this field but present high costs, ethical considerations and differences to humans. Stem cell-derived cardiomyocytes tissue engineering offers the promise of an alternative to animal models as well as potential personalised medicine. Although collagen and fibrin have been the two widely used hydrogel matrix in the cardiac tissue engineering field, few groups have studied their impact on the physiology of cardiomyocytes and the functional performance of the resulting tissues. Fibrin and collagen-based hydrogel have different stiffness, polymerisation time and permeability which could influence the physiology of the tissue construct. It is necessary to investigate those differences, in order to be able to choose the most suitable matrix depending on the focus of the study. No significant difference in sensitivity to isoprenaline and nifedipine were found between the two matrixes. The cell connectivity of the construct was improved with collagen strips having an average excitation threshold of 3.5 V compared to 5.0 V in fibrin. The maximum capture frequency average was also higher in the collagen strips with 4.5 Hz compared to 3.5 Hz in Fibrin. The generation of force was lower in fibrin matrix 14 days after casting with an average force of 89 µN in collagen constructs compared to 54 µN in fibrin. On the other hand, its variability was smaller in fibrin with a SEM of 15.4 µN compared to 8.7 µN in collagen. Contraction time was also

significantly different with an average of 200 ms for fibrin and 167 ms in collagen. Relaxation time on the other hand were not significantly different with 216 ms in fibrin and 241 ms in collagen. Collagen strips displayed a better cell connectivity of the tissue and most importantly superior contractility with great amplitude of developed force and faster force generation compared to fibrin based tissues. Further investigation is still required for a deeper understanding of the different matrix properties and to be able to optimise the matrix for desired tissue properties. These findings highlight the importance of the hydrogel matrix for engineered cardiac tissues used for disease-modelling and drug screening.

Funding source: Karolinska Institutet The Innovation and Technology Fund of the Hong Kong Government of the Special Administrative Region of the People's Republic of China

Keywords: Human pluripotent stem cells, Tissue engineering, Disease modelling

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CA120

DEVELOPING THE STABLE ON-CHIP VASCULAR BED SYSTEM TO CO-CULTURE A KIDNEY ORGANOID

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Current kidney organoids generated from human induced pluripotent stem cells (hiPSCs) have some structural defects. Especially, their nephron compartments, such as glomeruli and proximal tubes, are avascular and immature. These defects are caused by lack of vasculatures that are essential for supplying nutrients and oxygen. Blood flow through vasculature is also an essential factor to give mechanical stress which enhances some signaling. Recently, it was reported that kidney organoids were cultured under flow in a microfluidic device to enhance vascularization. However, this system lacks the exogenous vascular system. Thus, the in vitro co-culture system of a kidney organoid and perfusable vasculature is required to study the effects of vasculature or blood flow on kidney development. Here, we developed the "on-chip vascular bed (VB)" system which enables to co-culture a kidney organoid and vasculatures under perfusion mimicking blood flow. To fabricate VB stably, the device design and the concentration of the gel scaffold were optimized. We tried not only human umbilical vein endothelial cells (HUVECs), which are widely used to form vascular networks, but also human glomerular microvascular endothelial cells (hGMECs) for VB formation. Next, medium for the VB system was optimized. We cultured kidney organoids on a culture insert with three kinds of media; EGM2 (medium for vascular endothelial cells (VECs)), APEL2 (basal medium for a kidney organoid), and mixed medium (APEL2: EGM2 = 1:1) and evaluated the effect of these media on the differentiation of kidney organoids by immunostaning and real-time PCR. We succeeded to construct a perfusable VB stably by some optimizations and confirmed that hGMECs also had an ability to form VB. Next, we found that mixed medium induced development of kidney organoids at the similar level to APEL2. We also observed that growth factors of EGM2 enhanced the emergence of endogenous VECs. VB formed by hGMECs is expected to be more suitable for co-culture with a kidney organoid because hGMECs is a specific VECs of a kidney. Using optimized medium condition, we are trying to co-culture a kidney organoid and VB.

Funding source: AMED-MPS project Kyoto University Nano Technology Hub in "Nanotechnology Platform Project" sponsored by MEXT

Keywords: Kidney organoid, Organ on a chip, Vasculature

CA123

CIRCADIAN RHYTHM-CONTROLLING HEPATIC TISSUE CULTURE MODEL DERIVED FEOM MOUSE EMBRYONIC STEM CELLS

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Circadian rhythm influences drug effects and toxicities in animals. Hepatic culture model with circadian rhythm would be a comparable tool to the animal experiments for drug toxicity identification. The establishment of circadian rhythm in the process of embryo development has been still unclear. In this study, the gene of Shield1-dependent artificial transcription factor (DD-ZF-NLS-VP64-HA) which would bind with the upstream of Per1 gene and induce this expression, was introduced into the genome of mouse ES cells. The DD-ZF-NLS-VP64-HA gene carrying ES (ES(cr)) clones were confirmed to synchronize circadian rhythm after the Shield1 impulses. The time-dependent drug toxicity was evaluated by adding acetaminophen at two different time points with a 12 h interval after circadian rhythm synchronization. The ES(cr) cells were differentiated to hepatic tissues, consisting not only hepatocytes but also endothelial cells, and exhibited 24 h oscillating expression of clock genes Per1, Per2, Bmal1 and a drug metabolism-related gene Cyp2e1 after Shield1 impulse. The released LDH levels from the Shield1-synchronized ES(cr)-derived hepatic tissue changed in the circadian rhythm dependent manner. In conclusion, we succeeded to establish a novel culture system for circadian rhythm synchronization in mouse ES cell-derived hepatic tissues. The hepatic tissue model is expected to correspond laboratory animals with circadian rhythm. This circadian rhythm introduced culture model would be an expectable tool for drug toxicity identification in culture.

Keywords: ES cels derived-hepatic tissue model, artificial transcription factor, circadian rhythm

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

CA136

HEALTH ECONOMICAL RESEARCH FOR CELL THERAPY AGAINST ISCHEMIC STROKE

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Stroke is still a leading cause of death and disability, and despite intensive research, few treatment options exist. A recent breakthrough in cell therapy is expected to reverse the neurological sequelae of stroke. Since June 2017, we have also started the novel clinical trials, Research on advanced intervention using novel bone marrow stem cell(RAINBOW) study. It is a phase 1, open label, uncontrolled, dose response study. The primary purpose is to determine the safety of autologous BMSC product, HUNS001-01, when administered to acute ischemic stroke patients (Shichinohe H, et al. BMC Neurol. 2017;17:179). However, there are some problems to be solved before the clinical application, for examples, Ethical, Legal and Social Implications (ELSI) including Health Technology Assessment (HTA) for cell therapy. If the cost of cell therapy would be too expensive, should it be justified? National Institute for Health and Care Excellence (NICE) in UK proposed that less than £30,000 (about $40,000) per one quality-adjusted life year (QALY) would be appropriate. In our present study, we analyzed QALY using EQ-5D- 5L and the medical cost in subjects of RAINBOW study. Because we obtained the preliminary data of QALY from 7 subjects, we will report them.

Keywords: Health Technology Assessment, Stem Cell Therapy, Ischemic Stroke

EYE AND RETINA

CA142

PILOT PROOF OF CONCEPT STUDY FOR BULLOUS KERATOPATHY TREATMENT BY CORNEAL ENDOTHELIAL CELL SUBSTITUTES FROM HUMAN IPS CELLS IN A MONKEY MODEL

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In order to provide regenerative medicine for the unmet millions of patients waiting for corneal transplants globally, we have derived corneal endothelial cell substitutes from iPSCs (CECSi cells) for the treatment of corneal endothelial dysfunction (bullous keratopathy). We have improved the protocol to produce CECSi from iPS cells directly, omitting neural crest development, and performed a pilot proof of concept (POC) study using a monkey bullous keratopathy model. Hexagonal semi-confluent monolayer CECSi cells with Na,K-ATPase alpha 1 subunit (ATP1A1), ZO-1, N-cadherin, and PITX2 expression were produced from two iPS cell clones (Ff-I01s04, Ff-MH09s01). Ff-MH09s01-derived-CECSi cells were cryopreserved. One month after cryopreservation, CECSi cells were thawed, and transplanted on the posterior surface of crab-eating monkey corneas; host corneal endothelial cells were scraped, and 8 x 10^5 CECSi cells / eye were injected into the anterior chamber of two monkeys. After injection, monkeys were kept in a face down position for 3 hours so that cells attached on posterior surface of cornea. In the negative control group, host corneal endothelial cells were simply scraped without cell-injection (N=3). Seven days after transplantation, mean central corneal thickness of CECSi transplanted eyes was 840 µm, whereas that of negative control group was above measurement limit (>1200 µm), which suggested that CECSi cells functioned to recover corneal edema. In conclusion, CECSi cells injection therapy may be promising in the treatment of bullous keratopathy. Further repeatability is required to confirm POC.

Funding source: Joint research expenses from Cellusion Inc. Grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.

Keywords: iPS cells, Corneal transplant, Bullous keratopathy

CA247

A TISSUE ENGINEERED PRODUCT CONSISTING IN RPE DERIVED FROM HUMAN EMBRYONIC STEM CELLS DISPOSED ON HUMAN AMNIOTIC MEMBRANE ANIMAL MODELS FOR RETINITIS PIGMENTOSA CLINICAL TRIAL

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In developed countries, retinal degenerative diseases affecting Retinal Pigmented Epithelium (RPE), including Age-related Macular Dystrophy and inherited retinal diseases such as Retinitis Pigmentosa (RP), are the predominant causes of human blindness worldwide. Despite the scientific advances achieved in the last years, there is no cure for such diseases. In this context, we have developed a cell therapy medicinal product based on our expertise in tissue engineering and in the manipulation of pluripotent stem cells. This novel tissue engineered product (TEP) consists in RPE cells derived from clinical grade human embryonic stem cells disposed on a biocompatible substrate allowing the formation of a 3D functional sheet, suitable for transplantation. After functional validation in a rodent model of RP (Ben M'Barek et al., 2017), our purpose was to test the safety of the surgery and local tolerance in non-human primates (NHP). A specific device was developed in order to (i) embed the TEP in gelatin, (ii) allow its transport in a specific medium and (iii) cut the transplant at the right format. Non-human primates (NHP, n=6) were transplanted in one eye (right eye) with the TEP in the macular region. Left eye was left untreated. Retinal integrity and functionality were assessed at different time points (week 1, 2, 4, 6 and 7) through Eye fundus, Optical-coherence tomography (OCT) and electroretinography (ERG). Inflammation was also assessed using slit lamp. At the end of the experimental period, histological analysis was performed to evaluate the correct location and integration of the TEP within the host retina. We successfully developed a device allowing us to prepare and implant the TEP in the subretinal space of six NHP. We showed that ERG responses were not modified by the surgery and that it did not cause any long lasting inflammation. Moreover, transplanted cells were integrated in the retina and were able to phagocyte photoreceptor debris. We have shown in NHP that our surgical method of implantation was safe and did not provoke any local inflammation or retinal deterioration. Morphologic and histologic studies indicated that RPE cells were integrated into the host retina and were able to interact with photoreceptors. These results allowed us the start of clinical trial in sept 2019 (NCT NCT03963154).

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Keywords: retina, Cell therapy, preclinical

CA260

IDENTIFICATION OF AGE-RELATED MACULAR DEGENERATION RISK ALLELES IN CLINICALLY RELEVANT HUMAN EMBRYONIC STEM CELL LINES

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Age related macular degeneration (AMD) is the leading cause of blindness in the developed world. The disease is characterised by degeneration of the central macular region of the retina, due in part to damage to the retinal pigment epithelium (RPE). There are currently no clinical treatments that can restore sight or prevent vision loss in AMD patients, however a number of recent clinical trials, transplanting human embryonic stem cell (HESC)-derived RPE, offer new hope for patients. Increased risk for AMD is associated with advancing age, and environment and genetic risk factors. A number of AMD susceptibility genes have been identified in target gene and genome wide association studies, including ARMS2/HTRA1, APOE and members of the complement system (CFH, CFI, C2 and C3). HESC-derived RPE may provide healthy replacement cells, not affected by ageing and environmental risks, but little is known about the genetic AMD background of HESC prepared for clinical use.

Here, we have sequenced 17 AMD risk alleles in clinical grade HESC lines available from the UK Stem Cell Bank. We have also assessed risk alleles in two HESC lines currently being tested in HESC-RPE cell therapy trials for AMD (Shef1.3 - London Project to Cure Blindness and WA09(H9) - California Project to Cure Blindness). We found that all HESC lines tested contained at least one risk allele for AMD, and various combinations of alleles associated with increased/decreased predisposition to AMD. Genetic risk scores, calculated for each line, ranged from 0.59-0.84, suggesting that all HESC lines examined have genetic backgrounds associated with increased risk of AMD.

The implications of using therapies derived from stem cell lines with a genetic predisposition towards AMD is not known. The transplantation of HESC-RPE with genetic risk of AMD into an already compromised disease environment may affect donor cell viability, function and efficacy. Understanding the effects of individual genetic variations on stem cell-derived RPE should inform future research and help optimise effective therapies for AMD.

Funding source: Macular Society
The Michael Uren Foundation

Keywords: Age-related macular degeneration (AMD), genetic risk, HESC-RPE

HEMATOPOIETIC SYSTEM

CA154

DISSECTING EX VIVO HUMAN HSC EXPANSION BY SINGLE CELL RNA SEQUENCING AND CLONAL TRACKING IN XENOGRAFTS

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Hematopoietic stem cell (HSC) expansion remains an unmet goal for ex vivo gene therapy/editing. New assays are needed to reliably identify HSC in culture. We combined surface marker-based HSC enrichment (CD34+CD38-) from mobilized peripheral blood (mPB, n=3 donors) and CFSE marking before culture with serial monitoring of CD34+CD90+CD45RA- markers during culture to quantify the number of phenotypic HSC (phHSC) in the presence of different expansion compounds. On day 7 of culture, the median fold increase [range] of phHSC over the no compound condition was 3.6 [3.0-4.2] for UM171, 1.5 [1.2-2.7] for SR1 and 4.4 [2.9-5.0] for UM171+SR1, suggesting little benefit of adding SR1 to a 7 day expansion protocol. In this time-window, the majority (70-90%) of phHSC underwent 1 or 2 cell divisions. Single cell RNA sequencing (scRNAseq) confirmed the retention of stem cell signatures in phHSC, while CD90neg progenitors exhibited a skewing towards megakaryocytic and erythroid differentiation. Next, we performed scRNAseq on the progeny of CFSE-marked mPB CD34+CD38-CD90+CD45RA-CD49f+ cells, sorted after 7 days of UM171 culture into a quiescent/slowly proliferating (0 or 1 divisions) or highly proliferating fraction (2 divisions or more). The former homogeneously retained expression of stem cell signatures, while the latter upregulated lineage-defining markers, suggesting asymmetric divisions/differentiation and supporting the rationale for dynamic pHSC monitoring during culture. Last, we performed primary and secondary xenotransplantation of lentivirally-transduced mPB CD34+ cells, either directly after 36hr of transduction or following 7 days of ex vivo expansion with UM171. At saturating engraftment levels, mice showed a trend for higher multi-lineage engraftment in the UM171-expanded group. Lentiviral integration site (IS) analysis showed highly polyclonal grafts both in the expanded and non-expanded groups in primary (622 and 680 unique IS/mouse, respectively) and secondary recipients (92 and 85 unique IS/mouse), with similar abundance of individual clones, suggesting that ex vivo expansion did not lead to clonal selection. Remarkably, 4% of IS (vs. 0.07% in the non-expanded group) were shared between different primary mice providing robust molecular proof for symmetrical HSC division in vitro.

Keywords: HSC expansion for gene therapy, clonal tracking of HSPC in xenografts, single cell RNA sequencing

IMMUNE SYSTEM

CA160

DUAL-ANTIGEN TARGETED IPSC-DERIVED CHIMERIC ANTIGEN RECEPTOR-T CELL THERAPY FOR REFRACTORY LYMPHOMAS

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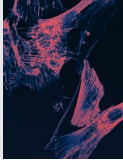
Chimeric antigen receptors (CAR) enhance effector T cell function in the tumor microenvironment. Clinical trials of T cells engineered to express CD19-directed CAR for relapsed and refractory acute B-lymphoblastic leukemia found ~90% remission rates. On longer observation, relapses nonetheless occurred in ~50% of patients who achieved remission, because of T cell exhaustion and tumor antigen escape. To address tumor escape, we generated dual-antigen receptor T cells from iPSCs. Extranodal NK/T-cell lymphoma, nasal type (ENKL), invariably expresses LMP1 and LMP2, which are good targets for CAR on the cell surface and for T cell receptor in association with HLA class I. Antigen-specific cytotoxic T lymphocytes (CTL) are functionally rejuvenated (rejT) by iPSC technology (Nishimura et al., 2013, Ando et al., 2015) and rejT can survive long-term in vivo as young memory T cells (Ando et al., 2019). To target both LMP1 and LMP2, we generated iPSC-derived dual-antigen receptor bearing CTL, LMP1-CAR/LMP2-rejT (LMP1/2rejT). LMP1/2rejT have 100% LMP1-CAR transgene expression, wtih 99% of native TCR recognizing LMP2 antigen. Cytotoxicity of LMP1/2rejT against ENKL was stronger than that of LMP1-CART with specific lysis in 51Cr release assays (86.1±5.9% vs 48.2±13.6% at an E:T ratio of 5:1). To evaluate the antitumor effects of LMP1/2rejT against ENKL in vivo, NOG mice were engrafted intraperitoneally with an ENKL cell line and tumor growth was monitored using IVIS Imaging System. After 4d, mice were treated with 3 once-weekly doses of LMP1/2rejT, LMP1-CART, or LMP2-rejT. All LMP1/2rejT treated mice survived >100d, with a significant survival advantage. To evaluate whether LMP1/2rejT actually persisted in vivo and were



still effective, we re-inoculated ENKL cells into mice that survived >110d after the first treatment. LMP1/2rejT rejected re-inoculated lymphoma cells, demonstrating that LMP1/2rejT persisted long-term in the treated mice. LMP1/2rejT may solve the issue of tumor escape. Our work constitutes proof-of-concept for this promising novel refractory-lymphoma therapy.

Funding source: These studies were supported by a grant from JSPS/KAKENHI (Grant Number16K09842 and 19K07781)

Keywords: Dual-antigen targeted CAR, iPSC-derived CAR, refractory lymphoma

MUSCULOSKELETAL

CA164

COLLAGEN VI SUPPLEMENTATION IMPROVES PATHOGENESIS OF ULLRICH CONGENITAL MUSCULAR DYSTROPHY BY MESENCHYMAL STROMAL CELL TRANSPLANTATION

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Mesenchymal stromal cells (MSCs) are multipotent progenitors for osteogenic, chondrogenic and adipogenic lineages and found in skeletal muscles. Recent studies reported that although MSCs normally function as supportive cells on skeletal muscle homeostasis through several secretory factors including collagen VI (COL6), they also respond to the fibrosis and fatty degeneration seen in diseased muscles. Moreover, mutations of Col6 genes cause Ullrich congenital muscular dystrophy (UCMD). UCMD patients show various phenotypes including muscle atrophy, ectopic fat deposition and muscle weakness. To investigate whether COL6 supplementation has a therapeutic effect for UCMD, we conducted in vivo and in vitro experiments using several types of MSCs: primary MSCs and MSCs derived from induced pluripotent stem cell (iMSCs) from healthy donors, COL6 knockout iMSCs (COL6KO-iMSCs), and UCMD patient derived-iMSCs (UCMD-iMSCs), which were COL6-deficient. All of these MSCs could engraft for at least 24 weeks when transplanted into the tibialis anterior muscles of immunodeficient UCMD model (COL6KO/NSG) mice. COL6 protein was restored by the MSC transplantation over a large region if the iMSCs were not COL6-deficient. Moreover, the phenotypes of COL6KO/NSG mice were ameliorated with the transplantation of the COL6-producing MSCs, and COL6 was restored in the limbs. Notably, myoblasts were activated and enhanced the myogenic regeneration, and myoblast fusion was enhanced and promoted the maturation of the regenerating myofibers. However, this was not the case with COL6-deficient iMSC transplantation. Co-culture experiments of skeletal muscle satellite cells derived from the COL6KO/NSG mice showed COL6-producing MSCs from healthy donors but not COL6KO-iMSCs improved the proliferation, differentiation and maturation of COL6KO-MuSCs. In summary, we demonstrated that COL6 supplementation ameliorated UCMD phenotypes. These results will help elucidate the pathology of UCMD and establish treatments in the future.

Keywords: induced pluripotent stem cells, mesenchymal stem/stromal cells, muscular dystrophy

NEURAL

CA166

INVOLVEMENT OF PDGF-BB AND IGF-1 IN ACTIVATION OF HUMAN SCHWANN CELLS BY PLATELET-RICH PLASMA

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Platelet-rich plasma contains high concentrations of growth factors that stimulate proliferation and migration of various cell types. Earlier experiments demonstrated that local platelet-rich plasma administration activates Schwann cells to improve axonal regeneration at a transected peripheral nerve lesion. However, the optimal concentration of human platelet-rich plasma for activation of human Schwann cells has not been determined, and mechanisms by which platelet-rich plasma activates Schwann cells remain to be clarified. Human Schwann cells were cultured with various concentrations of platelet-rich plasma in 5% fetal bovine serum/Dulbecco's Modified Eagle Medium. Cell viability, microchemotaxis, flow cytometry, and quantitative real-time polymerase chain reaction assays were performed to assess proliferation, migration, cell cycle, and neurotrophic factor expression of the human Schwann cells, respectively. Human Schwann cells were co-cultured with neuronal cells to assess their capacity to induce neurite extension. Neutralizing antibodies for platelet-derived growth factor-BB (PDGF-BB) and insulin-like growth factor-1 (IGF-1) were added to the culture to estimate contribution of these cytokines to human Schwann cell stimulation by platelet-rich plasma. An addition of platelet-

rich plasma at 5% strongly elevated proliferation, migration, and neurotrophic factor production of human Schwann cells. Both PDGF-BB and IGF-1 may be involved in mitogenic effect of platelet-rich plasma on human Schwann cells, and PDGF-BB may also play an important role in the migration-inducing effect of platelet-rich plasma. Neutralization of both PDGF-BB and IGF-1 cancelled the promoting effect of platelet-rich plasma on neurite-inducing activity of human Schwann cells. This study may suggest the optimal concentration of platelet-rich plasma for human Schwann cell stimulation and potential mechanisms underlying the activation of human Schwann cells by platelet-rich plasma, which may be quite useful for platelet-rich plasma therapy for peripheral nerve regeneration.

Keywords: SCHWANN CELLS, PLATELET-RICH PLASMA, PDGF-BB

CA171

DERIVATION OF ENTERIC NEURAL PROGENITORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Dysfunction of enteric nervous system (ENS) leads to severe motility disorder of gastrointestinal tract including rare diseases such as Hirschsprung's disease (HSCR). Regenerative medicine-based drug discovery and cell therapy are expected to be the next generation therapy for HSCR. Here, we introduce our novel culture condition that enables efficient induction and expansion of enteric neural progenitors (ENPs) from hiPSCs via neural crest lineage. Induced ENPs expressed key progenitor genes SOX10 and PHOX2B and showed high differentiation potential into subtypes of enteric neurons and glial cells in vitro. Expanded ENPs could engraft into intestinal muscle layer of immuno-deficient mice after transplantation and showed partial differentiation into neural and glial lineage. These characteristics of ENPs are suitable for both drug discovery platform and cell therapy application.

Keywords: Enteric nervous system, Neural crest cells, Hirschsprung's disease

CA172

APPLICATION OF SELECTIVE CYTOTOXIC VIRAL VECTOR FOR CONCENTRATION OF UNDIFFERENTIATED CELLS IN NEURAL PROGENITOR CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Cell-processed therapeutic products (CTPs) derived from human induced pluripotent stem cells (hiPSCs) have innovative applications in the regenerative therapy. However, since undifferentiated hiPSCs possess tumorigenic potential, there is a potential risk of tumor formation if the CTPs contain residual undifferentiated hiPSCs. The detection limit of the methods currently available for the residual hiPSCs is 1/105 (0.001%, undifferentiated hiPSCs/differentiated cells) or more, which could be insufficient for the detection of residual hiPSCs when CTPs contain more than 1 x 105 cells. To overcome this limit, we have previously constructed adenovirus (Ad) vectors expressing a suicide gene, iCaspase9, regulated by the CMV promoter, which is dormant in hiPSCs, for the selective expression of iCaspase9 in differentiated cells. The Ad vector possessed strong cytotoxicity to immortalized human neural progenitor cell (hNPC) but not to hiPSCs, which made possible the detection of a trace amount of hiPSCs in immortalized hNPC (0.0001%). In this study, we investigated whether the Ad vector concentrate residual undifferentiated cells in hiPSC-derived NPCs. The NPC differentiation of hiPSCs was confirmed by immunostaining for PAX-6 and TRA-1-60, a marker of NPC and hiPSC, respectively. Most of the hiPSC-derived NPCs showed PAX-6 positive and TRA-1-60 negative. Then, we examined whether the Ad vector possessed cytotoxicity to the hiPSC-derived NPCs and found that the Ad vector eliminated approximately 90% of the hiPSC-derived NPCs. Next, to identify cell characteristics in surviving cells after transduction with the Ad vector, we determined the expression of markers of NPC and hiPSC. The results revealed that PAX-6 positive cells in hiPSC-derived NPCs was decreased

only when induction of apoptosis by the Ad vector. In contrast, TRA-1-60 positive cells in hiPSC-derived NPCs was increased only when induction of apoptosis by the Ad vector. Therefore, we confirmed that the Ad vector selectively eliminated PAX-6 positive and TRA-1-60 negative cells, resulting in an increased proportion of undifferentiated cells in hiPSC-derived NPCs. This study suggested that the selective cytotoxic Ad vector is useful tool for decreasing the detection limits for residual undifferentiated cells in hiPSCs-derived CTPs such as hNPCs.

Keywords: neural progenitor cell, induced pluripotent stem cell, tumorigenicity

CA173

CHARACTERIZATION OF HUMAN IPSC-DERIVED ENGINEERED GUT WITH NEURAL CREST CELL-DERIVED ENTERIC NEURONS

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Enteric nervous system (ENS) is injured by several stresses and congenital disorders which lead to hypoplasia of ENS and causes severe gastrointestinal dysmotility. However, there are little platforms for research of human gut motility. Our group has developed the method for xeno-free differentiation of neural crest cells (NCC) from human iPS cells. By using the technology, we aim to establish human iPSC-derived engineered gut (iGut) with ENS from iPSC-derived NCC and evaluate the similarity to human gut. Vagal NCC differentiated into enteric neurons expressing markers for enteric neural subtypes in vitro. To establish engineered gut with ENS, human intestinal organoid (HIO) was combined with vagal NCC in vitro and transplanted into immunodeficient mice. In vivo matured engineered gut has small intestine-like structure with differentiated epitheliums and smooth muscle layers. Combined vagal NCC differentiated into enteric neural subtypes and glial cells between smooth muscle layers of iGut. Functional assay with organ bath assay system revealed that iGut was response to cholinergic stimulation, suggesting that it had matured smooth muscles. iGut showed electrical field stimulation (EFS)-regulated motility action and neurotransmitter-blockers inhibited the EFS-induced movement. These results indicate that NCC combined with HIO differentiated into functional enteric neurons and regulated gut motility.

Keywords: Engineered gut, Enteric nerve system, Neural crest cells

CA175

AXONAL EXTENSION ALONG CORTICOSPINAL TRACT FROM TRANSPLANTED HUMAN CEREBRAL ORGANOID

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Reconstruction of lost neural circuits by cell replacement is a possible treatment for neurological deficits after stroke and brain injury. Cerebral organoid derived from pluripotent stem cells is attracting attention as a novel cell source for the transplantation because cerebral organoids can generate three-dimensional structures of the cerebral cortex. Recent studies reported that the transplantation of human embryonic stem cell (hESC)-derived cerebral organoids into mouse cerebral cortex provided graft survival with vascularization and integration to the host brain. However, it remains unclear which developmental stage of the organoid is optimal for the transplantation. Additionally, it is unclear whether cerebral organoids provide axonal extension along the corticospinal tract (CST) of the host. To clarify these problems, we induced cerebral organoids from hESCs using SFEBq (Serum-free Floating culture of Embryoid Body-like aggregates with quick reaggregation) method. We transplanted the cerebral organoids at early and late developmental stages; the organoids at 6 or 10 weeks after differentiation (6w- or 10w-organoids) were transplanted into mouse cerebral cortex. Both 6- and 10w-organoids survived with vascularization and extended axons to the host brain. 6w-organoids extended more axons along the host CST but caused graft overgrowth with higher proportions of proliferative cells. Axonal growth along the host CST from 10w-organoids was smaller in number but could be enhanced by the host brain environment. Furthermore, we transplanted 10w-organoids into monkey motor cortex, which provided axonal extension without graft overgrowth. Our study is an important step for the reconstruction of motor pathways after stroke and brain injury.

Keywords: cerebral organoid, cell transplantation, corticospinal tract

CA177

DETECTION OF IMMUNE REACTION AFTER IPSC-DERIVED DOPAMINE NEURAL TRANSPLANTATION IN BRAIN

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Although the immune response in the central nervous system including the brain is less than in other organs, an immune response can still occur after cell transplantation. A physician-initiated clinical trial of cell therapy using induced pluripotent stem cells for Parkinson's disease has started in Japan. In the trial, positron emission tomography (PET) using a GE180 probe that responds to activated microglia and astroglia will be performed to detect graft-induced immune responses. However, PET examination is too expensive for wide practical use. Other methods are therefore needed to detect immune reactions for the purpose of prediction and control. Thus, cell transplantation into cynomolgus monkey brains was conducted. Blood samples from the monkeys after transplantation were collected periodically, and finally the animal brains were examined histologically. Using these blood samples, the detection of donor specific antibodies (DSAs) in serum and mixed lymphocyte reaction (MLR) tests with peripheral mononuclear blood cells (PBMCs) were performed. DSAs were detected in some of the samples after transplantation. The PBMCs from some animals showed positive reactions for the MLR test with PBMCs from donor animals. However, the MLR could not be induced with donor neurons. These results were compared with the results of PET imaging and histological examination. In the future, we will compare various human specimens and PET results in clinical trials to develop new methods for detecting immune reactions in the clinic setting.

Funding source: This study was supported by a grant from the Network Program for Realization of Regenerative Medicine from the Japan Agency for Medical Research and Development (AMED).

Keywords: induced pluripotent stem cell, transplantation, neuron

CA178

THE MECHANISM STUDY ON LINGO-1 KNOCK OUT PROMOTING THE CELL SURVIVAL IN A NEURAL STEM CELL-DERIVED NEURAL NETWORK SCAFFOLD TRANSPLANTED INTO INJURIED SPINAL CORD IN RODENTS

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Spinal cord injury (SCI) can cause the motorial and sensory dysfunction. The survival of cells is crucial for function recovery after transplanted into the injury site of spinal cord in rodent animals. Leucine rich repeat and immunoglobin-like domain-containing protein 1 (LINGO-1) expression is upregulated after SCI and it negatively regulates the survival, differentiation, axon growth and myelin formation of grafted neural stem cell-derived neurons and oligodendrocytes. The present study aimed to improve the cell survival of transplanted neural nerwork scaffold and to investigate related mechanism mainly through LINGO-1 reduction. LINGO-1 was knocked down (KD) in the neural stem cells (NSCs) overexpressing TrkC (a neurotrophin-3 receptor). Then these NSCs modified genetically were planted into neurotrophin-3 (NT-3)/fibroin coated gelatin sponge scaffold (NF-GS), constructing a neural network scaffold after culturing 14 days in vitro. Whereafter, the neural network scaffold was transplanted to the transected spinal cords of rodent animals (mouse and rat), and then the animals have survived for 8 weeks. The mouse is a LINGO-1 knock out (KO) model animal in the LINGO-1 KO group. The results showed that the cells in the grafted neural network scaffold survived more in the LINGO-1 KO group than that in the LINGO-1 KD and Control groups, and the Control group had the least cell survival. The recovery of motor function of paralysis hindlimb was positive correlation with the increase of cell survival in grafted neural network scaffold. Additionally, LINGO-1 binded to TrkC in the NSC-derived cells and inhibited the phosphorylation of TrkC, leading to obvious blocking of the PI3K/Akt pathway and ultimately causing donor cell apoptosis. The results demonstrate that activating PI3K/Akt pathway though NT-3 and TrkC interaction under the LINGO-1 knock out can promote the cell survival in grafted neural network scaffold through after SCI.

Funding source: National Natural Science Foundation of China (No. 81674064; 81891003) and The National Key R&D Program of China (2017YFA0104702) to Y.S. Zeng.

Keywords: Spinal cord injury, Neural stem cells, LINGO-1

CA185

AN EFFICIENT METHOD FOR DETECTING TRACES OF UNDIFFERENTIATED HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS) AMONG DIFFERENTIATED NEURAL STEM/ PROGENITOR CELLS DERIVED FROM HIPSCS

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Human induced pluripotent stem cell (hiPSC)-derived neural stem/progenitor cells (hiPSC-NSPCs) are promising sources of cells for regenerative therapies, such as those for the treatment of spinal cord injury and/or stroke. In cell-transplantation therapy, tumorigenicity of the grafted cells is a serious concern. Especially, the use of hiPSC-derived products may cause teratoma formation due to the presence of residual undifferentiated hiPSCs in hiPSC-derived cell products. Although teratomas with tissues belonging to the three germ layers is the best evidence of the pluripotency of hiPSCs, their development is not favorable in patients undergoing regenerative therapy. Therefore, the presence of hiPSCs in cell products should be assessed before transplantation. Since the clinical treatment of spinal cord injury and/or stroke requires the transplantation of millions of hiPSC-NSPCs, contamination of the population with even 0.1% hiPSCs may lead to the simultaneous transplantation of thousands of hiPSCs, thus rendering the procedure untenably dangerous. Moreover, the minimum number of hiPSCs sufficient for producing a tumor is reported to be 10 for a hiPSC line (201B7) in Matrigel after subcutaneous injection in NOG mice. Therefore, the number of residual hiPSCs must be below 0.001% (10/106) in a hiPSC-NSPC population, which can be determined by assays with a lower limit of detection (LLOD) of 0.0001%. This study aimed to detect such contamination of hiPSC-derived cell products based on an immunocytochemical detection method applicable to high-content screening system for analyzing millions of cells simultaneously. The method was quite simple, with the LLOD being below 0.0001%, comparable to that of the method for returning a cell product to hiPSC culture condition, and much more sensitive than quantitative reverse transcription-PCR (LLOD > 0.1%) or flow cytometry (LLOD > 0.001%). Our findings provide a feasible strategy for detecting residual hiPSCs, especially in a hiPSC-NSPC population, thereby avoiding the risk of teratoma formation during transplantation.

Keywords: Human induced pluripotent stem cell, Neural stem/progenitor cell, Tumorigenicity

CA189

DEVELOPMENT OF HIGHLY EFFICIENT DIFFERENTIATION METHODS FOR THE SPECIFIC BRAIN REGIONS USING RIGHT HUMAN IPS CELLS

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Human pluripotent stem cells, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), are a promising tool for not only regenerative medicine but also drug discovery, toxicity study, and disease modeling. To utilize them in various fields, we need to understand human early development (embryology) and properly differentiate them to a target cell type. We have investigated development of iPSCs with high differentiation ability (T-iPSCs) produced with Yamanaka factors and TET1, which is involved in epigenetic regulations. We selected this type of iPSCs based on the ability of “default differentiation”, which is a differentiation program evolutionally-preserved in vertebrate embryonic cells. When we differentiated T-iPSCs to a specific neuronal cell type, ventral midbrain dopaminergic neurons in a previous method, they had as high differentiation ability as that of ESCs. After carefully reviewing previous neuronal differentiation protocols, we modified them to induce T-iPSCs to target brain regions. We were able to induce them to specific regions in the forebrain, midbrain and spinal cord more efficiently than previously reported. In the future, the region-specific neuronal or glial progenitor cells induced using T-iPSCs with the modified methods will help perform reliable drug discovery and toxicity study.

Funding source: This research was supported by AMED under Grant Number JP19bk0104090h0001.

Keywords: Neuronal differentiation, Specific brain region, Cell source for toxicity study

CA249

MOUSE PHOTOTHROMBOTIC CEREBRAL INFARCTION MODEL FOR CELL TRANSPLANTAION

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Recent developments in organoid technology have made it possible to create cerebral tissue from pluripotent stem cells. These tissues are a promising source of cells for neural regeneration therapy after brain injury or stroke, but there are still no optimal animal models for regenerative therapy. Here we have developed a photothrombotic model using rose bengal for cerebral infarction, which is expected to be the next target of regenerative therapy, as an animal model for cell transplantation. In this study, we administered 100 mg/kg rose bengal intraperitoneally, and after 5 minutes, illuminated 2.5 × 3.0 mm region on the skull for 15 minutes. MRI confirmed cerebral infarction on the cerebral cortex, and the mise showed fine motor disability, which was assessed with a foot fault test. We tested the transplantation one week after the model was created. This model is simple and reproducible and is useful as a platform for the evaluation of cerebral tissue derived from pluripotent stem cells.

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

Keywords: Cerebral infarction, Cell therapy, Transplantation

NEW TECHNOLOGIES

CA205

C-STEM, A NEW TECHNOLOGY TO SCALE UP CELL THERAPY PRODUCTION

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Most stem cells, including Human pluripotent stem cells (hPSCs) are adherent cells. The historical standard of culture is in 2D in Petri dishes. This technology allowed for the first trials in cell therapy but is limited by: i) reproducibility issues, ii) scalability, iii) high cost due to labor intensive manipulations, and iv) genomic instability. For cell therapy applications, cell quality reflects the culture process and large quantities of cells are required for allogenic strategies. 2D automation enables reproducible clinical batch production but commercial batch sizes will require further scale up. Suspension culture in bioreactors is a straightforward choice for scale up with two main techniques: i) microcarriers, where the cells grow attached to their surface or ii) spheroids, where cells grow in 3D aggregates. In both culture conditions the cells' exposure to shear stress in bioreactors limits further scale up. The C-Stem™ technology addresses this challenge by encapsulating cells within hollow alginate hydrogel capsules, 250 µm in diameter with 30 µm thick walls. Capsules both protect cells form shear stress and let small molecules (smaller than 150 kDa), gas, nutrients and waste in and out. Inside the capsule, hPSCs are seeded in extracellular matrix to mimic in vivo conditions. The capsules are produced at high throughput (5000 per second) with proprietary microfluidics. The C-Stem™ technology allows for 100x fold expansion per week of hPSCs and their differentiation in traditional bioreactors. At the PSC stage, C-Stem™ significantly improves cell viability as compared to standard 2D PSC culture. C-Stem™ promotes pluripotency, yields biomimetic hPSC "epiblastoids" and maintains state of the art genomic integrity. The process is fully integrated from seeding to harvest, scalable, single use, closed and aligned with cGMP requirements. Most 2D and 3D protocols can be transposed in C-Stem™. Preclinical results will be presented for Parkinson's disease cell therapy. Fully mature dopaminergic neuronal microtissues are differentiated from hPSCs within capsules. They are then engrafted in Parkinson's disease rat model striata. This results in behavioral correction of rats within 6 weeks of surgery, thereby reducing by a factor 2-3 the recovery time as compared to standard progenitor engraftment.

Funding source: TreeFrog Therapeutics, IDEX Université de Bordeaux, CNRS, ANR, i-LAB, i-NOV, BPIfrance, Région Nouvelle Aquitaine, European Union (Horizon 2020 research and innovation program through the SME Instrument Phase 2)

Keywords: 3D stem cell culture, hiPSC mass production, Cell therapy scale-up

CA209

SINGLE-CELL CLONING OF INDUCED PLURIPOTENT STEM CELLS USING CELLCELECTOR INSTRUMENT AND NANOWELL PLATES

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Continuing progress in the generation and genetic manipulation of human induced pluripotent stem cells (iPSCs) is bringing them closer to the clinical application. One of the challenges in the manufacturing of clinical-grade iPSCs is single-cell cloning, the step relevant to the standardization and automation of iPSCs culture protocols, and essential in selecting cells with desired mutations of gene-edited cells. Here we report the results of our proof-of-concept study utilizing CellCelector, high-throughput nanowell-based image-verified single cell cloning platform (HT-NIC). The system consists of automated single-cell picking instrument, image analysis software, and nanowell plates, standard format culture plates containing 100,000 nanowells per plate. The nanowell plates facilitate isolation and identification of single cells, verification of monoclonality, assessment of the clones' outgrowth, and fast transfer of proliferating clones. Although the use of the system for single-cell cloning has been already reported, here we attempted to resolve challenges related to the requirement of iPSCs for the culture substrate and develop the protocol of cell dissociation suitable for the CellCelector system.

Keywords: single cell cloning, pluripotent stem cell, automation

CA211

EFFICIENT METHOD FOR DECELLULARIZATION AND RECELLULARIZATION OF TRACHEAL CARTILAGE

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The trachea is a hollow organ that connects the larynx to the lungs, allowing the channel of air. The common defects associated with trachea are tumor ablation, congenital anomalies and traumatic injuries and their treatment is fully dependent on the extent and size of the defect. The walls of trachea are highly rigid, so simple procedures like primary closure could only be used for small and partially defected tracheas. There is a boon in grafting technologies to overcome unmet clinical needs. However, there is a high risk of immune rejections, therefore autografts and allografts are limited and many constraints are faced while using synthetic materials. One such approach is to use the native trachea as scaffold for transplantation after decellularization, due to its non-immunological extracellular matrix (ECM). The decellularized trachea provides proper mechanical support and aid in stem cell differentiation and regeneration. In present study, rat trachea was decellularized using both enzymatic and non-enzymatic method. Histopathology, SEM and biocompatibility tests were performed for the decellularized trachea. The decellularized trachea was recellularized using mesenchymal stem cells (MSCs). Various biochemical and mechanical tests were performed for recellularized trachea. Additional in vivo experiments will be performed, where these recellularized trachea will be placed below the skin flap of the mice and cell differentiation will be studied.

Funding source: All INDIA INSTITUTE OF MEDICAL SCIENCES

Keywords: Trachea , Mesenchymal Stem Cells, Decellularization, Recellularization, Extracellular Matrix

CA215

EFFICIENT LARGE-SCALE PURIFICATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTES BY SYNTHETIC MICRORNA SWITCH AND MAGNETIC-ACTIVATED CELL SORTING

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Using pluripotent stem cell-derived cardiomyocytes (PSC-CMs) for clinical application of cardiac transplantation, large quantities of purified PSC-CMs are required. Moreover, genetic modifications and contamination of non-CMs should be avoided. Although cell sorting with antibodies against specific cell surface proteins is one of the promising strategies, it is time-consuming and harbors potential risk of immunogenic reaction and local inflammation. We have shown that synthetic mRNAs encoding a fluorescent protein tagged with complementary sequences against specifically expressed microRNAs (miRNA-switches) can be efficiently detected and used for purification. Combining miRNA-switch and magnetic-activated cell sorting (MACS) technologies, we evaluated the efficiency of large scale purification of human induced PSC-CMs (iPSC-CMs). We used CD4 as a selection marker for MACS and miR-208a as a specific miRNA of CMs. We synthesized a CD4 mRNA and transfected it into differentiated cells from iPSCs to confirm CD4 expressing on the surface of the transfected cells. We also synthesized a miRNA switch encoding CD4 tagged with the complementary sequence against miR-208a (CD4-208a switch) and transfected it into iPSC-derived differentiated cells. This CD4-208a switch allowed the distinction of CMs from non-CMs by the expression of CD4. Non-CMs could be removed by MACS while a co-transfected puromycin resistance carrying mRNA allowed the removal of untransfected cells with puromycin. Purified cells were then transplanted into NOG mouse hearts with myocardial infarction by direct injections into the myocardium to show their successful engraftment. After transfection of the CD4 mRNA into differentiated cells from iPSCs, 78±5% expressed CD4 on the cell surface. We also confirmed that the CD4-208a switch separated CMs and non-CMs as expected. Combination of MACS and puromycin selection purified iPSC-CMs from 69±5% to 97±2% as assessed by troponin T staining. Purified cells were shown to be engrafted in mouse hearts. We demonstrated that the synthetic CD4-208a switch efficiently purifies iPSC-CMs in large scale, suggesting that this technology can be clinically applied for stem cell-based cell therapy.

Funding source: Fellowship Program of Development of Young Researchers from iPS Cell Research Fund, and Health Labour Sciences Research Grant of the Ministry of Health Labour and Welfare

Keywords: purification, transplantation, cardiomyocyte

CA226

BIO THREE-DIMENSIONAL CONDUIT DEVELOPED FROM HUMAN IPSC-DERIVED MESENCHYMAL STEM CELLS PROMOTES RAT PERIPHERAL NERVE REGENERATION

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At present, many artificial nerve conduits have been developed to overcome limitations of autologous nerve grafting for peripheral nerve repair. However, the efficacy remains not ideal. Recently, a completely biological tube via Bio-3D-printer-based system using human fibroblast showed supportive effect on nerve regeneration. Based on this method, in this study we employed mesenchymal stem cells induced from iPSCs (iMSCs) by a xeno-free protocol. We generated clump of iMSCs (C-iMSCs) that consisting of iMSCs and self-produced ECM to form tough and functional units. We arranged the C-iMSCs by Bio 3D Printer, and cultured the generated Bio 3D structure with perfusion to construct scaffold-free nerve conduits. 8-week following iMSC Bio3D nerve conduit transplantation into a rat model of sciatic nerve injury, kinematic analysis/ electrophysiological studies/ histological and morphological studies were performed. The results show that supporting effects on peripheral nerve regeneration of iMSC Bio3D nerve conduit are significantly superior compared with silicone control group. iMSC Bio3D nerve conduit expressed neurotrophic and angiogenic factors. Besides, neovascularization was observed both inside and on the surface of the transplanted Bio 3D conduit. We thus concluded that the Bio 3D conduit fabricated from iMSCs is an effective strategy for nerve regeneration in a rat sciatic nerve injury model. This technology will be useful for segmental nerve defects in future clinical situations.

Keywords: Nerve regeneration, iPSCs, mesenchymal stem cells

CA229

DEVELOPMENT OF INNOVATIVE ANGIOGENESIS THERAPY USING INJECTABLE CELL SCAFFOLD

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Clinical trials demonstrate the effectiveness of cell-based therapeutic angiogenesis in patients with critical limb ischemia (CLI); however, their success remains limited. Maintaining transplanted cells in the ischemic tissue are expected to augment cell-based therapeutic angiogenesis. We have demonstrated that nano-hydroxyapatite (HAp) coating on medical devices shows marked cell adhesiveness. Using this nanotechnology, we generate HAp-coated poly(l-lactic acid) (PLLA) or 75:25 poly(l-lactide-co-epsilon-caprolactone) (PLCL) microspheres, named injectable cell scaffold (ICS), as a non-biological, biodegradable and injectable scaffold for cell-based angiogenesis. Here we report the effectiveness of ICS on therapeutic angiogenesis using mouse hindlimb ischemia model. After incubation of ICS with mouse bone marrow or human peripheral blood-derived mononuclear cells (MNC) with ICS, MNC+ICS were intramuscularly injected into mouse ischemic hindlimb. When GFP-positive mouse MNC were injected, GFP level in the ischemic tissue of mouse MNC+ICS group was approximate five-fold higher than that of mouse MNC group seven days after injection. Kaplan-Meier analysis demonstrated that MNC+ICS injection markedly prevented hindlimb necrosis ($P<0.05$ vs. BMNC). Compared to mouse MNC, mouse MNC+ICS markedly induced angiogenesis in the ischemic tissue and increased collateral blood flow confirmed by three-dimensional CT angiography. Enhanced angiogenesis in mouse MNC+ICS group was correlated with increased intramuscular levels of VEGF and basic FGF. Co-implantation of mouse MNC and ICS also prevented the apoptosis of transplanted cells, resulting in prolonged cell retention. A novel and feasible ICS potentiates cell-based therapeutic angiogenesis, which could be useful for the treatment of CLI. We are now preparing an investigator-initiated clinical trial of angiogenesis therapy by transplantation of autologous peripheral blood-derived MNC with ICS for patients with CLI refractory to endovascular treatment.

Funding source: This research was supported by AMED under Grant Number JP19hk0102059.

Keywords: Chronic limb threatening ischemia, Hydroxyapatite, Injectable cell scaffold

CA235

SPECIFIC GENE CORRECTION AND DIRECT CELL REPROGRAMMING: AN INNOVATIVE TREATMENT FOR PRYMARY HYPEROXALURIA TYPE 1, A RARE METABOLIC LIVER DISORDER.

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Nowadays the only definitive curative treatment for many inheritance metabolic liver disorders is organ transplantation. For this reason, it is necessary to develop new therapeutic approaches for the treatment of these patients. This is the case of Primary Hyperoxaluria Type 1 (PH1), a rare genetic disorder caused by alanine:glyoxylate aminotransferase (AGT) deficiency, a hepatic enzyme involved in the glyoxylate metabolism. Liver cell replacement therapy with primary hepatocytes or hepatocytes-like cells derived from iPSCs would be a promising alternative. However it is limited by the difficulty to generate and expand mature functional hepatocytes. Here, we propose the combination of site-specific gene correction and direct cell reprogramming for the generation of autologous phenotypically healthy induced hepatocytes (iHeps) from PH1 patient skin-derived fibroblast. Following this strategy it would be possible to obtain high amounts of corrected hepatocyte-like cells, avoiding the potentially tumorigenic steps of iPSC generation. We have already obtained AGXT gene corrected cells using two different CRISPR/Cas9 based strategies. First, accurate specific point mutation correction (c.853T-C) has been achieved by homology-directed repair (HDR) with ssODN harboring the wild-type sequence. In the second strategy, an enhanced version of AGXT cDNA has been inserted near the transcription start codon of the endogenous gene, constituting an almost universal correction strategy for PH1 mutations. Direct reprogramming of fibroblasts to iHeps is being conducted by overexpression of hepatic transcription factors and in vitro culture in defined media. Massive gene expression analysis of healthy iHeps has demonstrated their hepatic identity. PH1 patient fibroblasts and the gene corrected counterparts have also been reprogrammed to iHeps, showing similar general hepatic characteristics as healthy iHeps. These patient derived iHeps would be useful for PH1 disease modeling. Demonstration of their in vivo functionality in transplanted in NSG mice is ongoing. The development of these advanced therapies set up an alternative cellular source to replace endogenous deficient hepatocytes with autologous functional corrected cells for genetic liver disorders.

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Keywords: Liver inherited disease, Gene editing, Direct cell reprogramming

CA252

SCALING UP HUMAN INDUCED PLURIPOTENT STEM CELL CULTURE USING FUNCTIONALISED ALGINATE MICROCARRIERS IN SUSPENSION BIOREACTORS

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In its first project phase, the European Bank for induced pluripotent Stem Cells (EBiSC1) developed protocols for scaling up production of human induced pluripotent stem cells (hiPSCs), with a second project phase (EBiSC2) aiming towards long-term self-sustainability of the repository. To support the banking of the >800 well-characterised, high quality hiPSC lines and their derivatives in the EBiSC catalogue, a multi-site study was designed to investigate the robustness of the established up-scaling protocols on three catalogued lines (derived from a healthy donor, a donor with cardiac disease, and a gene-edited tool line). Same batches of hiPSCs were shipped to both study sites and initially thawed on Matrigel-coated plates in mTeSR-1 medium. Subsequently, hiPSCs were passaged in parallel onto Matrigel-coated plates (2D) and Matrigel-coated alginate microcarriers (3D microcarrier). Inoculated microcarriers were cultured in 50 mL tubes and maintained in suspension in a bench-top 3D cell culture incubator (CERO). After two passages, cells were harvested for analysis and cryopreserved (n≥3, per condition per line). The cells were analysed with flow cytometry, immunocytochemistry, and qRT-PCR; cryopreserved cells were thawed for viability and recovery measurements. At both study sites, across all three hiPSC lines, 3D microcarrier cultures exhibited higher volumetric efficiency, resulting in higher cell harvests per unit of consumed culture medium. Flow cytometry analysis revealed that 3D microcarrier-cultured hiPSCs were consistently positive for pluripotency-associated markers and did not significantly differ from 2D-cultured hiPSCs; qRT-PCR analysis indicated no significant difference in levels of spontaneous differentiation. Hence, upscaling protocols established in EBiSC1 were successfully applied to other hiPSC lines and could be used to reproducibly expand and maintain pluripotency of hiPSCs using 2D and 3D microcarrier approaches with reduced medium and plasticware consumption.

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Keywords: cell banking, EBiSC, bioprocessing

Theme: Modeling Development and Disease

CARDIAC

MDD127

GENETICALLY ENCODED VOLTAGE AND CALCIUM INDICATORS DELIVERED AS MODIFIED MRNA ALLOW ACCURATE FUNCTIONAL ANALYSIS OF HPSC-DERIVED CARDIOMYOCYTES

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Human PSC-derived cardiomyocytes (hPSC-CMs) provide a platform to investigate the mechanisms underlying cardiac disease, as well as to perform compound screens to identify not only potential new therapeutic strategies for these diseases, but also for toxicity studies. In this regard it is important to evaluate various aspects of cardiac physiology, namely the cardiomyocyte's electrical activity, intracellular calcium transients, and contractility kinetics. These can be measured optically using organic fluorescent dyes. However, the use of such dyes comes with certain limitations. For example many of these dyes cause cytotoxicity, and cannot be used for long term measurements due to bleaching effects. As an alternative to organic dyes, we have investigated the use of genetically encoded voltage and calcium indicators (GEVIs and GECIs). We find transfection of in vitro transcribed mRNA into hPSC-CMs to be efficient with low toxicity, and a rapid way to evaluate the sensitivity of different reporters. The signal from these genetically-encoded indicators is maintained at detectable levels in the hPSC-CMs for several days, and is less prone to photobleaching. While expression of these indicators results in some effect on the baseline functional characteristics of the hPSC-CMs, it does not affect the response of the cells to known pro-arrhythmogenic compounds. Furthermore, the GEVI, ASAP2f, also faithfully reported the expected electrophysiological phenotype in an hiPSC model of LQT2. These findings demonstrate not only the suitability of genetically-encoded indicators as reporters of cardiac physiological responses, but also highlights the potential of using mRNA to transiently express transgenes in hPSC-CMs and thereby avoid toxicity issues commonly observed with other gene delivery methods.

Funding source: This work was supported by a Starting Grant from the European Research Council under the European Union's Horizon 2020 Research and Innovation programme and a VIDI fellowship from the Netherlands Organisation for Scientific Research.

Keywords: modeling cardiac arrhythmias using hiPSCs, action potential and calcium transients, voltage and calcium indicators

MDD131

HOIL-1L RETAINS DIFFERENTIATION CAPACITY IN SKELETAL MUSCLES AND CARDIOMYOCYTES ASSESSED BY C2C12 AND HUMAN IPS CELLS.

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Recently, HOIL-1L deficiency has been reported as one of the causes of myopathy and cardiomyopathy. However, mechanisms of developing myopathy and cardiomyopathy have not been revealed at all. Only the fact reported previously is amylopectinosis in skeletal muscles (SM) and cardiomyocytes (CM). Aim of this research is to elucidate the mechanisms by using a murine skeletal myoblast cell line (C2C12) and human iPS cells (hiPS). First, to reveal myopathy mechanisms, HOIL-1L knockout C2C12 (KO-C2C12) was constructed by CRISPR/Cas9 system, and myotubes were differentiated both from C2C12 and KO-C2C12. RNA sequencing was performed to verify the difference of these myotubes. Next, hiPSs were generated from controls (C-hiPS) and a HOIL-1L deficiency patient (HOIL-hiPS). HOIL-1L knockout hiPS (HOIL-KO-hiPS) were created from C-hiPS by CRISPR/Cas9 system. CMs were differentiated from all these hiPSs, and morphological difference of these CMs were evaluated. On day 5 of myotube differentiation, fusion index, MHC density and expression levels of MRF4 and MHC were significantly lower in myotubes from KO-C2C12 compared

with myotubes from C2C12. Genes associated with SM function such as TNNC2, MB and MYH4 were down-regulated by RNA sequencing. In hiPS-CMs, morphology of HOIL-hiPS derived CMs showed larger CM size and more multi-nucleation compared with C-hiPS derived CMs. Experiments using HOIL-KO-hiPS and electrophysiological experiments of CMs from all hiPS cell lines are planned before ISSCR 2020, Boston. In conclusion, immature differentiation into SMs and CMs in HOIL-1L deficiency possibly lead to myopathy and cardiomyopathy.

Keywords: cardiomyopathy, HOIL-1L, CRISPR/Cas9

MDD473

SOLUBLE FACTORS RELEASED BY DEDIFFERENTIATED FAT CELLS REDUCE THE FUNCTIONAL ACTIVITY OF IPS-DERIVED CARDIOMYOCYTES

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The interaction of adipose tissue such as epicardial adipose tissue (EAT) and cardiomyocytes is regarded as important in the pathogenesis of heart failure. It is known that changes in adipose tissue properties in obesity or diabetes, there is impairing the differentiation of preadipocytes. Furthermore, proinflammatory cytokine secretion has been shown to be higher in preadipocytes than in mature adipocytes under diabetes and obesity previously. However, it is not yet well known how undifferentiated cells committed to an adipose lineage directly influence the cardiomyocytes. We used human-derived dedifferentiated fat (DFAT) cells as undifferentiated cells committed to an adipose lineage. We performed an indirect co-culture system of DFAT cells and iPS-derived cardiomyocytes and evaluate the effects of soluble factors interactions. As a result, we revealed that the interaction is predominantly an inflammatory response in the RNA sequencing. Furthermore, proinflammatory cytokines secrete from DFAT cells reduce myocardial function, such as contraction frequency ($p<0.001$) and catecholamine sensitivity. At the same time, these pro-inflammatory cytokines increased cardiomyocytes apoptosis ($p=0.0112$), decreased antioxidative stress tolerance ($p=0.0304$),

and oxygen consumption rate. These negative effects may be attributed to the secretion of pro-inflammatory mediators MCP1, CXCL1, CXCL 12, GCSF, IL6, IL8, MIF, and PAI1-A by DFAT cells. These results were thought to be useful in understanding the pathogenesis of EAT-related heart failure in terms of the involvement of undifferentiated cells committed to an adipose lineage. Furthermore, we suggest the importance of focusing on the surrounding adipose tissue as one of the strategies for maximizing the survival and function of the transplanted stem cell-derived cardiomyocyte.

Funding source: This work was supported by JSPS KAKENHI Grant Number18K16405

Keywords: Dedifferentiated Fat (DFAT) cell, iPS derived cardiomyocytes, epicardial adipose tissue (EAT)

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

MDD157

TISSUE ENGINEERED 3D LIVER ORGANOIDS FOR DRUG TOXICITY STUDIES AND BIOMARKER DISCOVERY

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Drug-induced liver injury is worldwide leading cause of acute liver failure. Preclinical methods of determining drug induced hepatocyte toxicity include immortalized or primary 2D cultures and also animal models, which do not completely recapitulate the in vivo human response to drugs and contribute to the high failure rate upon clinical trials. Recent developments in the field of in vitro hepatotoxicity include 3D tissue constructs, bioartificial livers, co-cultures of various cell types with hepatocytes or stem cell-derived hepatic lineage-like cells and also liver organoids. In this study, we aimed to establish bioengineered 3D liver organoid tissue model using primary hepatocytes or reprogrammed cells together with non-parenchymal liver cells and use it for drug toxicity studies. Different extracellular matrices (ECMs) including fibrinogen and PEG have been evaluated in 3D hepatocyte culture in an attempt to re-establish and subsequently maintain hepatocyte cell polarity. We engineered, expressed and purified several 3D matrix-bound growth factors, morphogens (VEGF, HGF, Jagged) and ECM–mimetic peptides/protein fragments in Expi293 system and used them for creation of favorable microenvironment for the growth and differentiation of cells. Using phosphospecific antibodies and microscopy we demonstrate that engineered growth factors activate VEGFR2, c-Met tyrosine kinases and induce cell migration. 3D organoids sustained secretion of albumin and urea, cytochrome CYP3A4 activity and viability of human hepatocyte cultures for more than three weeks. Gene expression and FACS analysis demonstrated expression of key hepatocyte specific markers. Experiments are under way to incorporate flow in the culture system that has been shown to increase and maintain a higher level of urea, albumin synthesis and glucose secretion. Thus, this model provides a unique platform to rigorously evaluate drug hepatotoxicity and also to study virus-host interactions, allowing the identification and validation of novel antiviral agents. These findings also will allow a better understanding of hepatocyte development, and consequently of their role in liver repair and regeneration.

Funding source: This research was supported by the Lithuanian Research Council grant P-MIP-19-521.

Keywords: Liver organoids, growth factors, fibrinogen

MDD161

MODELLING MODY3 USING HUMAN IPSC-DERIVED ORGANOIDS

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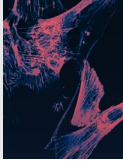
Maturity-onset diabetes of the young 3 (MODY3) is a rare type of diabetes caused by heterozygous mutations in the hepatocyte nuclear factor 1α (HNF1α). The most common HNF1α mutation in MODY3 is the insertion of a cytosine (C) in the polyC tract of codon 291(p291fsinsC), which induces an early stop codon and results in the expression of a truncated form of HNF1α protein. Strikingly, loss of one copy of HNF1α in mouse models or embryonic stem cells does not result in an impairment in beta cell function, suggesting that the truncated form has unknown functions pivotal for MODY3 development. Despite being the most common type of MODY, the molecular mechanism through which the truncated form of HNF1α causes diabetes remains elusive. In this study, we used induced pluripotent stem cells (iPSCs) derived from patients with MODY3 banked through the Human Induced Pluripotent Stem Cells Initiative (HipSci) to study the mechanism of p291fsinsC mutation in a human in vitro model. The effect of the mutation was investigated during the differentiation of iPSCs into pancreatic progenitors and beta-like cells using a novel 3D organoid system. Gene and protein



expression indicated that iPSCs derived from MODY3 patients show impaired pancreas progenitor formation and beta cell differentiation. Exome sequencing analysis of patient MODY3 iPSC lines revealed a striking heterogeneity which correlated with their differentiation potential. Induction of p291fsinsC mutation in wild-type iPSC lines using CRISPR/Cas9 technology recapitulated the impaired progenitor formation and beta cell differentiation observed in patient-derived MODY3 iPSC lines. RNAseq analysis of the pancreatic progenitor lines from the wild-type, patient and CRISPR/Cas9-engineered MODY3 iPSCs and biochemical analysis of HNF1α(WT)/HNF1α(p291fsinsC)/HNF1β interactions indicated that the p291fsinsC mutant impaired the key pancreatic development HNF1β transcriptional functions, resulting in impaired differentiation of MODY3 iPSC lines towards the endocrine fate. This study uncovers a novel mechanism of the p291fsinsC mutation during pancreas development in a new human model of MODY3. These results could help the development of personalized therapies for monogenic and polygenic diabetes, in which HNF1α mutations contribute towards disease pathogenesis.

Funding source: Wellcome Trust

Keywords: MODY3, organoids, human IPSC

MDD163

DECIPHERING THE ROLE OF BMP4 SIGNALLING AND GENE REGULATION IN THE SPECIFICATION OF HUMAN LIVER PROGENITOR CELLS

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BMP4 signalling, from cardiac mesoderm, is crucial for the development of the liver bud from foregut endoderm in early human development. Abolition of BMP4 signalling during anterior-posterior specification of the foregut endoderm prevents liver bud formation or expression of hepatic specific genes such as Albumin or AFP. However, the mechanism downstream of BMP4 signalling and the transcription factors responsible for the acquisition of the hepatic fate by foregut cells are not well known. In our study, we have used a stem cell-based model of human endoderm development to identify specific factors that facilitate cell fate changes during the specification of liver progenitor cells from the foregut. We differentiated human stem cells into hepatoblasts using our previously published protocol with or without BMP4 inhibition. We then completed Next Generation mRNA-sequencing which identified 47 transcription factors upregulated upon induction of BMP4 signalling, and 44 downregulated during BMP4 inhibition in the first 48 hrs during patterning of foregut endoderm. The majority of these transcription factors have not been previously described to have a function in the specification of liver progenitors. Using CRISPR/Cas9 and inducible shRNAs we have knocked down individual and multiple genes revealing previously unknown roles in hepatic specification, cell survival, proliferation and hepatocyte functionality. Our results reveal gene expression networks regulated by BMP4 signalling that are critical for the specification and maturation of stem cell derived hepatocytes. Ultimately, this will allow us to improve the differentiation efficiency and functionality of stem cell derived hepatocytes making them more suitable for regenerative medicine, disease modelling and toxicology applications.

Keywords: BMP4 signalling, Endoderm development, Specification of hepatoblasts

MDD177

IN VIVO ASSESSMENT OF SIZE-SELECTIVE GLOMERULAR SIEVING IN TRANSPLANTED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED KIDNEY ORGANOIDS

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Human induced pluripotent stem cells (hiPSC) can be differentiated to kidney organoids in vitro. This development holds promise for disease modelling, drug discovery and clinical application. The utility of kidney organoids in regenerative medicine will rely on the functionality of the glomerular and tubular structures in these tissues. Recent studies have demonstrated the vascularization and subsequent maturation of kidney organoids after renal subcapsular transplantation. Upon transplantation fenestrated glomerular endothelium, a glomerular basement membrane and extensive podocyte foot processes were formed, raising the question whether under these conditions the glomeruli also become functional. One of the basic functions of the kidney is its ability to produce ultrafiltrate in the glomerulus. The defining feature of an ultrafiltration membrane, such as in the glomerulus, is that it can sieve macromolecules on the basis of molecular size. We aimed to assess glomerular function of the transplanted hiPSC-derived kidney tissue by applying high-resolution intravital multiphoton imaging through an imaging window during intravenous infusion of multiple low- and high-MW fluorescently labelled dextran molecules or albumin. Glomerular sieving of low-MW dextrans could be observed as soon as 1 week after transplantation and displayed dextran and albumin size selectivity across the glomerular filtration barrier. Tubular presence of low-MW dextran molecules was also observed suggesting reuptake and endocytosis of these small dextran molecules in the proximal tubules, further corroborating the presence of an active filtration unit. Our approach demonstrated that hiPSC-derived glomeruli have developed an appropriate barrier function upon in situ maturation and discriminate between molecules of varying size, selectively restricting their passage into Bowman's space. For potential future clinical translation of kidney organoid transplantation, functional filtration will be key in the development trajectory to unlock further use of hiPSC derived kidney organoids both for clinical application as well as disease modeling.

Keywords: hiPSC-derived Kidney Organoids, Transplantation, Glomerular Filtration

MDD449

THE ROLE OF ERBB2/ERBB3 SIGNAL PATHWAY IN GENERATION OF CANCER STEM CELLS

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Cancer stem cells (CSCs) are suggested to be responsible for drug resistance and aggressive phenotypes of tumors. Mechanisms that regulating CSCs are still under investigation. Our lab has established a novel method to produce CSCs from iPSCs under cancer microenvironment using conditioned medium (CM) from cancer cell lines. By treatment iPSCs with CM, cells gain CSC characteristics. Here, we analyzed transcriptome from CSCs, which converted from iPSCs using CM from pancreatic ductal adenocarcinoma cells, and differentially expressed genes were identified. Pathway enrichment was analyzed by the Kyoto Encyclopedia of Genes and Genomes (KEGG). Comparing iPSCs with CSCs converted from iPSCS showed elevated expression of genes related to ERBB2, ERBB3 and PI3K, while KEGG pathways revealed enrich of pathways known to be involved in cancer including ERBB2/ERBB3 signal pathway by conversion of iPSCs to CSCs. Inhibition of ERBB2 by lapatinib induced cell proliferation arrest and losing of tumorgenicity of converted cells. This study shows a potential involvement of ERBB2/ERBB3 pathway in CSCs generation and could lead to potentially new options for cancer treatment and prevention.

Keywords: iPSCs, Cancer stem cells, Signal pathway

MDD456

TUBULAR LIVER: FABRICATED HUMAN LIVER-LIKE TISSUE INTEGRATED WITH LARGE VASCULAR CHANNEL AND CAPILLARIES

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Liver-like tissues such as spheroid and organoids have been developed for drug testing and as a graft for liver transplantation due to their superior functions compared to 2D-cultured cells. However, they still lack perfusable vasculature, which makes it difficult to maintain the inner part of the tissue by nutrition and oxygen supply and/or to inject test substances into the tissue and sample the metabolites for drug discovery. To overcome these difficulties, we propose the liver-like tissue containing a large main channel and capillaries (sinusoid-like structures) using a perfusion culture device, which is named tubular liver. To construct the tissue, we fabricated the device using 3D printer. Then, we set a needle across the device and filled the device with collagen solution containing hepatic cells (HepG2), vascular endothelial cells (HUVECs), and mesenchymal stem cells (MSCs). After gelation of collagen, we removed the needle to make a hollow channel which was subsequently inoculated with HUVECs. After the wall of the hollow channel was coated by HUVECs and a main channel was formed, we performed perfusion to induce the formation of the sinusoid-like structures. Regarding the morphology, the highly-dense aggregates around the channel were found around the channel in the perfused tissue by hematoxylin-eosin (HE) staining and immunostaining. This is quantitatively shown with measurement of the cell distributions around the channel. Moreover, the formation of the sinusoid-like structures branched from the main channel was visualized by immunostaining of CD31 and India ink perfusion, and the maintenance of the proper tissue functions were also confirmed by immunostaining of albumin and CYP2D6. Finally, we confirmed the feature of the main channel and the sinusoid-like structures for substance exchange by measuring the amounts of secreted albumin and CYP3A metabolization. Taken together, we believe that our method for fabricating a tubular liver can be useful in drug development and regenerative medicine.

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Keywords: vascular channel, liver, perfusion

MDD470

EPITHELIAL-DERIVED FACTORS AND MECHANICAL PROPERTY OF SUBSTRATE ORCHESTRATE THE EMERGENCE OF MUSCULARIS MUCOSA OF THE INDUCED PLURIPOTENT STEM CELL- DERIVED HUMAN GASTRIC ORGANOID

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The gastric muscularis mucosa (MM) is a thin layer of smooth muscle and contained in the gastrointestinal wall. It separates mucosa from the submucosa and enhances contact between the gastrointestinal epithelium and the contents of the lumen. MM is emphasized in cancer invasion, and the T classification of cancer TNM staging system progresses when cancer invades beyond MM. The human gastric MM has not been clarified how human MM emerges, although its development has been investigated in mouse and chicken. Recently, human gastric organoid (hGO) has been generated from induced pluripotent stem cells (iPSC) by using activin, noggin, and EGF. The epithelium of the organoid has been indicated gastric differentiation, in contrast, subepithelial cells have not shown smooth muscle differentiation in this article, and this hGO has not been accompanied by MM. In this study, we tried to induce hiPSC to differentiate into the hGO with MM at first. By culturing hGOs for a longer period

than reported manuscripts, subepithelial smooth muscle cells have been induced, and they have been considered to be accompanied by MM. The time-course analysis of hGO with MM revealed that subepithelial smooth muscle differentiation appeared after about day 21, and it followed epithelium. During differentiation into hGO with MM, we identified some signaling factors that were secreted by the organoid epithelium, and the emergence of MM was suppressed by inhibiting them. Furthermore, subepithelial smooth muscle differentiation was found to be promoted when the organoids protruded through the Matrigel layer and attached to the stiff bottom of the plastic plates. These findings suggested that epithelium-derived factors and mechanical property of substrate orchestrate the emergence of muscularis mucosa of the iPSC-derived human gastric organoid.

Keywords: iPSC, Gastric organoid, Muscularis mucosa

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

MDD191

SECRETED PROTEINS FROM IPS-DERIVED HUMAN LIVER ORGANOIDS WITH SINUSOIDAL VESSELS CORRECT COAGULATION DEFICIENT PHENOTYPE

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Seminal works in studying the orchestration of endoderm-mesoderm instructions in directing organogenesis are now begun translated into developing in vitro principles for human stem cell driven multi-cellular self-organization. Although it is well accepted that organ specialized vessels display specific anatomic, transcriptomic and functional features, the embryonic origins of tissue-specific vessels in human and the self-organizing mechanisms governing their formation are poorly understood. Here, we report self-organization of vascularized liver organoid with functional features of sinusoidal vessels from human pluripotent stem cell (PSC). We first directly differentiated PSC into endothelial cells with arterial and venous features. In the presence of hepatic lineages, PSC-derived hemogenic endothelial cells (HECs) is able to self-organize CD32b+ Factor VIII+ liver sinusoidal endothelial cells (LSECs), whereas arterial endothelial cells failed. Integration of LSEC components into liver organoids significantly facilitate balanced induction of multiple complement and coagulation pathway related proteins. Coagulation factors synthesis and activity persists in long-term culture of organoids with tissue-specific vasculature, which is sufficient for correction of in vitro clotting time with Factor V, VIII, IX and XI deficient patients plasma. Furthermore, infusion of the organoid-secreted coagulation factors without transplantation rescued the severe bleeding phenotype of hemophilia A mouse model. Given the safety concerns related to PSC-derived tissue transplant therapy, organoid-derived protein supplementation approach will be a potential approach to treat coagulation defective patients.

Keywords: Human Liver Organoid (HLO), Sinusoidal vessels, Hemophilia A

MDD487

A NOVEL PATIENT HIPSC-DERIVED IN VITRO BLOOD-BRAIN BARRIER MODEL OF COLLAGEN IV SMALL VESSEL DISEASE

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Cerebral Small Vessel Disease (SVD) causes 25% of stroke and 45% of dementia worldwide. Despite its prevalence, little is known about the mechanisms that cause or propagate the disease. However, neurovascular unit (NVU) dysfunction and perturbation of the matrisome are thought to play a key role in Blood-Brain Barrier (BBB) breakdown and SVD progression. Monogenic SVD can be caused by mutations in Collagen IV α1/α2 (COL4A1/2), a highly abundant matrisome protein. Using patient-derived human induced pluripotent stem cell (hiPSC) lines with two distinctive Collagen IV mutations (COL4A1G755R and COL4A2G702D), isogenic and multiple wild-type (WT) control lines, we have established an in vitro model of the BBB. This novel, tractable platform can be used to generate insights into SVD pathophysiology and elucidate a potential shared mechanism in SVD.

hiPSC were differentiated into brain microvascular endothelial cells (BMEC), astrocytes and mural cells (MC), using previously published protocols. The cell types were characterised for specific marker expression and response to functional tests, including transendothelial electrical resistance (TEER), permeability assays (4kDa FITC-Dextran and Sodium Fluorescein), LDL-uptake and tube formation. Cells were then combined into a Transwell® co-culture model of the BBB. hiPSC-derived BMEC exhibit high TEER of ~3000-4000Ωxcm2, which is increased and maintained over two weeks in co-culture with astrocytes and in tri-culture with astrocytes and MC. COL4A2G702D BMEC have reduced Collagen IV deposition and lower tight junction protein expression. Both COL4A1G755R and COL4A2G702D hiPSC-BMEC exhibit intracellular accumulation and defected p-glycoprotein efflux of Rhodamine123 and display discontinuous tight junction localisation of Occludin. Moreover, COL4A1G755R and COL4A2G702D BMEC and MC have increased levels of MMPs. These findings, which are consistent with what is seen in patients, strengthen the argument that matrisome alterations are important in Collagen IV SVD and suggest a role for MMPs in SVD pathogenesis.

Funding source: ACT-VAD (Stroke Association, British Heart Foundation, Alzheimer's Society) and The Rosetrees Trust

Keywords: Collagen IV, Blood-Brain Barrier, Matrix Metalloproteinases

EPITHELIAL

MDD216

A PAIR OF SOXS IN HUMAN LUNG DEVELOPMENT: COMBINING ORGANOID AND CRISPR TECHNOLOGY TO STUDY HUMAN BIOLOGY

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Organoid technology has the potential to transform research on multiple human diseases, but genetic manipulation in human organoids remains technically challenging. I have developed efficient pipelines for CRISPR-mediated gene targeting and inducible knock-downs in human organoid systems. These will be illustrated by investigating the functions of SOX proteins in developing human lungs. We have previously developed a human foetal lung organoid system in which epithelial progenitor cells can be expanded and subsequently differentiated to mature cell lineages. Here, I investigate the role of SRY-related HMG-box (SOX) family proteins-SOX2 and SOX9 in human lung development by combining the organoid culture system with CRISPR technology. Briefly, replacement of SOX2 with a Histone-H2B-GFP reporter does not influence organoid self-renewal, but does alter differentiation capacity. By contrast, when SOX9 was inducibly knocked down, organoid self-renewal was blocked and the epithelium transformed from a monolayer to multilayer, suggesting a polarity defect. These findings show the potential of organoid systems for genetic manipulation to build platforms for studying human development and disease.

Funding source: The Wellcome Trust & the Cambridge Trust

Keywords: Organoid, CRISPR, Human Lung Development

MDD463

DEVELOPMENT OF ALVEOLAR EPITHELIAL TYPE II CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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It is well known that some drugs cause pulmonary toxicities, such as interstitial pneumonia and it is important to minimize the risk of the drug-induced respiratory diseases for patient safety. In addition, COVID-19 is known to bind to ACE2 receptor following activation of the spike protein by TMPRSS2 and it is necessary to establish a good model for drug screening. However, it is difficult to obtain human lung cells and culture the cells for a long-term period to explore the mechanistic approach for the drug effects. In the present study, we have developed alveolar epithelial type II (AT2) cells from human iPSCs using two-dimensional culture method. Differentiation was performed by mainly two steps; the first step was to generate lung progenitor cells and the second step was to induce AT2 cells from lung progenitor cells. We found that the differentiated cells from human iPSCs expressed several AT2 cell markers, such as surfactant protein C, surfactant protein B, ATP binding cassette subfamily A member 3, and solute carrier family 34 member 2, suggesting the cells exhibit AT2-like properties. In addition, AT2 cells express both ACE2 and TMPRSS2 at higher levels to human lung tissue, suggesting that iPSC-derived AT2 cells is a good model for COVID-19 treatment. Taken together, these data suggest the potential of iPSC-derived alveolar cells as in vitro models for drug evaluation.

Keywords: alveolar epithelium, lung, ACE2

EYE AND RETINA

MDD227

DEVELOPMENT OF A HUMAN IPSC-DERIVED BLOOD-RETINAL-BARRIER ON-A-CHIP FOR MODELING AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is the major cause of blindness among the elderly in the Western world, but so far only limited treatment options are available. The key tissue implicated in the disease is the outer blood-retina barrier (BRB) composed of the retinal pigment epithelium (RPE), Bruch's membrane, and the choroidal capillaries, mostly formed by endothelial cells (ECs). Available in vitro models typically rely on simplified monolayer cultures that insufficiently capture the tissue dysfunction of AMD. In order to overcome this limitation, organs-on-a-chip represent a promising technology for the development of 3D in vitro models. The aim of this project is therefore to develop a physiologically relevant organ-on-chip model of the outer BRB, based on human induced pluripotent stem cell (hiPSC)-derived cells. We are currently generating ECs and RPE from hiPSC lines obtained from control and AMD-affected individuals. All individuals were genotyped for 52 AMD-associated variants, and 13 AMD-related genes were sequenced to detect rare coding variants. Differentiation and maturation of hiPSC-derived cells was confirmed by analyzing the expression of several markers specific to each cell type using RT-qPCR and immunostaining. Characterized cells were then incorporated into an organ-on-a-chip device containing a microchannel and an open top culture chamber, separated by a polyester membrane. ECs were seeded in the microchannel, where they were able to form a capillary-like structure. RPE cells were seeded in the open top culture chamber, were they formed a confluent pigmented monolayer in 15 days. Co-culture of hiPSC-derived RPE and ECs could successfully be established, and for both cell types survival and maturation in their respective microenvironment in the device were confirmed by immunostaining. As a next step, we will incorporate in the chip RPE and ECs derived from iPSC lines from AMD patients. This in vitro model will provide new knowledge on how various molecular, cellular and physical aspects interact in AMD, and can be used for personalized testing of new therapeutic molecules.

Funding source: Netherlands Organisation for Scientific Research (VICI grant 016.VICL170.024), Health Holland Top Sector Life Sciences and Health (PLURIMACULA)

Keywords: organ-on-a-chip, retina, endothelial cells

MDD228

IMPROVED TRANSERABILITY OF A DISEASE MODEL FOR AVASCULAR AGE-RELATED MACULAR DEGENERATION (AMD) TO EVALUATE CELL-BASED GENE THERAPIES USING AGED MICE

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Disease models for age-related diseases are mostly established in young animals; this condition may prevent transferability, distort pathophysiology and lead to failure in drug development. To mimic avascular AMD (aAMD), several models have been developed, which do not demonstrate the full range of aAMD symptoms. An immunological model in which mice are immunized with carboxyethylpyrrole (CEP) reflects more closely aAMD; however, this model uses young 2- 3-months old mice. Since the retina of young mice does not show the typical age-related vulnerability to oxidative stress or cellular changes, we have improved the model by using mice older than 1 year, to accelerate disease progression, intensify symptoms and improve translation of the model. 12 - 18-months old C57BL6JRj mice are immunized with serum albumin adducted with CEP (CEP-MSA); the optimized protocol consists of 1 subdermal hock injection of 200 µg CEP-MSA emulsified in 120 µl complete Freund's adjuvants (FA) and 3 booster neck injections at 10, 20 and 90 d with 100 µg CEP-MSA emulsified in 60 µl incomplete FA. The objective visual threshold ("acuity") was determined by measuring the optokinetic response that decreased significantly from 0.33 to 0.28 cpd ($p=0.002$) at 90 d after the first injection (post-immunization). Ocular coherence tomography and funduscopy at 213±30 d post-immunization showed increased retinal thickness, 0.6 AU vs 0.4 AU pre-treatment ($p<0.0001$) and an increase in the number of eyes showing drusen-like deposits, 68.8% vs 16.7% pre-treatment ($p=0.007$). Microscopic analysis at 3- and 8-months post-immunization revealed loss of neuroretinal integrity, degeneration, thickening, photoreceptor cell loss, retinal pigment epithelium alterations, increased Iba-1+ microglia and accumulation of drusen-like deposits containing vitronectin. In age-matched controls, similar pathological alterations were noted with increasing age, but to a substantially lesser extent. In summary, we have shown by imaging, functional and behavioral analyses that after short induction time the CEP aAMD model in old mice exhibits typical features of human aAMD including drusen-like deposits, retinal degeneration and decreased visual acuity. This optimized aAMD model will be used to investigate the efficacy of a cell-based gene therapy product.

Funding source: The project has been funded by the Swiss National Science Foundation grant no. 160195.

Keywords: age-related diseases, cell and gene therapy, neuroretinal degeneration

HEMATOPOIETIC SYSTEM

MDD246

CONDITIONAL EXPRESSION OF HOXA9 IN A HUMAN PLURIPOTENT STEM CELL DERIVED MODEL OF HAEMATOPOIESIS.

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The homeodomain-containing transcription factor HOXA9 has long been proposed to play a central role in haematopoiesis. Overexpression and knock-out mouse studies demonstrate it is the most important hoxa gene in haematopoietic stem cells (HSC), with roles in HSC self-renewal and in vivo engraftment. In human pluripotent stem cell (hPSC) differentiation to haematopoietic cells, expression of HOXA genes during mesoderm specification is proposed to replicate intra-embryonic haematopoiesis. Further, HOXA9 expression during differentiation follows the pattern of haematopoietic commitment, suggesting HOXA9 may act as a marker of the definitive haematopoietic program. Our current studies have similarly demonstrated HOXA9 is gradually upregulated post mesoderm, reaching highest levels in the haematopoietic CD34+ population. However, the mechanism, stage and extent to which HOXA9 influences haematopoietic cell fate, in particular definitive cell fate, remains unclear. Here, we examine the effects of continual and temporal HOXA9 overexpression during hPSC-based haematopoietic differentiation. A doxycycline-inducible HOXA9-mScarlet reporter system targeted to the AAVS1 locus was generated and verified by QPCR, immunocytochemistry and western blot analysis. Specific, temporal control of HOXA9-mScarlet expression during haematopoietic differentiation was feasible up until mesoderm induction. Following mesoderm specification inducibility was limited, potentially due to methylation within the system. In contrast, continuous stimulation of the reporter system led to overexpression of HOXA9-mScarlet throughout differentiation. Comparison of stage-specific and continual HOXA9-mScarlet expression on haematopoietic cell generation was monitored. Preliminary data indicates that the stage, level and duration of HOXA9 expression influences both haematopoietic cell formation and cell fate. Subsequent studies will focus on identifying the pathways and processes associated with HOXA9 overexpression by single cell RNA-seq.

Funding source: The Joan Browne Legacy

Keywords: Haematopoiesis, HOXA9, CRISPR/Cas9

IMMUNE SYSTEM

MDD441

FUNCTIONAL ANALYSES OF HUMAN IPS CELL- AND/OR PERIPHERAL CD14+ CELL-DERIVED AND IMMORTALIZED MYELOID CELL LINES (MYLC).

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We have a platform to establish immortalized myeloid cell lines (Mylc lines) from human iPS cells and/or primary CD14+ cells purified from human peripheral blood mononuclear cells (PBMC). Using this platform, we have established two Mylc lines from different iPS cells and four Mylc lines from different healthy donor-derived CD14+ cells, and examined the function of these Mylc lines from two perspective, 1) indicator cells for harmful substances and 2) host cells for virus infection. In many kinds of skin-sensitization tests and pyrogen tests, human-derived myeloid cell lines are used as indicator cells. First, we examined whether Mylc lines were utilizable as indicator cells in these tests. We cultured Mylc lines along with endotoxin, non-endotoxin or chemicals. Then the amount of inflammatory cytokines (IL-6 and IL-8) in the culture supernatants was measured. Upon the stimulation with endotoxin (LPS) or non-endotoxin (SAC, CpG-A, Resiquimod), Mylc lines produced these cytokines in a dose-dependent manner. Mylc lines were more sensitive than the representative human myeloid cell lines, Mono-mac-6 and THP-1. Some harmful chemicals could also induce the production of these inflammatory cytokines. All Mylc lines examined could respond to these pyrogens and harmful chemicals. Therefore, the test system using several Mylc lines might be more precise compared with the present tests using one particular cell line. We next examined whether Mylc lines before and after the differentiation into dendritic cell (DC) can be infected with dengue virus type 2 (DV2) 16681 strain. Mylc lines can be differentiated into DCs by culturing Mylc lines along with IL-4 for 3 days. Interestingly, all Mylc lines could be infected with DV2, especially after DC-differentiation (8 out of 8 lines examined). Some of DC-differentiated Mylc lines showed increased sensitivity to DV2 compared with Vero cells that are generally used for dengue virus study. Therefore, these results indicate that human Mylc lines may be useful and powerful tool

for virus isolation and research. Furthermore, this Mylc lineup including Mylc lines derived from patients would make functional assay and/or screening systems more reliable.

Keywords: myeloid cell, inflammatory cytokines, virus infection

MUSCULOSKELETAL

MDD453

USING HUMAN PLURIPOTENT STEM CELLS DERIVED MOTOR NEURONS TO ADDRESS THE PATHOGENESIS OF SPINAL MUSCULAR ATROPHY DISEASE

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Spinal muscular atrophy is the most common genetic cause of infant mortality characterized by the specific degeneration of lower motoneurons (MNs) in the spinal cord, leading to progressive paralysis and muscle atrophy. SMA etiology relates to an insufficient amount of SMN (survival motor neuron) protein, which results from mutations in the SMN1 gene. Despite the ubiquitous expression of SMN protein, it is still unclear why MNs are one of the most affected cell types. Understanding this specific cellular tropism is critical but requires access to the relevant cell type. In this present study, we demonstrated that the reduced expression of SMN lead to a decreased survival of hiPSC-derived MNs rather than a defect in their generation. We identified that this phenotype can be rescued by kenpaullone, an inhibitor of several CKDs as well as JNK, likely through a JNK dependent mechanism. By a transcriptomic approach, we identified SMA-specific changes in early MNs that include genes involved in synaptic plasticity. Interestingly, these genetic defects were rescued by kenpaullone treatment. These findings suggest that alteration in synaptic organization might be a new therapeutic target for SMA. Furthermore, several studies suggest that pathological changes of the neuromuscular junction (NMJ) precede the motor neuronal loss. Therefore, it is critical to evaluate the NMJ formed by SMA patients' MNs, and to identify drugs that can restore the normal condition. We thus developed an in vitro co-culture strategy to study the interaction between MNs and its skeletal muscle target. Altogether, our results demonstrate the potential offered by hiPSC to shed light on the cellular and molecular bases of selective MN vulnerability in SMA.

Keywords: Spinal muscular atrophy (SMA), Human induced pluripotent stem cells (hiPSCs), Co-culture system

MDD459

ROLE OF MBNL PROTEINS UN HUMAN MYOEGENSIS UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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Alternative splicing has emerged in the post-genomic era as the main driver of proteome diversity. The regulation of alternative splicing varies with cell type, during development or upon cellular differentiation, thereby participating in the fine-tuning of a gene signature temporally and spatially. To ensure this developmental and tissue-specific regulation, AS rely on complex mechanisms involving an intricate protein-RNA network. It has become apparent that these coordinated splicing networks have important physiological functions in different developmental processes in humans and that the disruption of these networks negatively impacts health and contributes to human diseases, including cancer, diabetes, and neuromuscular diseases. Since then, a precise understanding of the function of alternative splicing under physiological and pathological conditions should reveal new pathological mechanisms and reveal new targets for the discovery of more efficacious drugs. Illustrating this, Myotonic Dystrophy Type 1 represents one paradigmatic example of genetic disease in which loss of MBNL splicing factors is considered as the central pathological event in this neuromuscular disease. Despite this pathological contribution, relatively little is known about the functions of MBNL proteins during the development of the human neuromuscular system. For this purpose, and thanks to the CRISPR/ Cas9 technology, we generated human induced pluripotent stem cells depleted in MBNL proteins and assess their capacity to differentiate into skeletal muscle cells. Our results describe the involvement of MBNL proteins in the late stages of human myogenic differentiation while they are not required earlier on. Our results also highlight the importance of both MBNL1 and MBNL2 proteins to recapitulate the main molecular and cellular features associated with DM1 at the muscular level. By using co-cultures between skeletal muscle cells and hiPSC-derived motoneurons, we also identified a contribution of the MBNL proteins for the development of neuromuscular junctions. Altogether, our study illustrates the potential of using human pluripotent stem cells for deciphering the contribution of alternative splicing during development under phsyiological and pathological conditions.

Keywords: hiPSC-derived muscle cells, alternative splicings, development

NEURAL

MDD276

ASSESSING GLUTAMATE UPTAKE AFTER HYPOXIC INJURY IN ASTROCYTES DERIVED FROM HUMAN FETAL NEURAL STEM CELLS IN VITRO

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Birth hypoxia or maternal intra-uterine infections can lead to hypoxic injury to fetal brain cells. Astrocytes play a protective role in these conditions by mediating the uptake of excess glutamate from the synapse, thereby preventing excitotoxicity. The effect of hypoxic injury on the expression and function of glutamate transporters was evaluated in astrocytes differentiated from human fetal neural stem cells (FNSCs) in vitro. FNSCs isolated from the subventricular zone of human fetal brain (from aborted fetuses n=4, after Institute Ethics Committee clearance and informed consent from mothers) were expanded in culture, and evaluated for expression of neural stem cell markers Nestin and Sox2. Differentiation of FNSCs into astrocytes over 21 days was monitored by the expression of astrocyte specific GFAP, while differentiation into neuronal lineage was ruled out by checking expression of neuronal-specific DCX. Differentiated cells were exposed to different grades of hypoxia viz. normoxia (20% and 6%), moderate hypoxia (2%) and severe hypoxia (0.2%) for 48 hours. Exposure to hypoxia was validated by checking the expression of hypoxia-responsive genes. Glutamate uptake (fluorimetry) and expression of EAAT2 (western blot/qPCR) were assessed in astrocytes after hypoxia exposure. FNSCs showed neurosphere formation in culture and expressed neural stem cell markers Nestin and Sox2. As differentiation progressed, there was increased expression of astrocyte specific GFAP, with corresponding decrease in Nestin. Neuronal marker DCX was not expressed by differentiated cells. Differentiated cells also expressed the astrocyte-specific glutamate transporters EAAT1 and EAAT2. Differentiated astrocytes exposed to hypoxia, showed an upregulation of hypoxia markers VEGF, CA9 and PGK1. Glutamate uptake and transporter expression (EAAT2) remained unchanged after exposure to hypoxia. FNSCs were successfully differentiated into functional astrocytes in vitro. These cells expressed GFAP and glutamate transporters. When the differentiated astrocytes were exposed to various grades of hypoxia, EAAT2 expression and glutamate uptake were maintained, thereby indicating that astrocyte function is not affected by hypoxic injury.

Funding source: Authors acknowledge DBT, Govt of India, for financial support.

Keywords: Neural stem cells, astrocytes, hypoxia

MDD295

THE IMBALANCE OF EXCITATORY/ INHIBITORY NEURON DIFFERENTIATION IN NEURODEVELOPMENTAL DISORDERS WITH AN NR2F1 POINT MUTATION

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Recent studies have revealed an essential role for embryonic cortical development in the pathophysiology of neurodevelopmental disorders, including autism spectrum disorder (ASD). However, the genetic basis and underlying mechanisms remain unclear. Here, we generate mutant human embryonic stem cell lines (Mut hESCs) carrying an NR2F1-R112K mutation that has been identified in a patient with ASD features, and investigate their neurodevelopmental alterations. Mut hESCs overproduce ventral telencephalic neuron progenitors (ventral NPCs) and inhibitory neurons, and underproduce dorsal NPCs and excitatory neurons. These alterations can be mainly attributed to the aberrantly activated Hedgehog signaling pathway. Moreover, the corresponding Nr2f1 point mutant mice display a similar excitatory/inhibitory neurons imbalance and abnormal behaviors. Antagonizing the increased inhibitory synaptic transmission partially alleviates their behavioral deficits. Together, our results suggest that the NR2F1-dependent imbalance of excitatory/inhibitory neuron differentiation caused by the activated Hedgehog pathway is one precursor of neurodevelopmental disorders and may enlighten the therapeutic approaches.

Keywords: Neurodevelopmental disorders, Neuron differentiation, NR2F1 point mutation

MDD316

IN VITRO DIFFERENTIATION OF EPO-PRODUCING HINDBRAIN NEURAL CREST CELLS FROM HUMAN IPS CELLS

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Erythropoietin (EPO) is a glycoprotein cytokine that stimulates the proliferation and differentiation of erythroid cells. EPO was produced mainly by kidney interstitial fibroblast in adulthood. When the kidney was injured (for example, by chronic kidney disease), EPO-producing kidney interstitial cells turn to myofibloblast which secrete many extra-cellular matrix proteins and cause fibrosis of kidney. Recently, it is reported that P0-Cre (neural crest) lineage cells are positive for EPO-producing kidney interstitial cells in adult mice1. These cells differentiate into myofibloblast after kidney injury1, suggesting that at least a cell-of-origin of renal fibrosis is neural crest cells (NCCs). Meanwhile, EPO-producing cells find neuroepithelium and hindbrain neural crest in mid-gestational stage of mouse embryos. Therefore, we hypothesize that embryonic EPO-producing NCCs differentiate into adult EPO-producing kidney interstitial cells. We challenge to induce EPO-producing kidney interstitial cells from human iPSCs via hindbrain NCCs for making in vitro kidney fibrosis model. First, we tried to differentiate embryonic EPO-producing neural crest cells from human iPSCs. Our previous NCCs differentiation method mostly generate midbrain NCCs. To induce posterior NCCs, we added retinoic acid (RA) which determine anterior/posterior axis during embryonic development, for NCC differentiation. RA treated NCCs were express hindbrain regional marker HOXB4 and EPO. Furthermore, secreted EPO protein was detected in hindbrain NCCs culture media and which was increased by hypoxia condition.

Keywords: Erythropoietin, Neural crest, Retinoic acid

MDD500

FUNCTIONAL CHARACTERIZATION OF AXONS IN HUMAN IPSC-DERIVED NEURONS MODELING BRAIN DISEASES BY HIGH-DENSITY MICROELECTRODE ARRAYS

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Axons play a central role in neuronal pathophysiologies of Parkinson's Disease (PD) and Amyotrophic Lateral Sclerosis (ALS). Human neurons with functional axons that can be used to model brain diseases can be generated in vitro by induced-pluripotent-stem-cell (iPSC) technology. High-Density Microelectrode Arrays (HD-MEAs) enable label-free and long-term recording of the electrical activity of axons. In this study, we compared the action-potential-propagation velocity along axons between human control neuron lines and isogenic neuron lines that have been genetically modified to model PD and ALS. Our findings indicate that axonal action-potential-propagation velocity can be used as an indicator for (1) electrophysiological phenotype characterization of multiple human iPSC-derived neuronal lines and for (2) testing the effects of drugs on axon physiology. We have used HD-MEA technology featuring 26'400 electrodes at 17.5 um pitch (MaxWell Biosystems AG). Commercially available human dopaminergic and motor neuron lines were plated on HD-MEA chips: (1) iCell® DopaNeurons, (2) MyCell® DopaNeurons α-synuclein (A53T) modeling PD, (3) iCell® Motor Neurons and, MyCell® Motor Neurons TDP43 (Q331K) modeling ALS, (Fujifilm Cellular Dynamics International). All neuronal lines were co-cultured with human astrocytes iCell® Astrocytes (Fujifilm Cellular Dynamics International). Each HD-MEA chips was plated with 100'000 neurons and 20'000 astrocytes. Axonal action potential velocities were compared across neuronal cultures by customer-written software in MATLAB. We found that control dopaminergic neurons and control motor neurons had significantly different axonal action potential velocities at DIV 28. Dopaminergic neurons featured average velocities of 500 mm/s, whereas motor neurons featured average velocities of 600 mm/s. Furthermore, we found that PD dopaminergic neurons showed a decrease of 15% in average velocity as compared to the control dopaminergic neurons. Finally, we found that ALS motor neurons showed an increase of 10% in velocity as compared to the control motor neurons. HD-MEA systems enable to access novel electrophysiological parameters of iPSC-derived neurons, which can be potentially used as biomarkers for functional phenotype studies and drug screening.

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Keywords: Axon, Amyotrophic Lateral Sclerosis, Parkinson's Disease

NEW TECHNOLOGIES

MDD404

THE POTENTIAL OF STIRRED-TANK BIOREACTORS IN EXOSOME PRODUCTION

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Exosomes are a population of naturally occurring mobile, membrane-limited, 30 – 100 nm in diameter, extracellular vesicles containing a large amount of proteins, lipids, messenger and micro-RNAs. It was shown that they play a role in the mediation of intercellular communication, the modulation of immune-regulatory processes, tumor metabolism and regenerative as well as degenerative processes. In recent years, there is increasing interest in the therapeutic potential of exosomes produced by Mesenchymal Stem Cells (MSCs). High quantities of high-quality exosomes are required for therapeutic applications, which are produced in a defined process. Stirred-

tank bioreactors offer the scalability, as well as the possibility to tightly control and monitor the production of exosomes, however downstream manufacturing steps like purification remains critical and labor intensive. Cultivating cells on a growth matrix like Fibra-Cel disks could overcome this challenge. Fibra-Cel disks have already shown their capacity in vaccine and stem cell production approved for therapy applications. Packed-bed glass and single-use bioreactors are available, enabling the cultivation of MSCs under controlled conditions in a stirred-tank bioreactor. In our talk, we will highlight the potential and benefits of stirred-tank bioreactors in the cultivation of stem cells for exosome production and will give an outlook on exosome bioprocessing.

Keywords: Bioreactor, Exosomes, Bioprocess

MDD405

ESTABLISHING A SUSTAINABLE EUROPEAN BANK FOR HUMAN INDUCED PLURIPOTENT STEM CELLS: EBISC2

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The European Bank for induced Pluripotent Stem Cells (EBiSC, https://cells.ebisc.org) is established as a centralised, non-profit repository and distribution hub ensuring long term storage and safeguarding of deposited human induced pluripotent stem cell (iPSC) lines and their distribution to academic and commercial researchers worldwide. Since 2015, >19 depositors across Europe and America have deposited iPSC lines into EBiSC from >35 disease areas such as cardiac, neurodegenerative, neurological, metabolic and eye disorders, with associated familial and isogenic controls also available. To further strengthen the iPSC landscape, in 2019, EBiSC started a second project phase to reach long-term sustainability (EBiSC2). An innovative and flexible approach allows collection, qualification and distribution of iPSC lines from a multitude of international sources. Robust review criteria ensure collection of appropriate consent and if present, clear communication of use restrictions to users. Novel processes for iPSC high throughput culture, banking and differentiation using a benchtop bioreactor increase efficiency and throughput. A robust Quality Control (QC) screening regime ensures that iPSC lines from varying sources perform comparably in the hands of users and are of a high quality and integrity. The human Pluripotent Stem Cell Registry (hPSCreg, https://hpscreg.eu/) streamlines iPSC line data management. Key data is publically available prior to purchase via the EBiSC website, with genomic datasets available via the EBiSC data access committee. A simplified access procedure allows users to access iPSC lines from multiple sources simultaneously under a single access agreement. EBiSC's second project phase guides iPSC research projects to design a project framework with clarity on iPSC ownership, support with biosample collection and guidance on advised QC at critical time points. Additionally, EBiSC2 performs iPSC reprogramming, gene-editing, custom banking and QC for users, upon request. Further developments include generation of iPSC derived differentiated cells delivered in formats ready for use with extensive characterisation datasets and protocols supplied, in addition to assessing feasibility of distributing hiPSCs for commercial use.

Funding source: The EBiSC and EBiSC2 projects have received funding from the Innovative Medicines Initiative Joint Undertaking (JU) and EFPIA under grant agreement No 115582 and No 821362 respectively.

Keywords: iPSC, Gene-editing, Differentiation

MDD421

A VERSATILE BASE MEDIUM TO MAINTAIN A STABLE AND XENO-FREE CELL CULTURE ENVIRONMENT DURING PSC DIFFERENTIATION

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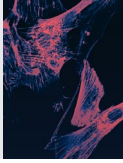
Human pluripotent stem cells (hPSCs) play an important role in disease modeling, drug discovery and cell therapy applications. Their ability to differentiate into cell types of all three germ layers makes them attractive to study specific cell development, cellular functions or cellular behavior of certain cell types in response to different treatments. hPSCs are sensitive to changes in their culture system in terms of medium composition, in particular with respect to nutrient, biosynthetic precursor and vitamin supply, pH and osmolality. Therefore, PSC benefit from a well-defined, steady medium environment to remain viable and functional during maintenance cultivation and subsequent differentiation experiments. Here, we developed a xeno-free and cytokine-free base medium which can be used for various differentiation purposes. The formulation is based on our xeno-free PSC maintenance medium and minimizes the risk of adaption stress when changing from maintenance to differentiation culture. The medium efficiently supports spontaneous EB formation and can also be used as basis for several 2D or 3D directed differentiation assays. As proof of principle, hPSCs were spontaneously differentiated using the cytokine-free base medium, first as embryoid bodies (EBs) in 3D suspension culture, followed by 2D



adherent maturation. Subsequently, immunocytochemistry was used to successfully identify cells of the mesoderm, ectoderm and endoderm lineage. Additionally, directed EB differentiation and directed 2D differentiation was performed by adding lineage-specific cytokines and small molecules to the base medium. The quantitative flow cytometry analysis confirmed a high differentiation efficiency both in 2D and 3D into all three germ layers. Therefore, the medium provides a good basis for a variety of differentiation protocols in 2D and 3D culture conditions.

Keywords: differentiation, embryoid bodies, spontaneous

MDD432

CRISPR-SNIPER - A RELIABLE GENOME EDITING METHOD FOR ROUTINE GENERATION OF SNP-CONVERTED HUMAN IPS CELLS

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Human induced pluripotent stem cells (iPSCs) are attractive target cells for genome editing to engineer in vitro disease models that recapitulate development of many genetic diseases. While gene knockout of human iPSCs can be achieved in a relatively simple manner, modification of specific nucleotides by knock-in, for example single base conversion in disease-associated single nucleotide polymorphism (SNP) often requires considerable technical know-how, and is a laborious task due to the isolation and analysis of a large number of clones. Here, we present a few case studies of successful SNP conversions using a combination of CRISPR with GenAhead Bio's genome editing detection system, SNIPER (specification of newly integrated position and exclusion of random-integration). Our workflow enables the estimation of gene editing efficiency one week after transfection and prior to the single cell cloning step, from which successful isolation frequency of homozygous and heterozygous mutant clones can be predicted. In case of a low knock-in efficiency, No-Go decision can be made early on to evade laborious downstream clone isolation and screening process. In turn, further optimizations can be made to increase knock-in efficiency, resulting in a higher success rate of target clone expansion. Collectively, compared to conventional CRISPR, SNIPER is a reliable, rapid and cost-effective option to routinely creating iPSCs with disease-related SNPs, accelerating iPSC-based translational research and cell therapy development.

Keywords: Genome editing, disease models, CRISPR

MDD464

A DUAL CELL CYCLE REPORTER HIPSCELL LINE FOR THE LIVE-IMAGING OF THE CELL CYCLE IN IPSC-BASED SYSTEMS.

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We generated human induced pluripotent stem cell line with a dual fluorescent reporter to support the development and validation of a microfluidic platform for testing biomaterials in an organ-on-chip setting. The iPSC should allow the analysis of the cycling state and proliferation rate over a period of time using real-time imaging. Information gathered with this line will help to gain insight into the spatial and temporal behavior of cells in iPSC-based differentiation protocols. In addition, real-time imaging features will be instrumental in the validations of the sensors developed to measure cell viability in response to different biomaterials in an organ-on-chip setting. In a hiPSC parental line we first introduced a doxycycline(DOX) regulatable Histone 2B(H2B)-mTurquoise2 into one of the AAVS1 alleles using CRISPR/Cas9 and subsequent FACsorting of the mTurquoise2 positive cells. We show that the mTurquoise2 is efficiently induced (up to 99,7%) and maintained in the majority of cells during stem cell maintenance in the presence of DOX. Upon removal of doxycylin H2B-turquoise slowly dissipates. To improve the detection of the cell cycle stage we also wished to equip the generated H2B-mTurquoise2 iPSC line with a Geminin(GMNN) reporter. Geminin is expressed in the S phase and expression increases during G2 and M phases before instantly disappearing at the metaphase/anaphase transition. We have tried to generate a GMNN/mScarletI fusion through targeting of GMNN at exon 7. Unfortunately, none of our attempts was successful. Consequently, we switched to the insertion of an ubiquitously expressed mScarletI/truncated GMNN(1-110) fusion into the other allele of the AAVS1 locus using CRISPR/Cas9. Positive cells that were isolated by FACsorting demonstrated ScarletI positivity that was cell cycle stage specific. Currently, we are in the process of further validation of the generated cell cycle reporter lines.

Funding source: Interreg European funding for regional development (Flanders-the Netherlands) by the European Union

Keywords: Cell Cycle Reporter line, CRISPR/cas9, hiPSC cell line

MDD472

GENERATION OF HUMAN BRONCHIAL ORGANOIDS FROM PRIMARY BRONCHIAL EPITHELIAL CELLS FOR SARS-COV-2 RESEARCH

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COVID-19 (coronavirus disease 2019) is a disease that causes fatal disorders including severe pneumonia, and development of a therapeutic drug is desired. To develop a therapeutic drug for COVID-19, a model that can reproduce the viral life cycle and can evaluate the drug efficacy of anti-viral drugs is essential. SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), which is the causative agent of COVID-19, is known to be present in large numbers in the bronchi of COVID-19 patients. Therefore, in this study, we generated bronchial organoids from primary human bronchial epithelial cells and examined whether they could be used as a model for SARS-CoV-2 research. The primary human bronchial epithelial cells were embedded in Matrigel, and then cultured in Advanced DMEM/F12 medium containing Noggin, R-Spondin 1, FGF7 (fibroblast growth factor 7), and FGF10. The bronchial organoids were found to contain basal, club, ciliated, and goblet cells. Also, ACE2 (angiotensin-converting enzyme 2), which is a receptor for SARS-CoV-2, and TMPRSS2 (transmembrane serine proteinase 2), which is an essential serine protease for priming spike protein of SARS-CoV-2, were highly expressed. After bronchial organoids were infected with SARS-CoV-2 (7.0×104 TCID50/organoid), remarkable amplification of the viral genome was confirmed at day 5 by performing qPCR analysis. In addition, LDH (lactate dehydrogenase) release increased 2.19 times due to the virus infection suggesting that bronchial organoids were impaired. Furthermore, treatment with 10 µM Camostat, an inhibitor of TMPRSS2, reduced the viral copy number to 2% of the control group. These results suggest that our bronchial organoids are acceptable for SARS-CoV-2 infection and replication, but also can be used as a model for COVID-19 drug discovery.

Keywords: COVID-19, SARS-CoV-2, bronchial organoid

MDD492

CANCER STEM CELL AND EPITHELIAL-MESENCHYMAL TRANSITION-RELATED MOLECULAR NETWORK PROFILING IN MESENCHYMAL STEM CELLS AND GASTRIC CANCER

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Cellular phenotype alters from epithelial to mesenchymal states upon epithelial-mesenchymal transition (EMT). EMT plays an important role in the acquisition of cancer stem cell (CSC) feature and drug resistance. Some of the CSC population exhibits the EMT feature, in which the detailed cellular mechanism has not been fully revealed. To elucidate molecular network pathways interacting with each other and regulating cellular phenotypes in EMT and CSCs, gene expression, as well as the molecular networks, in mesenchymal stem cells (MSCs), diffuse- and intestinal-type gastric cancer (GC) were profiled. The gene expression of cadherin 1 (CDH1), epidermal growth factor receptor (EGFR), erb-b2 receptor tyrosine kinase 2 (ERBB2), ERBB3 were up-regulated in diffuse-type GC compared to MSCs. The gene expression of growth factor receptor bound protein 7 (GRB7), Wnt family member 5A (WNT5A) and ERBB2 were up-regulated, while that of slit guidance ligand 2 (SLIT2) was down-regulated in intestinal-type GC compared to diffuse-type GC. Wnt/beta-catenin pathway, ERBB pathway and patched 1 (PTCH1) pathway were involved in EMT molecular network. In conclusion, the molecular networks related to EMT and CSCs have been revealed in MSCs and GC.

Keywords: cancer stem cell, epithelial-mesenchymal transition, molecular network

PLACENTA AND UMBILICAL CORD DERIVED CELLS

MDD435

ANTI-FIBROTIC AND ANTI-INFLAMMATORY EFFECTS OF FATTY ACID ETHANOLAMIDES SECRETED FROM AMNION-DERIVED MESENCHYMAL STEM CELLS

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We previously demonstrated that administration of conditioned medium (CM) from amnion-derived mesenchymal stem cell (AMSC) culture led to the improvement of colitis and strictures after endoscopic submucosal dissection in animal models. Mass spectrometry detected bioactive lipids such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) in CM from AMSCs. The aim of this study was to investigate anti-fibrotic and anti-inflammatory effects of these bioactive lipids. We used a liver fibrosis model for PEA and a colitis model for OEA. In vitro experiments of PEA, activation of hepatic stellate cells (HSCs) induced by transforming growth factor (TGF)-β1 was significantly inhibited by PEA, and in PEA treatment inhibited the TGF-β1-induced phosphorylation of SMAD2 and upregulated the expression of SMAD7. The reporter gene assay demonstrated that PEA downregulated the transcriptional activity of the SMAD complex. In vivo experiment of rats, liver fibrosis was induced by an intraperitoneal injection of carbon tetrachloride. Intraperitoneal administration of PEA significantly reduced the fibrotic area, deposition of type I collagen, and activation of HSCs and Kupffer cells in rats with liver fibrosis. In vitro experiments of OEA, the expression of tumor necrosis factor (TNF)- α and the phosphorylation of inhibitor of kappa (Ik) B± induced by lipopolysaccharide was inhibited by OEA in human embryonic kidney cells. Moreover, OEA downregulated the expressions of interleukin (IL) -8 and IL-1^2 and inhibited the phosphorylation of IkB± induced by TNF-± in human enterocytes. This effect of OEA was blocked by peroxisome proliferator-activated receptor-± antagonist. In vivo experiments of rats, colitis was induced by the oral administration of dextran sulfate sodium. Intraperitoneal administration of OEA significantly ameliorated the decrease in body weight, the increase in disease activity index score, and shortening of colon length. OEA administration also decreased the infiltrations of macrophages and neutrophils and tended to decrease expressions of inflammatory cytokines in the rectum. In conclusion, fatty acid ethanolamides secreted from AMSCs demonstrated anti-fibrotic and anti-inflammatory effects in rats with liver fibrosis and colitis.

Keywords: amnion-derived mesenchymal stem cell, fatty acid ethanolamide, anti-fibrotic and anti-inflammatory effect

Theme: Tissue Stem Cells and Regeneration

ADIPOSE AND CONNECTIVE TISSUE

TSC102

METABOLIC ANALYSES OF MESENCHYMAL STEM CELLS DURING EXPANSION IN PLATELET LYSATE

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Platelet derived plasma products are increasingly being used during preparation of cellular therapy products. Here we describe metabolic characteristics of mesenchymal stem cells (MSCs) and the soluble environment exposed to different supplements (platelet lysate/PL, fetal bovine serum/FBS, platelet poor plasma/PPP or human bone marrow plasma/BMP). Bone marrow MSCs generated from healthy transplant donors were used. PLs were prepared from blood donors by pooling of 10 samples after repeated freeze-thaw cycles. Intracellular analysis of MSCs revealed 479 significant metabolites, 242 were identified. Extracellular analyses were performed on medium samples with serum/plasma additives and MSC-conditioned mediums. 605 extracellular metabolites showed significant changes, 116 of them have been identified. When MSCs expanded in the presence of FBS were compared with other plasma additives (PL, PPP, BMP) the main affected pathways were valine, leucine, isoleucine biosynthesis, citrate (TCA cycle), cysteine and methionine metabolism, aminoacyl tRNA biosynthesis and phenylalanine metabolism. On the other hand sphingolipid, tyrosine metabolism, glycolysis, glucogenogenesis and propanoate metabolisms were only affected in PL group compared to FBS. MSCs exposed to PL showed evidence of increased glycolysis and glutaminolysis. The late-occurring metabolites of TCA cycle including fumarate and succinic acid were higher in FBS-MSCs ($p<0.01$ both) suggesting more active TCA cycle. The differences between the extracellular metabolite levels at baseline and after MSC expansion (conditioned medium) indicated consumption or synthesis of certain metabolites, especially when the intracellular levels were considered as well. Aspartic acid and glutamic acid consumption was significantly increased in PL cultures whereas L-alanine consumption was higher in FBS cultures. Significant changes in lipid metabolism were also noted. The levels of lysophosphatidyl choline closely involved in immunomodulation were found higher in PL-MSCs, in contrast lysophosphatidyl ethanolamin levels were increased upon exposure to FBS. In summary, due to the significant metabolic changes caused by different serum/plasma additives, the choice of the supplement may have clinical implications.

Funding source: These studies were supported by the projects of 014 D07 940 001 -593 and THD-2018-17187 by Hacettepe University Scientific Research Coordination Unit.

Keywords: Mesenchymal stem cells, Platelet lysate, Metabolic

TSC110

MECHANICAL STRETCH INDUCES PHENOTYPE CHANGE OF DERMAL FIBROBLASTS

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Soft tissue expansion is a step in classical reconstructive surgery in which mechanical stretching induces the cell proliferation to regenerate skin. It was found that the dermis changed significantly during the process of expansion. Little has been known of the change in expanded dermis. This study aimed to explore the fibroblasts (Fbs) subpopulations upon mechanical stretching induced skin regeneration, and study the role of papillary fibroblasts (Fps) and reticular fibroblasts (Frs) in soft tissue expansion. Human skin samples were collected from patients underwent skin expansion and were divided into well-regenerated group and poorly-regenerated group based on clinical observation, and verified by histologic examination. Skin samples underwent histologic examination after H&E, picrosirius red, Masson staining and anti-PCNA staining. The Fps lineage markers and the Frs lineage markers were analyzed by staining in the two groups by qPCR and staining, which revealed different locations of the Fb subpopulations in the dermis. We examined and verified the differences of Fps and Frs in rat skin expansion model by histological and immunohistochemical staining and mRNA quantification. The well-regenerated skins and poorly-regenerated skins yielded different in histological results, notably changes in the papillary dermis of the poorly-regenerated skin, with a flattened basement membrane, a thin papillary dermis, and an increased type III/I collagen ratio. The expression of the Fps marker increased in the well-regenerated skin but decreased in the poorly regenerated skin, and the Frs markers increased was also seem in the well-regenerated skin but not in poorly-regenerated skin. Additionally, the Lrig1 expression was observed in the reticular dermis. The mRNA expression of genes related to Fps and Frs was consistent in the expanded rat skin and human skin samples, with an increase in early expansion skin and a decrease in late expansion skin. Mechanical stretching can change the papillary dermis structure and the characteristics of Fb subpopulations in skin. Under stretching stimulation, the papillary dermis in poorly-regenerated skin was greatly different and showed as “aging” skin.

Keywords: papillary fibroblasts, Mechanical stretch, reticular fibroblasts

CARDIAC

TSC114

A CRISPR/CAS9 IN VITRO HUMAN INDUCED PLURIPOTENT STEM CELL MODEL TO INVESTIGATE THE ROLE OF PERLECAN IN CARDIOVASCULAR FIBROTIC DISEASE

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Perlecan is a modular heparan sulphate proteoglycan of basement membranes and the basal laminae. The N-terminal domain I is decorated with heparan sulphate (HS) and the C-terminal, domain V contains an a1b2 integrin binding site, implicated in the renal fibrosis. This project aims to use the CRISPR/Cas9 gene-targeting system to target perlecan domains in hiPSCs to investigate functions within cardiovascular fibrosis. CRISPR/Cas9 targeting plasmids (designed/constructed with Snapgene”) were transfected into hiPSCs. Successful targets clones selected by assessing the expression of perlecan using immunocytochemistry and qPCR. After transfection and selection, we isolated heterozygous and functional homozygous clones with reduced perlecan mRNA (~50% by qPCR), and protein expression by immunofluorescence. Targeted clones maintained pluripotency and capacity to differentiate into cardiomyocytes, demonstrating that a reduced expression of perlecan had no significant effect on the ability of hiPSCs to respond to differentiation cues. Our results show that we can use CRISPR/Cas9 to successfully target the perlecan gene (HSPG2) to create clonal hiPSC lines with reduced expression, but maintain differentiation ability. These cell lines will be used to investigate fibrotic phenotypes, including activation, migration, ECM secretion/composition, as the cells differentiate into cardiomyocytes/cardiac fibroblasts and respond to fibrotic stimuli.

Keywords: Cardiac Fibrosis, CRISPR/Cas9, HSPG2

TSC119

DYNAMIC TISSUE CULTURE IN A ROTATING WALL VESSEL BIOREACTOR YIELDS FUNCTIONAL ENGINEERED CARDIAC CONSTRUCTS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS VIA ACTIVATED MTOR SIGNALING PATHWAY

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Cardiac tissue engineering aims to construct functional massive cardiac tissues for disease model or replacing severely damaged myocardium. It is crucial to culture these tissues to keep or improve their function by supply nutrient and oxygen to the whole tissue. We hypothesized that a rotating wall vessel (RWV) bioreactor can grow functional massive cardiac constructs. We

made engineered 3D cardiac tissue by seeding 2.0 × 10^6 human induced pluripotent stem cell derived cardiomyocytes on the PLGA fiber sheet. It was cultured in the RWV bioreactor for seven days (RWV group). For the control, static culture has been done. After cultivation, these tissues were evaluated by molecular biological, mechanical, and electrophysiological analyses. The RWV group demonstrated maturation of cardiomyocytes evidenced by significantly higher expression of Troponin T (TnT), sarcomeric α actinin (SAA), connexin 43 (Cx43) and myosin heavy chain 7 (MYH7) than the control by Western blots (TnT; 2.7±1.0 vs. 1.0±0.4, p<0.01, SAA; 2.1±0.7 vs. 1.0±0.2, p<0.01, Cx 43; 2.0±0.6 vs. 1.0±0.1, p<0.05, MYH7; 10.9±2.7 vs. 1.0±0.1, p<0.01). QT-PCR analysis also demonstrated that the expression of cardiac-related genes was more up-regulated in the RWV group. Motion analysis revealed that contraction velocity and deformation distance were significantly faster and longer in the RWV group than in the control (velocity; 55.2±15.3 vs. 30.1±7.4 μm/s, p<0.01, deformation distance; 5.86±1.4 vs. 3.37±0.76 μm, p<0.01). Microelectrode array demonstrated that conduction velocity was significantly faster in the RWV group than in the control (17±3.3 vs. 10±3.4 m/s, p<0.01). mTOR-related signaling pathway was more up-regulated in the RWV group than in the control (phosphorylated/total mTOR; 2.3±0.3 vs. 1.0±0.4, p<0.01, phosphorylated/total S6K; 3.2±0.9 vs. 1.0±0.3, p<0.01). RWV bioreactor produced more functional cardiac constructs with cardiac maturation related with up-regulation of mTOR-related signaling pathway.

Keywords: Cardiac tissue engineering, Induced pluripotent stem cells, Dynamic tissue culture in a rotating wall vessel bioreactor

TSC381

CRYOPRESERVATION OF HIPSC-DERIVED CARDIOMYOCYTES DOES NOT COMPROMISE THEIR MOLECULAR AND FUNCTIONAL PROPERTIES

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a powerful platform for in vitro modelling of cardiac diseases, safety pharmacology, and drug screening. All these applications require large quantities of well-characterised and standardised batches of hiPSC-CMs. Cryopreservation of hiPSC-CMs without affecting their biochemical or biophysical phenotype is essential for facilitating this, but ideally requires the cells being unchanged by the freeze-thaw procedure. We therefore compared the in vitro functional and molecular characteristics of fresh and cryopreserved hiPSC-CMs generated from multiple independent hiPSC lines. While the frozen hiPSC-CMs exhibited poorer recovery than their freshly-derived counterparts after replating, similar proportions of cardiomyocytes were retrieved when this was factored in, though for 3 out of 4 lines a greater proportion of ventricular cardiomyocytes were recovered upon cryopreservation. Moreover, electrophysiological and contractile properties of hiPSC-CMs were typically unaffected by freezing, though for one line the cryopreserved counterparts exhibited longer action potential durations. These results provide evidence that cryopreserved hiPSC-CMs retain their in vitro molecular, physiological and mechanical properties. It also establishes cryopreservation as a convenient method for stockpiling hiPSC-CMs, thereby allowing the same batch of hiPSC-CMs to be used for multiple applications and evaluations.

Keywords: hiPSC-derived cardiomyocytes, Cryopreservation, Cardiac electrophysiology

TSC386

REGENERATION OF INFARCTED SWINE HEART MUSCLE AND ABSENCE OF VENTRICULAR TACHYCARDIA FOLLOWING TRANSPLANTATION OF HUMAN EMBRYONIC STEM CELL DERIVED CARDIOVASCULAR PROGENITORS

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Human heart muscle does not regenerate after infarction. Clinical trials with adult stem cells have been deemed futile, but treatment with pluripotent stem cell (hPSC) derived cardiomyocytes (CM) has caused arrhythmia and inconsistencies in protocols and large scale falsifications have caused a crisis in regenerative cardiology (Chien et al., 2019). Multipotent cardiovascular progenitors (CVPs) have shown improvements in animal models and some early-phase clinical trials (Fernandes et al., 2015; Menasche et al., 2015). We have developed a highly reproducible differentiation protocol that allows generation of CVPs from hPSCs in a fully-defined, human laminin-based differentiation system and regenerated infarcted mouse hearts with significant improvements in cardiac function (Yap et al., 2019). The procedure when applied to infarcted pig hearts has yielded promising results. The CVPs were transplanted into the infarcted myocardium and cardiac function was measured by MRI at 1, 4 and 12 weeks post-transplantation. Significant improvement in the ejection fraction of transplanted hearts was measured as compared to controls (P<0.05). Endocardial voltage mapping revealed that left ventricle of post-transplantation pigs,

observed both normal as well as abnormal voltages in treated animals. Interestingly, the damaged regions are not completely blocked of electrical activity as evidenced by its contractility. In contrast, we measured absence of voltages in large areas in the untreated animals, suggesting complete tissue infarction and formation of a dense scar. Daily ECGs were recorded and ventricular tachyarrhythmia was observed in untreated animals, but transient atrial and sinus tachyarrhythmia were seen in treated animals. The hearts were stained using human nuclear antibody, human cardiac markers (cTNT, alpha-actinin), CD31 and Ki67 to visualise human cell organisation, vascularisation and proliferation. No tumour formation was observed as analysed by CT scans. The results demonstrated formation of new functional human muscle and absence of graft-associated tachyarrhythmia, significant improvement in cardiac function after progenitor transplantation in large animal. We are convinced that this cellular therapy is effective, safe and ready for initial clinical trials.

Funding source: National Medical Research Council (NMRC) Singapore Translational Research Investigator (STaR) Award and Tanoto Foundation to K.T. and a Khoo Postdoctoral Fellowship Award to L.Y.

Keywords: Cardiovascular Progenitor, Stem Cell Therapy, Arrhythmia

TSC403

DEFINING CELLULAR DYNAMICS OF HUMAN CARDIAC VENTRICULAR PROGENITORS DURING HEART TISSUE REPAIR

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Given the global burden of heart disease and its increasing prevalence in aging populations, the development of strategies to regenerate the human heart is among the most important challenges. The majority of cardiomyocytes in the heart exit from the cell cycle soon after birth, which limits its regenerative capacity. Under disease conditions, cardiac fibroblasts begin to remodel the myocardium by depositing excess extracellular matrix, resulting in reduced compliance of the tissue. Medical therapies that target remuscularization and fibrosis remain limited. Here, by combining human pluripotent stem cells and native non-human primate heart bio-mimics, we established a molecular roadmap of ventricular lineage committed cardiac progenitors for myocardial regeneration. We demonstrate that human ventricular progenitors specifically migrate to sites of injury, replace damaged cardiac muscle, electro-mechanically integrate, and target fibrosis by repelling fibroblasts. Single-cell RNAseq captured distinct modes of action, revealing that chemo-attraction of progenitors is mediated by CXCL12/CXCR4 signalling pathway, while fibroblast repulsion is regulated by SLIT2/ROBO1 guidance in organizing cytoskeletal dynamics. Moreover, transplantation of human ventricular progenitors in hypo-immunogenic CAG-LEA29Y transgenic pig hearts after injury substantiated their chemotactic response and their ability to generate a re-muscularized scar without arrhythmogenesis in vivo. Our study demonstrates that inherent development programs within human ventricular progenitors are sequentially activated in the context of disease, enabling the cells to identify and counteract injury. Taken together, these human ventricular progenitor cells may represent an ideal bio-therapeutic for functional heart rejuvenation.

Funding source: This work was supported by grants from the Swedish Research Council, Knut and Alice Wallenberg's Foundation, European Research Council, Jean Le Duc Foundation, and the German Research Foundation.

Keywords: Cardiac regeneration, Fibroblast, Cardiac Injury

EARLY EMBRYO

TSC133

EXOSOME-MEDIATED PHENOTYPIC SYNCHRONY OF DIFFERENTIATING CELLS

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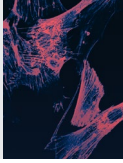
Embryonic development proceeds in a highly orchestrated manner. It is assumed that synchronization of a timing of differentiation and cell fate among neighboring cells is necessary for proper tissue development. However, the mechanism of synchronization is still largely unknown. A mouse ESC line (PKA-ESCs), in which constitutively active protein kinase A (PKA)



can be expressed with doxycycline removal (Dox-OFF), rapidly differentiated into mesoderm after PKA activation. Co-cultured control ESCs and PKA-ESCs rapidly differentiated in synchrony when PKA was activated only in PKA-ESCs, a phenomenon we named “Phenotypic Synchrony of Cells (PSyC)”. PSyC was blocked by inhibitors of exosome secretion. On the other hand, exosomes from activated PKA-ESCs specifically promoted the differentiation of control ESCs. The exosomes also promoted mesoderm differentiation in postimplantation-stage mouse embryos. We also found that miR-132 included in the exosome mediate PSyC. This study suggests a novel role of exosomes in regulation of development.

Funding source: This work was supported by JST CREST Grant Number JPMJCR17H5, Japan.

Keywords: Exosomes, Microvesicles, Extracellular vesicles, PLGA nanoparticles, Cell-cell communication, Synchronization

TSC406

HUMAN IPS CELLS EMBRYONIC BODY FORMATION AS AN ALTERNATIVE IN VITRO MODEL FOR METYLMERCURY CHLORIDE TERATOGENICITY TESTING

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Human induced pluripotent stem cells (hiPSC) provide unique non-controversial model of early development to study the effect of drugs and toxins on three germ layer formation. Embryonic Bodies (EB) obtained in 3D hiPSC culture spontaneously differentiate into the mesoderm, endoderm and ectoderm lineages. In this study we have investigated teratogenic effect of MeHgCl, well-known developmental neurotoxin on germ layer formation in 3D hiPSC culture. We have identified selected developmental markers present in growing EBs which might be vulnerable to MeHgCl during germ layer formation. In silico gene network analysis with gene function prediction performed in Genemania (https://genemania.org) identified: POU5F1, FOXA2 and PAX6 as sequence-specific DNA binding transcription factors, which play an important role in cell fate commitment. ACTA2 with PAX6 participate in of epithelial development, while NEFH and NES, together with FOXA2, PAX6 are involved in the neuron fate commitment. Above markers were chosen in this study for developmental toxicity test performed during EBs formation. The strongest cytotoxic effect of MeHgCl for EBs (as compared to the undifferentiated stage of hiPSC) was detected after 24h exposition to dose of 1µM and 0,5µM. In each tested doses of MeHgCl in EBs culture, the ROS level was significantly up-regulated (DCF-HDA) while mitochondrial membrane potential (MitoTracker Red CMXRos) was down-regulated. The low dose MeHgCl (0,001 µM) was not cytotoxic for EBs but strongly disturbed EBs three germ layer formation. The gene expression of NES (ectoderm), SOX17 (endoderm), and TBXT (mesoderm) were strongly down-regulated. Teratogenic effect of 0,001µM MeHgCl was confirmed immunocytochemically by POU5F1, FOXA2, ACTA2, PAX6, NEFH and NES markers level. DNA damage analysis and apoptosis were measured in EB by by immunodetection of gamma H2AX and Casp3 markers respectively and in low doses showed no significant difference between EB derived from control or experimental cultures. Data indicate, that during early human development mimicked by EBs, the process of three germ layer formation is sensitive to very low, not cytotoxic doses of developmental neurotoxins. Our experiments showed that markers selected in this study can be used to set up effective alternative teratogenocity test.

Funding source: This work has been supported by National Science Centre, PRELUDIUM 9 grant no UMO-2015/17/N/NZ7/04096 and statutory funds to MMRC.

Keywords: Human iPScs, Embryonic Bodies, Teratogenicity testing

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

TSC136

GALECTINS FROM HEPATIC STELLATE CELLS IN HEPATIC STEM CELL NICHE CONTRIBUTE TO LIVER REGENERATION

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As an important cellular component in the hepatic stem cell niche, hepatic stellate cells (HSCs) play critical roles in regulating the expansion of hepatic stem cells, liver regeneration or fibrogenesis. However, the signaling between HSCs and hepatic stem cells, especially that promoting hepatic stem cell expansion remains unclear. Galectins overexpression is revealed after liver injury, and their roles involved in the cell-cell interactions in the hepatic stem cell niche is suggested. In this study, 2-acetylaminofluorene plus 70% partial hepatectomy treated model rats was used to generate a liver regeneration microenvironment with hepatic oval cell response. Following liver injury, the cytokeratin 19-positive ductal cells were robustly induced near the periphery of portal tracts together with the emergence of OV6+/CD44+/CD133+/EpCAM+ hepatic stem cells. The coactivation of HSCs was identified by the recruitment of desmin-positive cells in the periportal area together with an expansion of the laminin sheath. qPCR revealed that galectins were dominantly detected and dramatically enhanced in non-parenchymal cell fraction during liver regeneration, 50.0-fold of Gal-1, and 163.7-fold of Gal-3 increased respectively at week 1, and were significantly accompanied with oval cell response. Interestingly, their expression pattern was consistent with the genes related to stemness of hepatic stem cells involved in

hepatic regeneration. Additionally, the expression of galectin-1 was enriched in HSCs localized in the periportal area. The HSCs isolated from rat liver sustained the expression of galectin-1 and -3, and enhanced the clonal colony-forming efficiency and colony size of c-Kit−CD29+CD49f+/lowCD45−Ter-119− hepatic stem cells when co-cultured in vitro. The supplement of galectin-1 recombinant protein promoted colony forming efficiency while the addition of galectin-1 or -3 blocking antibody significantly suppressed the stem cell colony formation. Our finding for the first time demonstrated that galectins derived from activated HSCs contribute to hepatic stem cell expansion during liver regeneration, suggesting that galectins serve as important stem cell niche components.

Keywords: Galectins, Hepatic stellate cells, Stem cell niche

TSC141

INVESTIGATING THE CONTRIBUTION OF LENTIVIRAL VECTOR TARGETED LIVER CELLS TO POST-NATAL GROWTH FOR IN VIVO GENE THERAPY APPLICATIONS

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Liver-directed gene therapy with adeno-associated viral (AAV) vectors delivering a clotting factor transgene into hepatocytes has shown successful results in adults with hemophilia. However, because of their non-integrating nature, AAV are diluted during liver growth, thus challenging their use in pediatric patients. We developed lentiviral vectors (LV), which integrate in the host cell genome, which achieve stable and therapeutic levels of coagulation factor IX (FIX) transgene expression in the liver of adult mice, dogs and non-human primates (NHP), after intravenous (i.v.) delivery. In view of a potential use of LV in pediatric patients, we treated 3 2-4 month-old hemophilia B dogs by i.v. administration of LV and showed long-term efficacy for up to 3.5 years, after an initial decrease of FIX activity. Longitudinal studies in mice treated as newborns with LV-luciferase showed stable total signal over time, which decreases around 3 weeks if normalized on mouse weight, similarly to what observed in dogs. By contrast, treated newborn mice with LV-GFP showed a continuous increase in size of GFP+ clusters of cells by 3D imaging, showing local proliferation and long-term maintenance of transduced hepatocytes, thus suggesting a decrease in transgene expression per cell rather than reduction of targeted hepatocytes during growth. We confirmed the same pattern in LV-FIX treated newborn mice, while administration in 2-week old mice resulted in stable and 3-fold higher FIX output compared to mice treated as neonates. We evaluated clonal proliferation of LV-transduced and untransduced hepatocytes in Alb-Cre/Rosa26-Confetti mice during growth and showed that only a fraction of cells generates continuously growing clusters, while the others appear quiescent. Moreover, we showed in vivo gene transfer in NHP ductal cells after i.v. administration of LV. These cells maintain their capacity to give rise to LV-positive organoids, which transdifferentiate in hepatocytes and produce the coagulation factor encoded by the LV. Our work will provide a rationale for the application of LV-mediated liver gene therapy to pediatric patients in order to guarantee life-long transgene maintenance and may shed light on the role of different cell populations involved in post-natal liver growth.

Keywords: Liver Stem Cells, Gene Therapy, Lentiviral Vectors

TSC146

INFUSED BONE MARROW DERIVED CELLS HAVE IMPORTANT CAPACITIES THAT REPAIR OF FIBROSIS AND PHAGOCYTOSIS OF DAMAGED CELLS

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Recently, 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 and function of infused BMC in liver cirrhosis using Electron Microscopy (EM) in recipient liver. C57BL/6 mice were injected with CCl4 twice a week for 4 weeks to make the

liver cirrhosis. GFP-positive BMC were infused from tail vein and sacrificed at 4 weeks after BMC infusion. The liver sample was fixed using both paraformaldehyde+glutaraldehyde and made epon section. We analyzed the characterization of the infused GFP-postive BMC using both EM and Immune EM (IEM). We analyzed the image of IEM, comparing with the character of positive cells by immunohistochemistry and double fluorescent staining(Antibody:GFP, EpCAM, A-6, Liv8-CD44,Liv2-hepatoblastmarker,MMP9,MMP13,AK4,CXCR4,p62,CD68,TGF-beta,alfa-SMA,transcription regulator-maternal of inhibitor of differentiation-Maid). We analyzed some kind of gene by Real-TimePCR(Gene:p16,p21,p62,EpCAM,AFP,A-6,HNF4,Sirt1,2,3,6,AK-4,Hmox,Ncam,ATF, XBP1 etc). We detected two kinds of GFP positive important BMCs in recipient cirrhosis liver using IEM method. One group of GFP positive BMCs was similar to hepatocyte in size(15-30um) and located around fiber. MMP9 positive cells, Liv8-CD44 positive cells, Maid positive cells, CXCR4 positive cells were same. These cells were round forms and different from stellate cell or Kupffer cell in feature and had the increase of lysosome structure in cytoplasm. These cells were located on fiber in hepatic cord and repaired fibrosis. The other group cells were small size (2-5um) and located in destructive area and A6 positive cells, Liv2 positive cells, EpCAM positive cells were same. These cells had high N/C ratio and smaller than hepatocyte. These cells migrated into damaged cell area and had the phagocytic capacity. These cells were few F4/80 positive cells and smaller than Kupffer cell in size. We detected two kind of infused BMCs. The round BMCs repaird liver fibrosis and the small BMCs worked the phagocytized damaged hepatocyte and maintenance of liver.

Keywords: the autologous bone marrow cells(BMC), two kind of BMCs (the round BMCs,the small BMCs), Electron Microscopy (EM)

TSC152

PROMOTING PANCREATIC KRT5+ CELL DIFFERENTIATION TO BETA CELLS THROUGH NOTCH INHIBITION IMPROVED ENDOCRINE DYSFUNTION FOLLOWING ACUTE PANCREATITIS

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In clinical circumstances, patients who survive severe acute pancreatitis (SAP) may develop prediabetes or diabetes mellitus after discharge. Thus, the recovery of endocrine function following acute pancreatitis is of great importance. In our previous study, we have demonstrated the possible role of Krt5+ cells as pancreatic stem cells in the regeneration of pancreas in murine model, thus promoting conversion of Krt5+ cells into functional beta cells may be a novel method to mitigate the development of diabetes mellitus after AP in vivo. In this study, we aimed to study the activation and differentiation of pancreatic Krt5+ cells in SAP human pancreatic tissue and AP murine model as well as to explore the role of Notch signaling on pancreatic Krt5+ cells differentiation and endocrine function recovery. Human pancreatic tissue with acute pancreatitis were obtained. In murine AP model, acute pancreatitis was induced by six hourly intraperitoneal injection of cerulein (100ug/kg) dissolved in 0.9% saline administered on 4 consecutive days. In DAPT group, the Notch inhibitor DAPT (50ug/kg) was administered right after last cerulein injection and continued for the next six days. Mice tissue collection and intraperitoneal glucose tolerance test were performed at indicated time points. Pancreatic tissue of AP patients showed necrosis and dedifferentiation of beta cells. Expanded Krt5+ cells (7.48%±1.48%) was noticed along with up-regulated activated-Notch1 and Hes1 expression, which are downstream targets of activated Notch signaling. In murine AP model, similar massive activation of Krt5+ cells were observed in both AP and DAPT group. Increase Krt5+insulin+ co-expression cells in DAPT group suggested a possibly more active conversion of Krt5+ cells to beta cells compared to AP group (12.10%±1.76% vs 8.29%±1.25%, P=0.001). Increased insulin+ cell clusters (ICCs) in DAPT group were noticed compared with AP group. Further more, intraperitoneal glucose tolerance test demonstrated a better glucose tolerance of DAPT group on day 7 and 15 day post AP compared with AP group. Our study demonstrated that Notch inhibition improved glucose tolerance probably through promoting differentiation of pancreatic Krt5+ cells to beta cells which provide a new way to attenuate the development of diabetes mellitus after AP.

Keywords: Krt5, beta cell, Notch signaling

EPITHELIAL

TSC175

CHRONIC SENESCENCE IN HUMAN MESENCHYMAL STEM CELLS REDUCED THEIR WOUND HEALING PROPERTIES AFTER EXPOSURE TO THE CHEMICAL WARFARE AGENT SULFUR MUSTARD

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Wound healing is extremely complex. A disbalance of single contributing mechanisms can result in chronic wounds especially after exposure to sulfur mustard (SM). Since mesenchymal stem cells (MSCs) are essential for wound healing, we hypothesize that SM impairs MSCs. MSCs in chronic senescence may

persist over long time periods and create a proinflammatory microenvironment. In this study, we analyzed if SM can induce senescence in MSCs and thereby affecting wound healing properties. Additionally, we investigated a possible treatment option by using already available senolytic drugs. The quality of human bone marrow derived MSCs was determined via specific cell surface markers as well their differentiation potential. After single dose exposure to SM or hydrogen peroxide, senescent cells were stained. Migration and scratch closure of a wounded area, the secretome of the cells and mRNA levels were determined 21 days post exposure. Senescent and non-senescent MSCs were treated with different senolytics and cell viability was assessed. Senescence induction was time and concentration dependent. Three weeks after single dose exposure stable senescence could be verified by various senescence markers. Morphological changes as well as reduced clonogenic and migration potential were observed. Senescent cells had prolonged scratch closure times and secreted increased levels of proinflammatory cytokines. The senolytic drug ABT-263 showed a reduced viability for senescent cells compared to non-senescent controls. In conclusion, SM single dose exposure is sufficient to induce a chronic senescence in human MSCs. Senescent MSCs may be unable to fulfil their regenerative role and be part of the wound healing disorder. Selective removal of these cells by senolytic drugs might possibly become an innovative treatment strategy for SM poisoning.

Keywords: mesenchymal stem cells, senescence, sulfur mustard

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

TSC426

PUBLIC ASSESSMENT ON STEM CELL RESEARCH: USES, AWARENESS, ATTITUDE, AND KNOWLEDGE IN THE KINGDOM OF SAUDI ARABIA

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Stem cell treatment is still in the phase of clinical trials and is not ready to be used by the public for skin regeneration, hair growth, or reducing the signs of aging. Marketable and commercial products, as well as treatments, of unproven stem cells use is an expanding issue in Saudi Arabia. We see the increasing number of products that claim to have useful stem cells for skin regeneration and to delay the signs of aging. Not only that, but also the rising number of beauty salons and centers that offer stem cell treatment (for skin or hair) with a high price. Therefore, there is a need to assess the level of public awareness regarding this issue. This survey-based report shows that a high number of people in Saudi Arabia are willing to try stem cell treatments/ products and around half of the people who took the survey are prepared to travel to countries that offer un-approved stem cell treatment. Therefore, we aim to set a baseline for health believes and to administer better behavior and knowledge which will improve the public choices and minimize the risk of stem cell tourism and commercial marketing deception.

Keywords: stem cell treatment, false advertising, unproven treatments

EYE AND RETINA

TSC195

EFFECTS OF WNT SIGNALING IN DIRECT RETINAL CELL REPROGRAMMING FROM FIBROBLASTS

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Age related macular degeneration (AMD) is an untreatable form of macular dystrophy that progresses with the death of retinal pigment epithelium (RPE). Over 200 million people will be suffering from AMD by 2020; therefore, a high quality, fast/efficient and affordable RPE cell therapy will provide a long-lasting treatment for such patients. Current ongoing clinical pluripotent stem cell (PSC)-derived RPE tests, famously from our group, are prohibitively expensive. Our group has sought affordable alternatives, one of which induces RPE directly from fibroblasts, termed iRPE. Such iRPE are generated in short time and may be potentially safe for biomedicine. We are trying to improve our iRPE system because globally iRPE direct cell reprogramming for biomedicine has not yet been meaningfully achieved. WNT signaling is a major signal transduction pathway in mammals and affects gene regulation and cell proliferation. We have tested several WNT pathway molecules and other small molecules in our iRPE system and some molecules may have improved reprogramming with respect to cell yield in terms of colony counts and diameter. Uncovering the role of WNT signaling in our current iRPE direct cell reprogramming system may improve upon cell output and quality and inform broader cell reprogramming studies.

Keywords: Direct reprogramming, Retinal pigment epithelium (RPE), Biomedicine

TSC197

A NEW ANTERIOR CHAMBER TRANSPLANTATION MODEL OF IN VIVO TUMORIOGENENICITY TEST TOWARDS IPSC DERIVED CELL THERAPY

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Since the discovery of induced pluripotent stem cells (iPS cells), stem cell research has advanced rapidly and stem cell products are now seeing therapeutic applications. The tumor forming potential of human iPSC derived cells should be examined using a suitable animal model prior to clinical administration. The current protocol for assessing tumoriogenecity in vivo involves subcutaneous transplantation, which requires over 3 months for tumors to form. Here, we established a novel tumoriogenecity test in vivo by using the anterior chamber of immunodeficient rats. Taking advantage of the unique transparent characteristic of the cornea, this method enabled us to detect the onset of tumors rapidly and accurately. First, we injected iPS cells (1x10^6 cells) mixed with Matrigel directly into the anterior chamber and found teratoma formation with in 4 weeks. We also examined the difference in teratoma formation between two iPS cell lines. To examine the sensitivity of this method, we injected 0.01%-10% Hela cells and iPS cells spiked with fibroblasts and injected into the anterior chamber of rats. During the observation period, Hela cells tumor formation was detected in 83.3% of 0.01% Hela group (1x10^2 injected) in contrast to teratoma formation of 83.3% in the iPS 1% group (1x10^5cells injected) Since transplantation of cells to the anterior chamber of the eye requires only 1 minute and can be applied to any type of cell, this model should prove useful for investigating the safety of iPSC-derived cells.

Keywords: iPSC, tumoriogenicity test, eye

TSC199

DIFFERENTIAL ACTIVITIES OF THREE BHLH TRANSCRIPTION FACTORS IN STIMULATING PROLIFERATION AND REPROGRAMMING OF MULLER GLIA IN MOUSE RETINA

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Sight is one of the most precious senses for human. In a number of retinal degenerative diseases, including glaucoma, age-related macular degeneration and retinitis pigmentosa, vision impairment is caused by irreversible retinal neuron loss. Amazingly, lost retinal neurons can be spontaneously replenished by one type of glia cells, Müller glia (MG), in fish and other lower vertebrates. Although MG plays a quiescent role in mammalian retina and goes through gliosis in response to the injury, researchers recently found that MGs of mammalian retina can be stimulated to regenerate neurons by supplementing additional growth factors and/or forced expression of certain transcription factors (TFs). Our study is focused on three proneural basic helix-loop-helix (bHLH) family TFs, Atoh7, ASCL1 and NeuroD1, which direct neurogenesis and cell type specification in retina development. Here we hope to compare their activities in stimulating MG regeneration after the completion of retina development. The TFs were packaged separately in adeno-associated virus (AAV) under the control of a MG-specific promoter, and AAV vectors were injected into the eyes of young pups or adult mice. We found that overexpression of Atoh7 or ASCL1 can stimulate MG proliferation in an ex vivo retinal degeneration model as well as in healthy mouse retinas in vivo. We used Glast-creER; Rosa-CAG-LSL-tdTomato mouse to trace the cell fate of MGs and found that as a result of Atoh7 overexpression, MGs migrate to other nuclear layers, decrease the expression of MG marker Sox9, and express early neuronal marker Otx2 in young mouse retinas. However, de-differentiation of MGs also disrupts retina morphology and causes degeneration of retinal neurons. In comparison to Atoh7, ASCL1 and NeuroD1 have more limited and no activity in driving MG de-differentiation, respectively. Our results suggest that Atoh7 can stimulate proliferation and de-differentiation of MGs, but we did not observe any specific type of retinal neurons regenerated from MGs by overexpressing Atoh7 alone. Driving MGs to terminal differentiation of retinal neurons and minimizing adverse effect caused by MG reprogramming are the main issues to be investigated.

Keywords: bHLH Transcription Factors, Müller Glia, Retina Regeneration

TSC201

GAMMA SECRETASE INHIBITOR-INDUCED HAIR CELLS GENERATION IN COCHLEAR ORGANOIDS AND ORGANOTYPIC CULTURES BY TRANSDIFFERENTIATION OF SUPPORTING CELLS

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Mechanosensory hair cells in the cochlear sensory epithelium are responsible for sound perception. These are organized in a stereotyped pattern and intercalated to supporting cells. The two cell types are specified from a common prosensory progenitor during cochlea development and Notch signaling plays a pivotal role in this fate specification. Supporting cells can act as “facultative tissue progenitors” in non-mammalian species and restore sensory perception after tissue damage. In mammals instead, hair cell are not replaced spontaneously after loss or damage, resulting in permanent hearing loss. Regenerative strategies aiming at reactivation of developmental pathways to induce tissue repair recently showed some promising effect in young postnatal animals. Therapeutic

translation of these strategies relies on a detailed understanding of tissue development, of the changes occurring during tissue maturation, as well as on the establishment of cellular assays to validate drug- or gene- based therapies . We have recently devised organoid culture methods for the expansion and in vitro differentiation of human fetal prosensory progenitors and optimized methodologies for 3D culture of somatic progenitors from the rodent sensory epithelium. Here we exploited cochlear organoids to study the efficacy of chemical inhibition of Notch signaling by gamma secretase inhibitors (GSI) to induce hair cell differentiation and regeneration after damage. GSI treatment induced an increase in hair cell numbers obtained from in vitro differentiation of supporting cells. Hair cells showed F-Actin-rich and Espin positive bundles, as well as the ability to uptake FM1-43, a surrogate readout of functional hair bundles. Moreover, they displayed susceptibility to aminoglycoside-induced damage. Interestingly, hair cell numbers were restored in GSI-treated cultures after sisomicin exposure. Similar responses were observed in explant cultures of the sensory epithelium, where GSI treatment increased outer hair cell number in the apical cochlear turn, through transdifferentiation of supporting cells. We aim to further implement this technology to obtain mature and aged phenotypes in vitro to better mimic the adult cochlear tissue and its pathologies and to assess regenerative strategies in more relevant scenarios.

Funding source: This work was supported by a Eurostars project (HEARit, Project ID: E!10491) through Innosuisse (Swiss Innovation Agency), and by the Swiss National Science Foundation (grant 162583).

Keywords: Cochlear organoids, Sensorineural hearing loss, Hair cell regeneration

TSC204

DEFINING STEM CELL DYNAMICS IN THE MOUSE OCULAR SURFACE EPITHELIUM

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Adult tissues contain label-retaining cells (LRCs), which are relatively slow-cycling and considered to be tissue stem cell (SC) s. The ocular surface, the outermost layer of the eye, is covered by the corneal and conjunctival epithelium, which is continuously renewed by SCs. LRCs in the corneal epithelium are detected in the limbus, a boundary between cornea and conjunctiva. The conjunctival epithelium contains LRCs in the fornix, the folding territory of the conjunctiva. However, the character of LRCs and the identity of SCs have been controversial due to the lack of appropriate molecular markers. Here we show that the ocular surface epithelium accommodates highly heterogeneous and spatially distinct stem/progenitor populations. By combining EdU pulse-chase analysis and lineage tracing with three CreER transgenic mouse lines: Slc1a3CreER, Dlx1CreER and K14CreER with low TM, we detect at least three distinct dynamics of epithelial cells in the cornea and conjunctiva. In the central cornea, cells are mostly non-LRCs and the number of clones declines after a short period of time, suggesting their property as short-lived progenitor cells. In contrast, the limbus has Slc1a3CreER+ long-lived SCs, which constantly produce progenitor cells to replenish the central cornea. In the conjunctiva, which consists of bulbar, fornix and palpebral, each territory is regenerated by their own SCs without migrating one region to another. Upon limbus injury, a corneal population around the limbus expands laterally but cells in the central cornea or conjunctiva do not contribute to the regeneration of limbus region. In contrast, the chemical damage of cornea leads to the activation of conjunctival cells to migrate toward the cornea, causing conjunctivalization of the eye. Taken together, our work defines multiple SC/progenitor populations in homeostasis and their behavioral changes in response to injury.

Funding source: This work was supported by AMED-PRIME, AMED (JP19gm6110016) and research grants from The Ichiro Kanehara Foundation for the Promotion of Medical Sciences and Medical Care to A.S.

Keywords: Ocular surface epithelium, Corneal stem cells, Lineage tracing

GERMLINE

TSC206

CRACKING THE DIFFERENTIATION POTENTIAL: BIOLOGICAL MODELS TO ADVANCE STEM CELL RESEARCH

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The pluripotent nature of stem cells makes them a valuable resource for research and regenerative medicine. Induced pluripotent stem cells (iPSCs), neural stem cells (NSCs) and mesenchymal stem cells (MSCs) differentiated along various lineages can provide insights into proteins involved in disease or developmental processes, which would otherwise be inaccessible for research. The extensive application of these models from developmental biology to gene therapy generates a consistent need to efficiently characterize them. We have differentiated human iPSCs and NSCs to detect and characterize proteins present in their most relevant biological models: Alpha-actinin 2 and Troponin I in cardiomyocytes, insulin in the beta cells of the pancreas, and nestin, optineurin and GFAP in neural cells. We also developed 3D organoid models of intestine and kidney which have helped to develop and validate antibodies against proteins like Sox9 and podocalyxin. Sox9 is an important transcription factor which regulates the proliferation and differentiation of mammalian intestinal epithelial stem cells. Thus, intestinal organoid was used as the most relevant biological model for Sox9 antibody validation. Similarly, since podocalyxin is expressed by the podocytes of kidney, which help in the formation and maintenance of podocyte foot processes, and is otherwise absent in undifferentiated iPSCs, the antibody

against podocalyxin was validated using kidney organoid. We have also differentiated MSCs into adipocytes, osteocytes and chondrocytes in order to study the change in expression of proteins involved in the various stages of differentiation. For example, we studied the differential expression of the key transcription factor RUNX2 in the commitment of mesenchymal progenitors to osteoblast lineage. Given their importance and wide use in stem cell research, there is an absolute need for extensively tested and specific antibodies for key markers of pluripotency and differentiation. Our data demonstrate the utility of using antibodies to characterize stem cell progeny and conversely to use differentiated cells to validate antibodies in the proper cell model. Through this holistic understanding of the target protein biology, we have developed antibodies and validated their use to support stem cell research broadly.

Funding source: ThermoFisher Scientific

Keywords: Stem cell differentiations, Antibody, Organoid, Neuron, RGC, Cardiomyocytes

HEMATOPOIETIC SYSTEM

TSC221

LSD1/KDM1A AND GFI1B ORCHESTRATE HEMATOPOIETIC EMERGENCE FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Hematopoietic development during embryogenesis is a complex and not well understood process in humans, taking place during multiple distinct waves and in different hematopoietic organs. The final, permanent site of hematopoiesis is the bone marrow (BM) responsible for lifelong blood supply. Definitive developmental sites give rise to blood cells derived from hematopoietic stem and progenitor cells (HSPCs) that arise from hemogenic endothelium (HE) through a process termed endothelial to hematopoietic transition (EHT). In mice GFI1B and its cofactor, the chromatin demethylase LSD1/KDM1A are essential to produce functional HSPCs through this transition, but their role in humans during this process is not clear. With the limited access of pre-natal human material, iPSC differentiation provide a good alternative to further understand hematopoietic emergence. Here, we aimed to unravel the role of GFI1B and LSD1 during EHT. We used patient-derived iPSCs that express a dominant negative dysfunctional GFI1B-Q287*, as well as wild type iPSCs treated with an irreversible LSD1 inhibitor. GFI1B-Q287*-iPSCs nor inhibition of LSD1 affected the formation of HE cells (CD144+/CD309+) during iPSC to hematopoietic differentiation. However, the hematopoietic committed population as well as the yield of hematopoietic progenitors were severely reduced in GFI1B-Q287*-iPSC and absent upon LSD1 inhibition. To reveal the molecular mechanisms underlying EHT related LSD1 inhibition, we performed single cell RNA sequencing (scRNAseq) on the HE population with or without LSD1 inhibition. scRNAseq showed a complete block of hematopoietic transition upon LSD1 inhibition and the absence of dynamical expression of genes associated with EHT. Furthermore, within the uncommitted HE population, we could identify gene modules that are specifically attributed to LSD1 inhibition. Based on the results we suggest that the endothelial program during EHT is partly controlled by LSD1/GFI1B. In conclusion we showed the crucial role of the LSD1/GFI1B axis in early hematopoiesis from iPSC and our scRNAseq dataset will provide further insights of the molecular events that drive hematopoiesis from iPSCs. The data paves the way towards the development of novel therapeutic/medicinal products including BM-repopulating HSCs and blood products.

Keywords: iPSC, hematopoiesis, LSD1

TSC235

TRACING ACUTE MYELOID LEUKEMIA STEM CELLS IN LONGITUDINAL PATIENT SAMPLES BY MICRORNA REPORTERS AND SINGLE CELL RNA SEQUENCING

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Acute myeloid leukemia (AML) is a hematologic malignancy with marked intra-tumoral heterogeneity (ITH). A minor fraction of blasts with stem-like features (LSC) propagates the disease and may drive relapse. We traced LSC in AML patient samples at diagnosis (n=11), early after chemotherapy (CTX) (n=6) and at relapse (n=3) by single cell RNA sequencing (scRNAseq). We chose NPM1c+ AML as a model since this mutation is reliably detectable in scRNAseq data, allowing the distinction of AML from non-leukemic hematopoietic progenitors. We previously showed that miR-126 expression closely correlates with LSC activity (Lechman et al, Cancer Cell 2016). We confirmed by limiting-dose xenotransplants that leukemia-initiating potential is strongly enriched in miR-126 high fractions of NPM1c+AMLs (n=3), allowing us to derive a transcriptional signature (miR-126S) that could serve as a surrogate LSC marker. Next, we mapped the miR-126S onto scRNAseq data. At diagnosis, miR-126S highlighted a subset of immature, quiescent blasts. Interestingly, patients with adverse outcome displayed higher abundance of miR-126S cells compared to those maintaining complete remission. Pseudotime trajectory located miR-126S cells opposite to differentiated, monocyte-like blasts, separated by an

intermediate cluster of actively cycling cells, likely representing a transitioning state between the two subsets. RNA velocity confirmed the directionality of gene-expression programs driving intermediate proliferating cells towards monocyte blast clusters along the trajectory. To investigate ITH across longitudinal samples, we performed scRNAseq of residual AML early after CTX (day 14 and 30). At day 14, blasts segregated into an LSC-like miR-126S cluster and one of actively cycling blasts, possibly a snapshot of AML re-growth. Blasts at day 30 were enriched for miR-126S and displayed cell cycle quiescence and induction of oxidative phosphorylation genes, a known mechanism of CTX resistance. At relapse, AML exhibited strong enrichment for miR-126S cells, confirming their role in the natural history of the disease. In summary, this work highlights the importance of AML ITH in clinically relevant patient samples and explores novel transcriptomic features of LSC at diagnosis and after CTX.

Keywords: acute myeloid leukemia stem cell, intra-tumoral heterogeneity, single cell RNA sequencing

IMMUNE SYSTEM

TSC237

COMPARISON OF MIGRATION AND CYTOKINE PRODUCTION ABILITIES DEPENDING ON THE ORIGIN OF HUMAN MESENCHYMAL STEM CELLS

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Human mesenchymal stem cells (MSC) are currently the only stem cell that has been approved for marketing authorization as a regenerative medical product in Japan, and is expected to be further used as starting materials for regenerative medical products in the future. The expected therapeutic effects of MSC stem from several properties, including their ability to: 1) differentiate into various cell lineages, 2) secrete soluble factors important for cell survival and proliferation, 3) modulate the immune response, and 4) migrate to the site of injury. In this study, we compared the migratory abilities and cytokine production in hypoxic state of MSCs derived from three different origin and investigated for their regulators. The migration of bone marrow-derived (BM), adipose-derived (AD), and amniotic-derived (AM) MSCs were measured by gap closure migration assay, and comprehensive analysis of gene expression and pathway analysis were performed to search for the regulatory factors of migration. Evaluation of vascular endothelial growth factor (VEGF), matrix metalloproteinase-1 (MMP-1), monocyte chemotactic protein-1 (MCP-1) and interleukin-6 (IL-6) proteins expression of each MSC in a hypoxic (1% O2) state was done using ELISA methods. The migration ability was higher in the order of AD> BM> AM. Investigation of the characteristics of the genes whose expression levels were higher in this order showed that the genes involved in cell migration and angiogenesis significantly contributed to cell migration. We have also found enhanced TGFβ signaling and altered expression levels of MMP1, involved in cell migration, angiogenesis, wound healing, and tissue remodeling. Thus, it was shown that the migration ability was different depending on the origin of MSC, and the difference in the gene expression level indicated a part of the functional characteristics of each MSC. Regarding cytokine production, all MSCs derived from three tissues increased VEGF production in response to stimulation by hypoxia. VEGF production was BM> AD, and AM was significantly affected by donor. Since each function of MSC differs depending on their origin, it may be necessary to select the origin and donor of MSC to be used depending on the purpose of treatment.

Keywords: human mesenchymal stem cells, migration, cytokine production

MUSCULOSKELETAL

TSC240

AN ENDOGENOUS MECHANISM THAT REGULATES MEMBRANE POTENTIAL OF CELLS AND PROMOTES THE COORDINATED CONTROL OF SEVERAL DEVELOPMENTAL SIGNALS TO SCALE ENTIRE FISH APPENDAGES

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Bioelectricity is demonstrating to be prominently involved in several tissue formation phenomena. However, the molecular mechanisms through which bioelectric signaling regulate these phenomena are poorly understood. We show that changes in membrane potential due to changes in potassium conductance of cells is sufficient to induce developmental programs that include the morphogen-mediated pathways shh and wnt in zebrafish adult fins, larva and embryos, indicating significance of electrophysiological changes in several biological contexts. We also provide evidence that the induction of these developmental pathways by increasing potassium conductance is cell autonomous, suggesting that the regulation of these gene in each cell is directly due to changes potassium conductance of the cell. Moreover, we observe from mosaic transplants that the ability of potassium conductance to induce developmental gene expression occurs in different and diverse tissue cell types. Furthermore, we provide a mechanism through which the calcineurin phosphatase specifically regulates the activity of the potassium channel Kcnk5b, which scales zebrafish appendages, and we show that the regulation of the Kcnk5b-mediated in vivo scaling occurs through the serine 345 in the cytoplasmic tail of the Kcnk5b. Thus, we uncovered an endogenous mechanism that controls a K+-mediated electrophysiological signal to

activate several important morphogen pathways in different contexts, including the scaling of adult fish appendages, which we offer as an important in vivo paradigm in which membrane potential acts as potent regulator of coordinated developmental signaling.

Funding source: ShanghaiTech University, Deutsche Forschungsgemeinschaft

Keywords: Bioelectricity, Membrane potential, Developmental signaling

TSC241

EFFICIENT DERIVATION AND DIFFERENTIATION OF EXPANDABLE CHONDRO-/ OSTEOGENIC-PROGENITOR CELLS (ICOPS) FROM HUMAN IPSCS USING CHEMICALLY-DEFINED AND ANIMAL ORIGIN-FREE CULTURE MEDIUM

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Cartilage has a limited capacity to regenerate itself because chondrocyte, the main constituent cell, has a limited potential to proliferate. Alternative approach to replace the cartilage by injecting chondrocytes derived from stem cells, like pluripotent stem cells (PSCs), are under development. However, long derivation period and safety issues in using animal-derived or undefined components in cell culture media remain to be solved for this approach to be applicable for clinical treatment. In this study, we characterized a novel progenitor cell, named as iPSC-derived chondro-/ osteogenic-progenitor cell (iCOP), which has highly improved long-term proliferative capacity and can be differentiated into chondrocyte in a relatively short period. iCOP was produced through sclerotome, a transient status in the course of chondrocyte formation following mesodermal somite development, using a chemically-defined and animal origin-free (CD-AOF) culture medium. iCOP could be expanded for more than 10 times per passage for over 10 passages in the expansion medium. Furthermore, the chondrocyte differentiated from iCOP showed the hyaline cartilage markers within a week. iCOP could also be cryopreserved while maintaining its proliferative capacity as well as differentiation potential, suggesting that iCOP could be a convenient intermediate for manufacturing a large number of chondrocytes for clinical use. In conclusion, we successfully established the protocol to produce chondrogenic progenitor cells from iPSCs using CD-AOF media. iCOP would be useful to develop cartilage substitutes for clinical applications in the future.

Keywords: human iPSCs, novel chondro-/ osteogenic-progenitor cells, CD-AOF culture medium

TSC244

EFFICIENT MYOGENIC DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS ON NEXT GENERATION LAMININ

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The in vitro differentiation of human induced pluripotent stem cells (hiPSCs), which have high therapeutic potentials, was designed by recapitulating embryogenesis. However, more robust methods for skeletal muscle lineage differentiation are still under development. Here, we describe a system for differentiating hiPSCs on the next generation laminin (NGL) which is a recombinant form of a laminin-421 E8 fragment conjugated to the heparan sulfate chains (HS) attachment domain of perlecan. In this system, HS bind to fibroblast growth factor-2 (FGF2), form stable high-affinity HS-FGF2-FGFR complexes on the cell surface, then strongly stimulates the FGFR signaling pathway. Recapitulating embryogenesis in hiPSCs differentiation, NGL time-dependently increases the marker genes expression of the primitive streak and paraxial mesoderm lineage, therefore, remarkably increases the population of skeletal muscle progenitors, myocytes, myotubes, and muscle stem cells. These effects depend on the unique structure of NGL, that could not be replaced by treating with the high dose of FGF2 or coating the mixture of laminin-421 E8 and perlecan. Summary, using this xeno-free matrix, we establish a highly efficient differentiation system for hiPSCs induced myocytes and muscle stem cells, thus providing an infinite source for disease modeling and regenerative medicine.

Keywords: Next generation laminin, Myogenic differentiation, Muscle stem cells

TSC255

CHARACTERIZATION OF MUSCLE STEM CELL DERIVED FROM HUMAN IPS CELLS REVEALS A NEW SURFACE MARKER FOR MYOGENIC CELL POPULATION

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Skeletal muscle tissue possesses a highly regenerative capacity, mainly because the presence of specific muscle stem cells (MuSC) called satellite cells, which can be activated after injury and regenerate the muscle. Transplantation of healthy MuSC is one of the most promising treatments for skeletal muscle diseases such as Duchenne Muscular Dystrophy (DMD). To produce cells for transplantation, induced pluripotent stem cells

(iPSCs) are a useful tool because of their expansion capacity and differentiation potential. Sakurai lab has stablished a step wise differentiation protocol to obtain iPSCs derived MuSC (iPSCs-MuSC), recently we showed that these cells resemble to fetal MuSC. Unlike their adult counterpart, fetal MuSC has not been deeply studied yet, hence we decided to study the muscle progenitor population of cells from our step wise protocol. Pax7 and Myf5 are well known myogenic cells markers and expressed in MuSC. Therefore, we stablished two reporter cells lines: Myf5+tdTomato and Pax7+venus. Pax7+ and Myf5+ cells were obtained at early stage (day 42) and late stage of myogenic differentiation (day 84). Cells were characterized in-vitro, in-vivo and at RNA level by RNA seq. We found that late stage cells were more myogenic than their early counterpart. Additionally, late stage cells showed an increase in muscle regeneration capacity in-vivo, and higher expression of fetal MuSC markers in comparison with early stage cells. Furthermore, Myf5+ late cells were more myogenic in-vivo than pax7+ late cells. By analysing the RNA seq result we were able to identify a new surface marker to sort a myogenic population of cells in our differentiation protocol. Moreover, we found that this surface protein has functions relative to cell proliferation.

Keywords: iPSC, Satellite cells, Surface marker

NEURAL

TSC286

CELL THERAPY FOR SPINAL CORD INJURY USING HUMAN IPSC-DERIVED SPINAL CORD-TYPE NEURAL PROGENITOR CELLS

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It has been demonstrated that transplanted neural progenitor cells (NPCs) derived from fetal spinal cord exerted tissue regeneration and functional recovery in spinal cord injury (SCI) compared with those from other neural regions. Therefore, original identity of the transplanted cells is critically important for spinal cord regeneration. We have previously reported a culture system to control the region-specific NPCs from human iPSCs. We generated regionalized NPCs with the forebrain (FB) and spinal cord (SC) identity from human iPSCs. The aim of this study is to examine the efficacy of these NPCs on spinal cord regeneration. We induced both FB- and SC- type NPCs, by using the neurosphere culture system as previously described with slight modifications. Contusive SCI was induced in immunodeficient mice at the level of Th10. Nine days after injury, both FB-, SC-type NPCs and PBS (Control) were injected into the lesion epicenter. Motor function was evaluated until nine weeks after injury. Histological analysis was performed using immunohistochemistry and tracing analysis. After NPCs transplantation, FB-type NPCs mainly expressed the FB marker FOXG1, whereas the SC marker HOXB4 was predominantly expressed in SC-type NPCs, suggesting that both FB- and SC-type regionalized NPCs retained their regional identities. Grafted cells mainly differentiated neurons. SC-type NPCs transplanted group had better functional recovery than PBS group. By using tracing analysis, the host BDA-labeled CST fibers integrated into the grafts and contributed to synapse formation with SC-type transplanted cells. Meanwhile, FB-type NPCs grafted mice did not display functional improvement. Histologically, the connection between FB-type NPCs and the host CST could not be detected, which could be the reason for the insufficient recovery of FB-type NPCs engraftment. We showed that the SC-type NPCs derived from human iPSCs maintained its original characteristics even after transplantation, and contributed to motor functional improvement by forming synapses between host-derived CST and transplanted cells. The present study underscores the importance of the regional identity of iPSC-derived NPCs for cell therapy towards spinal cord regeneration, and also provides practical instructions to develop the complete treatment for SCI.

Keywords: Spinal cord injury, Stem cell therapy, Regional specificity

TSC312

EFFECTS OF TRANSREPRESSION-SELECTIVE LIVER X RECEPTOR (LXR) LIGANDS ON INFLAMMASOME ACTIVATION OF MOUSE MICROGLIA AND NEURAL PROGENITOR CELLS

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It is recently reported that sustained activation of inflammasomes (cytosolic protein complexes) in microglia and neural progenitor cells (NPC) may induce neuroinflammation and impaired neurogenesis in neurodegenerative diseases. While liver X receptor (LXR) activation induces transcription of lipid metabolism related genes through a mechanism called transactivation, the activation suppresses expression of genes, such as interleukin-6 (IL-6) and IL-1beta, a mechanism called transrepression. We have developed transrepression-selective LXR ligands which have anti-inflammatory actions without causing hypertriglyceridemia. We determined the effects of transrepression-selective LXR ligands on inflammasome activation of mouse microglia and NPC. Activation of inflammasomes in 6-3 mouse microglia cell clone or mouse NPC stimulated by TNF and LPS was examined by the expression of inflammasome components (NLRP3 or caspase-1), IL-1beta, or IL-18 using Western blot analysis. The

differentiation potential of NPC into neural cells was evaluated by NeuN expression using Western blot analysis. A transrepression-selective LXR ligand (AA70 or M2-76) was pretreated prior to the stimulation by TNF and LPS. Stimulation with TNF and LPS induced caspase-1 activation and production of IL-1beta and IL-18 in mouse microglia or NPC. Pretreatment with either AA70 or M2-76 significantly inhibited the inflammasome activation. In addition, stimulation with TNF and LPS significantly suppressed NeuN expression in the differentiated NPC. Pretreatment with either AA70 or M2-76 also significantly inhibited the suppressive effect. Pretreatment of mouse microglia or NPC with the transrepression-selective LXR ligand inhibited inflammasome activation and suppression of neural differentiation by TNF and LPS.

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Keywords: inflammasome activation, transrepression-selective LXR ligand, neural differentiation

TSC315

EFFICIENTLY DIRECT CONVERSION OF HUMAN FIBROBLASTS INTO NEURONS AND DOPAMINE NEURONS BY COMBINATIONS OF TRANSGENES AND SMALL MOLECULES

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Parkinson's disease (PD) is mainly caused by the selective loss of dopamine neurons in substantia nigra of the middle brain. Cell replacement represemt a great potential for the treatment of PD. The cell sources of human neural progenitor cells (hNPCs) of fetal brain and human embryonic stem cells (hESCs) have the immuno-rejection and ethical issues. Even though the recently developed human induced pluripotent stem cells (iPS cells) overcome the immune-rejection problem, the differentiated neural stem cells and dopamine neurons of iPS cells might contain some undifferentiated cells to produce the teratoma and carcinogenicity. Recent studies have shown that human somatic cells could be directly reprogrammed to the neurons and dopamine neurons by overexpression of specific neural transcription factors. But the lentiviral vectors could be integrated into the genome of the transformed neurons and induce the carcinogenicity. In this study we constructed non-integrated episomal plasmids to overexpress Ascl1,Nurr1,Pitx3 and Fox2a and sceened for different combinations of small molecules (VPA, CHIR99021, and Repsox, Forskolin, SP600125, GO6983, and Y-27632) to directly reprogram the PD patients-derived fibroblasts to the neurons and dopamine neurns during 6-8 week inducton. We characterized the regrogrammed neurons and dopamine neurons by RT-PCR and immuno-fluerescence. We were able to convert skin fibroblasts to the neurons with efficiency of 50-70% whereas the efficiency of converted dopamine neurons is only 5-10%. These regrogrammed neurons and dopamine neurons have the similar electrophysiological functions to the fetal brain-derived neurons and dopamine neurons by the whole-cell patch clamp analysis. We are working to improve conversion of dopamine neurons from the fibroblasts by testing different combinations of transgenes and small molecules. We are also using the epigenetic modifications,RNA-seq, immuno-precipatation and chromatin immunoprecipitation (ChIP) to explore the epigenetic mechanisms of the directly regprogramed neurons and dopamine neurons from fibroblasts. This study will provide the theoretical and experimental evidence for cell transplantation therapy of PD by directly reprogrammed neurons and dopamine neurons from human fibroblasts.

Funding source: Natioanl Natural Science Foundation of China

Keywords: Parkinson's disease, direct reprogramming, Fibroblast

TSC376

TRANSPLANTATION OF HUMAN DENTAL PULP STEM CELLS AMELIORATES DIABETIC POLYNEUROPATHY VIA ANGIOGENIC AND NEUTROPHIC FACTORS

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We previously demonstrated that transplantation of cryopreserved rat DPSCs (rDPSCs) ameliorated diabetic neuropathy equal to freshly isolated rDPSCs. Human DPSCs (hDPSCs) can be obtained from teeth extracted for orthodontics reasons at a young age and can be cryopreserved until use. The aim of this study is to reveal the therapeutic mechanism of hDPSCs transplantation on diabetic polyneuropathy. We collected human impacted third molars from adults at Aichi-Gakuin University hospital. Identification of hDPSCs was analyzed by FACS and differentiation capabilities. Diabetes was induced by injection of STZ in 6 week-old BALB/cAJcl-nu/nu mice. Eight weeks after STZ injection, hDPSCs were transplanted into unilateral hindlimb skeletal muscles of normal and diabetic mice. Sciatic blood flow (SNBF), sciatic motor /sensory nerve conduction velocity (MNCV/SNCV) and current perception threshold (CPT) were measured four weeks after the transplantation. To elucidate the therapeutic mechanisms of DPSC transplantation, diabetic mice were treated with neutralizing antibody soon after transplantation. Immunohistological and gene expression analysis of hindlimb skeletal muscles were also performed at the end of the experiments. Flow cytometric analyses with two-color immunofluorescence staining showed that hDPSCs were positive for CD29, CD73, CD90 and CD105 and negative for CD45. We confirmed the differentiational ability of hDPSCs to adipocyte osteoblasts. Diabetic mice showed significant reductions in SNBF, MNCV and SNCV and increase in CPTs in the control side compared with normal mice. Transplantation of hDPSCs significantly ameliorated the impaired SNBF, MNCV, SNCV and CPTs in the hDPSCs-injected side. The transplanted hDPSCs were located around the muscle bundles and expressed the human VEGF and NGF genes. Capillary/muscle bundle ratio was also increased in the hDPSCs-injected side. Furthermore, the effects of hDPSCs transplantation were cancelled by neutralizing antibodies of VEGF and NGF. These results suggest that hDPSCs transplantation may be useful for treatment of diabetic neuropathy via the angiogenic and neurotrophic factors secreted from hDPSCs.

Keywords: dental pulp stem cells, diabetic polyneuropathy, cell transplantation

TSC384

SELECTIE DERIVATION OF HUMAN PERIPHERAL NEURONS IN THE AUTONOMIC NERVOUS SYSTEM FACILATE PRECISE CONTROL OF CARDIOMYOCYTE BEATING

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The autonomic nervous system (ANS) play pivotal roles in controlling tissue homeostasis and remodeling through opposite effects of sympathetic and parasympathetic neurons. However, the induction protocols for only sympathetic neurons from human pluripotent stem cells (hPSCs) have been developed until now. Here, we developed the stepwise induction protocol without usage of genetic engineering, in which we recapitulated the guidance signals during ANS development in vivo. Following our highly standardized stepwise protocol, both sympathetic-like neurons and parasympathetic-like neurons were simultaneously generated from human embryonic stem cells and human iPS cell (hiPSC) lines. Bulk RNA-seq analysis in the stepwise induction, single cell RNA-seq analysis, and functional assays demonstrated that these neurons acquired distinct sympathetic and parasympathetic marker expressions, and pharmacological and electrophysiological properties. In addition, we identified that cell density and neurotrophic factors were key parameters for selective differentiation of sympathetic and parasympathetic neurons. These neurons controlled beating rates of hiPSC-derived cardiomyocytes in opposing manner. These novel induction methods provide an advanced peripheral neuron specification protocol and consequently lead to precise control of multi-organ tissue functions.

Funding source: AMED under Grant Number JP19be0304324

Keywords: human pluripotent stem cells, Parasympathetic neurons, Sympathetic neurons

TSC408

IDENTIFICATION OF A NOVEL STEM/ PROGENITOR POPULATION OF THE MOUSE ADRENAL MEDULLA

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The adrenal glands regulate multiple physiological processes including the stress response, the immune system and metabolism. The adrenal is composed of an outer cortex that produces steroids, and an inner medulla that produces catecholamines. Tissue-specific stem/progenitor populations have been identified in the adrenal cortex, while the presence of a functional stem/progenitor population in the adrenal medulla is unclear. The adrenal medulla derives from the neural crest and contains chromaffin cells, neurons and sustentacular (support) cells. Establishing cell hierarchy and elucidating mechanisms of regulation of the different cell types is important to understand normal homeostasis and disease pathogenesis, such as of pheochromocytomas. Using genetic approaches in mouse, we have established that a subpopulation of sustentacular cells express the stem/progenitor marker SOX2. Through genetic lineage-tracing using the Sox2-CreERT2 strain, we demonstrate that these are an expanding population, capable of giving rise to chromaffin cells and neurons throughout life, consistent with a stem/progenitor role in vivo. We further demonstrate the self-renewal and differentiation potential of SOX2+ cells through in vitro isolation and expansion. Through analysis of FFPE sections of human adrenals, we confirm the presence of SOX2+ cells in the normal adult organ, as well as in pheochromocytomas. Taken together, our data support the identification of a previously undescribed stem/progenitor cell in the mammalian adrenal medulla, and confirm its functional relevance.

Funding source: DFG CRC/TRR205 "The Adrenal Gland: Central Relay in Health and Disease"

Keywords: adrenal, medulla, endocrinology

TSC420

A NOVEL SILK BIOENGINEERED 3D-CULTURE MODEL REPRODUCIBLY RECAPITULATES MATURE AND FUNCTIONAL HUMAN DOPAMINE NEURON IN HUMAN BRAIN ORGANOIDS

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Parkinson's disease (PD), one of the most common neurodegenerative disorders, is characterized by progressive loss of dopamine (DA) neurons in midbrain. Although the relatively focal degeneration in PD makes it a good candidate for cell-based therapies, the inaccessibility of functional human brain tissue and the inability of two-dimensional in vitro cultures to recapitulate the complexity and function of dopaminergic circuitries have made the study of human midbrain functions and dysfunctions challenging. Three-dimensional (3D) neural culture systems provide an opportunity to understand complex organization in a physiologically relevant cellular context and to better obtain functional maturity of neurons in vitro. In this study, we designed a new protocol for differentiating human pluripotent stem cells (hPSCs) into 3D human regionalized organoids that, when patterned towards a ventral midbrain (VM) fate, results in the formation of authentic and functional DA neurons. By combing CRISPR-Cas9 gene editing with transcriptional profiling at single-cell resolution, we showed that tyrosine hydroxylase neurons exhibit molecular and electrophysiological properties of mature DA neurons, expressing functional receptors of A9 and A10 neurons as well as being able to release dopamine. However, the use of a conventional 3D methodology resulted in high variability in terms of morphology, cellular composition, and inability to fully recapitulate late and functionally mature stages of human brain development. We therefore established a novel technological approach using recombinant silk protein functionalized with a cell-binding fibronectin motif to create a bioengineered scaffold that arranges hPSCs in an organ-like configuration while maintaining their self-organizing property. The silk microscale internal scaffold creates natural channels within organoids that enable the delivery of oxygen and nutrients, maintaining health of cells and preventing necrosis regardless of their size and geometric shape. In contrast to traditional 3D culture systems, bioengineered silk organoids supports highly reproducible DA organoid differentiation, leading to long-term expansion of subtype specific VM DA neurons that are transcriptionally and functionally similar to human fetal DA neurons.

Keywords: organoid, stem cell, Dopamine Neuron

NEW TECHNOLOGIES

TSC326

OOCYTE EXTRACT REPROGRAM MDA-MB-231 NOT MCF-7 BREAST CANCER CELLS TO A MORE BENIGN PHENOTYPE

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“Tumor Reversion” is a promising approach that aims to reprogram the tumor cells to lose their malignant state. Tumor reversion aims to induce growth arrest, apoptosis, or differentiation of cancer cells by means of environmental cues, and is based on restoring the epigenetic status of the cells to express tumor suppressor genes and to shut the expression of proto-oncogenes. The embryonic environment presents unique milieu for tumor reversion by inducing the loss of malignant phenotypes and/or growth arrest of cancer cells. Recently, oocyte extract from Axolotl and Xenopus species has been shown to effectively reprogram cancer cells to less malignant phenotype. In this paper, we assessed the reprograming potential of human oocyte extract (OE) on MCF-7 and MDA-MB-231 breast cancer cell lines. Our data showed that OE differentially affected breast cancer cell lines. OE induced dormancy in MDA-MB-231 as shown by the longer G0 phase, the down-regulation of G1/S cell cycle regulators, and the upregulation of cell cycle inhibitor. This was also confirmed by the increase in cellular quiescence and immunological dormancy markers, the increase in reactive oxygen species, and the inhibition of epithelial to mesenchymal transition (EMT). Furthermore, dormancy was shown by the increase of matrix stiffness and the increased formation of heterochromatin. However, OE increased the migration in MCF-7 cells as shown by the induction of EMT, the down-regulation of SMAD7, the increase in autophagosome formation, and the suppression of IL-8 and Il-10 expression. These differential effects were shown to be attributed to the TGF-beta pathway and to the change of matrix stiffness. These data are relevant for developing quiescence-promoting drug using purified oocyte extract-enriched factors to limit the symptoms of MDA-MB-231 breast cancer.

Funding source: Science and Technology Development Fund 5300

Keywords: oocyte extract, dormancy, tumor reversion

TSC331

A CHEMICALLY-DEFINED AND ANIMAL-ORIGIN FREE(CD-AOF) CULTURE SYSTEM FOR OSTEOGENIC AND CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Human mesenchymal stem cells (hMSCs) are multipotent cells that could be a promising source for regenerative medicine. hMSCs are capable of differentiating into osteogenic, chondrogenic, and adipogenic lineages. hMSC culture in vitro is typically performed in the presence of undefined components, such as fetal bovine serum (FBS), that could have large lot-to-lot variations in their biological activity. In this study, therefore, chemically-defined and animal-origin free (CD-AOF) protocols for osteogenic and chondrogenic differentiation were established using a new supplement StemFit For Differentiation (StemFit-Diff). Human bone marrow-derived MSCs cultured in the osteogenic differentiation medium including StemFit-Diff showed increased mineral deposition with higher expression of osteogenic markers in 2 weeks comparing with the cells in the conventional medium using FBS. Likewise, hMSCs differentiated into chondrocytes in the CD-AOF medium demonstrated increased Alcian blue stain with higher expression of chondrogenic markers in 3 weeks than those in the FBS medium. StemFit-Diff, combined with the “StemFit For Mesenchymal Stem Cell”, a novel CD-AOF expansion medium for hMSC, can promote clinical applications of hMSC by providing the CD-AOF culture systems for both hMSC expansion and differentiation.

Keywords: mesencymal stem cell, differentiation medium, chemically defined animal origin free

TSC339

ROLE OF FLUOROQUINOLONE CLASS OF ANTIBIOTICS IN PRECONDITIONING OF RAT BONE MARROW MESENCHYMAL STEM CELLS FOR IMPROVED THERAPEUTIC POTENTIAL

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Mesenchymal stem cells (MSCs) are known for their potential to differentiate into various cell types. However, poor viability at the injured site as well as risk of differntiation into undesired lineages are the major dilemma faced by these transplanted cells. Among various strategies utilized to overcome this problem, ex vivo preconditioning of stem cells with pharmacological agents have demonstrated great promise. Antibiotics can also be used as preconditioning agents owing to their role in immune system, complement activation and cell proliferation. The objective of this study is to examine the role of fluoroquinolone class of antibiotics in preconditioning of rat bone marrow derived MSCs. Ciprofloxacin, levofloxacin and moxifloxacin were selected respectively from second, third and fourth generation of fluoroquinolone antibiotics. Treated MSCs were subjected to oxidative stress (OS) by 10 µM dexamethasone for 4 hours, and evaluated for reduction in ROS level, cell migration and homing ability, effect on apoptotic pathway and inflammatory responses. DCF-DA assay and RT-PCR of OS responsive genes, Nrf2, SOD1, SOD2, SOD3, PRDx1, PRDx2, GPx1 and GPx2, showed significant reduction in ROS levels in case of fluoroquinolone pre-treated cells. Downregulation of IL-1β and IFNγ genes implies reduced inflammatory response to OS in preconditioned MSCs. Scratch assay also showed improvement in migration potential of preconditioned MSCs under OS after 24, 48 and 72 hours. Considerable decrease in the number of early and late apoptotic cells after 3 days of treatment suggests negative modulation of apoptotic pathways. As hypothesized, fluoroquinolone preconditioning of MSCs increased survival and homing of stem cells during oxidative stress. Consequently, this preconditioning strategy may help in optimizing clinical application of stem cell therapy to maximize its potential in regenerative medicine.

Keywords: Preconditioning, MSCs, Fluoroquinolone

TSC341

SOFT SUBSTRATES PROMOTE SELF-RENEWAL OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN BFGF REDUCED CONDITION

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To achieve the cell therapies using patient cells, we have to prepare the large number of cells. However, the somatic cells have a growth limitation. As a cell source for the cell therapy, many researchers have focused on human induced pluripotent stem cells (hiPSCs). The hiPSCs has two characters, 1) un-limited growth capability, 2) pluripotency. Although the hiPSCs are promised as a cell source, maintaining undifferentiated state with low cultivation cost has been a major challenge as hiPSCs cultured in basic fibroblast growth factor (bFGF) conditions is costly, spontaneously differentiation, unstable expression of pluripotency genes. In resent year, many researchers have reported the physical stimulation can be altered of stem cell fate and behaviors. In this study, we cultured of the hiPSCs on the various stiffness substrates as a physical stimulation. Interestingly, the hiPSCs cultured on soft substrate without bFGF for 5 days were maintained Alkakine Phosphatase (AP) activities, expression of pluripotent makers and differentiation capability to the three germ layers. Our findings demonstrate the possibility that hiPSCs self-renewal and pluripotency can be maintained using physical stimulations without expensive cytokines.

Funding source: This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion Science, Japan (Nos. 18K14063).

Keywords: Human induced pluripotent stem cell, Soft substrate, Mechanobiology

TSC349

NANOFIBRILLAR CELLULOSE AS A MATRIX FOR 3D HUMAN STEM CELL MAINTENANCE, PROLIFERATION AND DIFFERENTIATION

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In the pursuit of biologically relevant in vitro cell models, new materials are being developed for their ability to provide a well-defined three-dimensional (3D) extracellular environment with improved functionality and application for the model in question. Nanofibrillar cellulose (NFC) hydrogel,GrowDex® and anionic NFC (aNFC), GrowDex®-T derived from the Birch tree, are animal free hydrogels which have been shown to provide an effective support matrix for culturing various cell types in 3D, namely hPSCs and MSCs. 1) Cell colonies from hESCs (WA07) and hiPSCs (iPS(IMR90)-4) were embedded in 0.5% GrowDex (UPM) in mTeSR1 (STEMCELL Technologies) media and culture expanded in 96-well plates for up to 26 days. Pluripotency was analysed with OCT4 and SSEA-4 marker expression, in vitro EB-mediated differentiation, and teratoma assay. hESCs and hiPSCs proliferated in GrowDex without feeder cells, formed spheroids with 100-200 µm diameter and cells retained their pluripotency throughout the 26 day study. With the use of an atomic force microscope (AFM)-based colloidal probe microscopy (CPM), negligible interactive effects of NFC with hESCs was observed which allowed more dominant cell-cell contact, 3D spheroid formation and maintainance of pluripotency. 2) Adipose tissue derived hMSCs were embedded in 0.2% GrowDex-T in DMEM media and transferred to 24-well tissue culture inserts. Adipogenic and osteogenic differentiation were induced with StemPro™ differentiation kits (ThermoFisher) for 21 days. Analysis with Oil Red O and Alizarin Red S staining as well as ICC for the presence of osteocalcin and osteopontin by confocal microscopy was performed. Additionally, high resolution SEM and confocal microscopy was used to examine the morphology and interaction of hMSCs with aNFC. Cells within aNFC were seen to have extensive cell-matrix anchor points, with a typical fibroblast-like morphology. hMSCs also successfully differentiated along both adipogenic and osteogenic lineages in GrowDex-T. The wood-derived NFC hydrogels, GrowDex and GrowDex-T, offer a well-defined, tunable 3D culture matricies for various regenerative medicine applications. Authors would like to thank Yan-Ru Lou and Riina Harjumäki for performing the hPSC experiments.

Keywords: Nanofibrillar Cellulose, Stem Cell Maintenance, Stem Cell Proliferation

TSC375

LONG-TERM SHAKING CULTURE MAINTAINS MULTIPOTENCY AND ANTI-INFLAMMATORY PROPERTY OF MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are promising candidates for cellular therapy. However, prolonged conventional culturing as adherent cells can alter their phenotype. A previous study reported that MSC spheroids generated using suspension culture systems improved many of their properties, such as differentiation potential and inflammatory function; however, such effects on MSC spheroids maintained for long periods are unclear. Culturing cells in three-dimensional (3D) systems more closely mimics the in vivo environment, which may prevent changes in MSC characteristics that occur during long-term culture. Thus, we hypothesized that MSC spheroids could maintain their characteristics steadily in a long-term 3D culture. The objectives of this study were to establish a novel 3D culture method for MSCs and investigate their differentiation potential and inflammatory cytokine profile after long-term shaking culture. Low-affinity nerve growth factor receptor (LNGFR)+/thymus cell antigen-1 (THY1)+ human MSCs were prepared and expanded as adherent monolayers for a short period (s-MSCs: 5-9 passages) and long period (l-MSCs: 16-18 passages). Morphological changes and a reduction in proliferation and differentiation potential were observed in the long-term adherent cultures. After expansion, s-MSCs and l-MSCs were transferred to a flask and cultured under shaking 3D conditions for 21 days in neural stem cell medium to generate spheroids. Interestingly, l-MSC spheroids recovered their differentiation potential, even when generated from l-MSCs with impaired differentiation potential. VCAM-1, an MSC marker with high multipotency, and Sox2, a stemness marker, were upregulated in both s-MSC and l-MSC spheroids, suggesting that 3D culture methods improved MSC multipotency. IL-10, an anti-inflammatory marker, was upregulated in both s-MSC and l-MSC spheroids, while the pro-inflammatory marker IL-6 was down-regulated in s-MSC spheroids. These data demonstrate that MSC spheroids would have anti-inflammatory properties which may increase their therapeutic potential. In conclusion, we established a long-term shaking culture of MSC spheroids that maintains their characteristics, including multipotency and anti-inflammatory properties, even by using long-term expanded MSCs.

Funding source: Grand-in-Aid for JSPS Research Fellow Grant number JP18J21198

Keywords: Mesenchymal stem cells, 3D culture, Multipotency

PLACENTA AND UMBILICAL CORD DERIVED CELLS

TSC364

VITRONECTIN MEDIATED CELL CYCLE ARREST PREVENTS APOPTOSIS OF MESENCHYMAL STEM CELLS UNDER SERUM DEPRIVATION STRESS.

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Mesenchymal stem cell (MSCs) based therapy is currently limited due to massive cell death and low retention of exogenously administered MSCs, possible reason being the harsh microenvironment comprising of nutrient deprivation, hypoxia, inflammation, etc at the site of injury or inflammation. Hence, identifying the pro-survival factors and understanding their underlying mechanism of action is essential for improving the efficacy of MSC based therapy. In the current study, we have investigated the role of vitronectin (VTN), a multifunctional glycoprotein in the survival of umbilical cord-derived MSCs (UC-MSCs) under the condition of serum deprivation. UC-MSCs adopted thin and flattened morphology with increased adhesion as demonstrated by de-adhesion kinetics under serum deprivation. Serum deprivation led to G0/G1 phase cell cycle arrest of UC-MSCs with no remarkable apoptotic change. Upregulation in the expression of VTN was noted both at mRNA and protein levels in the serum-deprived UC-MSCs. Immunofluorescence staining also depicted increased expression, and redistribution of VTN to cytoplasm and ECM, while it was majorly localized in the nucleus under control condition. Inhibition of pro-survival PI3K pathway, further upregulated VTN expression and cells remained in G0/G1 arrest with no change in apoptotic status. VTN knockdown under serum deprivation led to G0/G1 arrest reversal with a significant loss of viability. Furthermore, knockdown of VTN under serum-deprived treatment along with PI3K inhibition led to substantial cell death. p65 knockdown resulted in downregulation of VTN and led to similar changes as shown with VTN knockdown, demonstrating an association between NF-κβ pathway and VTN and indicating NF-κβ pathway as a positive regulator of VTN expression . To summarize, VTN was demonstrated to play a novel pro-survival role in maintaining the viability of UC-MSCs by mediating cell cycle arrest under serum-deprivation stress.

Keywords: Umbilical cord-derived mesenchymal stem cell, Serum deprivation, Vitronectin

POSTER SESSION IV
14:00 – 16:00

Theme: Cellular Identity

CARDIAC

CI106

MOLECULAR MEDIATORS REGULATING SPECIFICATION OF CARDIOMYOCYTE SUB-TYPES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Precise control of lineage commitment is critical to ensure proper development in all metazoans and faulty regulation of this process underpins many diseases. In most cases, however, we lack fundamental knowledge of how cell fate. Human induced pluripotent stem cells (hiPSCs) are a powerful model for understanding the molecular basis of cell fate determination; however, in vitro differentiation of pluripotent cells results in immature cardiac phenotypes. Currently, the lack of quantitative tools has limited our ability to measure the dynamic process of cardiac lineage commitment. We generated transgenic hiPSC lines engineered for cardiac-specific expression of voltage reporters (Archon1) and calcium sensors (NIR-GECO1) in order to learn the regulatory code to distinguish specific cardiac muscle (CMs) sub-types under a range of conditions. We are now leveraging these quantitative hiPSC (qhiPSC) tools to perform CRISPR screens to identify genes that promote lineage specificity and maturation. Our recent work has focused on uncovering the metabolic signaling pathways that drive cardiomyocyte maturation. For example, CMs undergo a major metabolic shift toward fatty acid beta oxidation after as the cells become post-mitotic and lose their ability for repair in response to injury. Using our innovative qhiPSC tools combined with genetic and genomic approaches, we have begun to develop a systems level framework that will reveal the key decision points as early cardiac progenitors commit to distinct cardiac muscle sub-types. More broadly, our work will uncover key mechanisms for heart development that will facilitate new strategies to promote maturation of hiPSC-derived cell types for potential therapeutic applications.

Funding source: CBET: 0939511 National Science Foundation "NSF Science and Technology Center: Emergent Behaviors of Integrated Cellular Systems" MIT Skoltech Seed Fund "Leveraging Developmental Principles for Engineering Cardiac Tissue."

Keywords: Cardiomyocyte maturation, Voltage reporters (Archon1), Calcium sensors (NIR-GECO1)

CI110

MULTI-DIMENSIONAL MAPPING OF CELL STATES BY INTEGRATING GENE EXPRESSION AND STRUCTURAL ORGANIZATION IN HUMAN IPS CELL-DERIVED CARDIOMYOCYTES

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The Allen Institute for Cell Science is developing a state space of stem cell structural signatures to study changes in cellular organization as human induced pluripotent stem cells (hiPSCs) differentiate into cardiomyocytes. We used CRISPR/Cas9 to generate a collection of >40 endogenous fluorescently tagged hiPSC lines (www.allencell.org), each expressing a mono- or biallelic FP-tagged protein that localizes to a particular cellular structure or organelle. In addition, we have developed methods for: 1) scarless GFP-tagging of late expressing cardiomyocyte genes, including ACTN2, ssTNNI1, MYL2, MYL7 and TTN, to study the organization and morphogenesis of the contractile apparatus; 2) a robust protocol for differentiation of hiPSCs into cardiomyocytes and methods for preparing cells for imaging; and 3) image-based assays and segmentation algorithms that enable single-cell analyses of structural organization in differentiated cardiomyocytes. To investigate whether subcellular structure is related to gene expression in cardiomyocytes, we performed single-cell RNA sequencing to identify differentially expressed genes across cardiomyocyte populations collected at D12, D24 and D90. We identified a subset of genes that could distinguish the stage of differentiation, which included known myosin heavy chain isoforms MYH6 and MYH7. We also developed machine learning methods for classifying and quantifying sarcomeric organization in populations of single cells across populations. To determine whether these changes in gene expression correspond to changes to structural changes during sarcomere organization, we combined high resolution imaging using the mEGFP-ACTN2 cell line with multiplexed RNA-FISH, cell by cell, across these cell populations. Interestingly, we found that structural organization does not correlate well with gene expression state (ie. of MYH6 or MYH7), suggesting that gene expression is not predictive of structural organization. Our study highlights the power of integrating single cell transcriptomic data with quantitative structural imaging to classify cell states.

Keywords: sarcomere organization, RNA FISH, cell states

EARLY EMBRYO

CI116

LIVE VISUALIZATION OF ERK ACTIVITY REVEALS LINEAGE SPECIFIC SIGNALING DYNAMICS IN THE MOUSE BLASTOCYST

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Extracellular stimuli and cell intrinsic regulators together modulate signaling activity to pattern tissues during development. Genetic and pharmacological evidence indicates that FGF/ERK signaling is crucial for self-organized patterning of the mouse blastocyst, but ERK activity has never been directly visualized in this context. We therefore generated a targeted mouse line expressing an ERK-kinase translocation reporter (KTR). Live imaging of this biosensor in embryos revealed ERK activities in unprecedented detail and at single-cell resolution. Spatially graded ERK activity was observed in the polar and mural trophectoderm prior to overt differentiation. Within the inner cell mass (ICM) all cell types relayed FGF/ERK signals. Primitive endoderm cells had higher overall levels of ERK activity, while pluripotent epiblast cells exhibited lower basal activity with sporadic pulses. These results constitute the first instantaneous direct visualization of the signaling events underlying self-organized mammalian pre-implantation development and reveal the existence of spatial and temporal lineage specific dynamics.

Funding source: This work was supported by grants from the National Institutes of Health (NIH) to A.-K.H. (R01DK084391, R01HD094868 and P30CA008748) and NYSTEM (C029568) and C.S.S. was supported by a training award from NYSTEM (C32599GG).

Keywords: Blastocyst, ERK, Biosensor

CI123

GENETIC SCREEN IN HUMAN PLURIPOTENT STEM CELLS IDENTIFIES A MAJOR EPIGENETIC FACTOR REGULATING EXIT FROM PLURIPOTENCY AND TUMORIGENICTY

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Chromatin regulators play fundamental roles in controlling pluripotency and differentiation. We examined the effect of mutations in 703 genes from nearly 70 chromatin-modifying complexes. The vast majority of chromatin-associated complexes are essential for embryonic stem cell (ESC) growth, however, the only complexes that showed growth advantage upon mutation of their members, were the repressive complexes LSD-CoREST and BHC. Both complexes include the most potent growth-restricting chromatin-related protein, ZMYM2. Interestingly, while ZMYM2 expression is rather low in human blastocysts, its expression peaks in primed ESCs and is again down-regulated upon differentiation. ZMYM2-null ESCs over-express pluripotency genes and show genome wide promotor localized histone-H3 hyper-acetylation. These mutant cells were also refractory to differentiate in vitro and essentially failed to produce teratomas upon their injection into immunodeficient mice. Our results suggest a central role for ZMYM2 in the transcriptional regulation of the undifferentiated state and in the exit-from-pluripotency of human ESCs.

Keywords: Pluripotency, Teratoma, epigenetics

CI125

MAINTAINING PLURIPOTENCY BY ACTIVE REGULATORY ELEMENT INACTIVATION

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Pluripotency is a short-lived state in vivo and cultured pluripotent stem cells rapidly differentiate upon removal of stringent culture conditions. Chromatin studies revealed these pro-differentiation regulatory elements are not entirely silenced but maintained at a poised state to be rapidly activated. However, the pluripotency regulatory network transcription factors (TFs) responsible for maintaining these regulatory sequences inactive remain obscure. We developed a functional CRISPR-based screen to identify TFs required to maintain pluripotency categorizing them into core and accessory genes. The screen identified ZBTB11 and ZFP131 (C2H2 Zinc Finger BTB/POZ repressor TFs) as core pluripotency TFs, receiving the same priority score as Oct4 and Nanog. ZBTB11 and ZFP131 are expressed in the early human and mouse embryo, pluripotent stem cells, and induced during reprogramming. ZBTB11 and ZFP131 mutations cause rapid embryonic stem cell (ESC) differentiation into all germ layers, suggesting the simultaneous repression of several differentiation pathways. ZBTB11 and ZFP131 do not bind with core pluripotency TFs, nor at Polycomb-repressed domains. These TF bind to accessible regulatory elements of pro-differentiation genes along with histone deacetylase complex members. Loss of ZBTB11 and ZFP131 cause pro-differentiation genes to lose their posed bivalent state, acquiring active histone modifications. Of note, genetic ablation or chemical histone deacetylases inhibition induces embryonic stem cell differentiation phenocopying these TF mutations. Together, our data is consistent with ZBTB11 and ZFP131 actively deactivating pro-differentiation genes that can then be rapidly activated upon differentiation. Traditionally, the core pluripotency gene regulatory network is viewed as an interlocked positive feedback loop. We believe ZBTB11 and

ZFP131 are the repressive arm of the pluripotency regulatory network that recruits negative chromatin modifies repressing differentiation.

Funding source: 1R01HD079682

Keywords: PLURIPOTENCY, TRANSCRIPTION FACTOR, GENETIC SCREEN

CI129

AUTHENTIC TROPHECTODERM LINEAGE SEGREGATION FROM HUMAN NAÏVE PLURIPOTENT STEM CELLS

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Trophectoderm (TE) is the first cell lineage formed in the early mammalian embryo. Morphological segregation of trophectoderm and inner cell mass (ICM) marks the initiation of blastocyst development. Within the ICM the naïve epiblast subsequently emerges. Classical mouse embryology has established a sequence of lineage bifurcations underpinning early mammalian development. Consistent with this paradigm, mouse embryonic stem cells originating from the epiblast have lost the capacity to generate extraembryonic trophectoderm. However, we report here that human naïve pluripotent stem cells (PSCs) readily produce trophoblast. Inhibition of the Erk pathway is central to naïve stem cell self-renewal, but remarkably is also instrumental in trophectoderm induction. Nodal inhibition enhances differentiation and BMP signalling is not engaged. Transcriptome analyses authenticate trophoblast fate and furthermore expose a trajectory via reversion to early inner cell mass. It has been reported that conventional primed and expanded potential human PSCs may be induced to differentiate into trophoblast. We show by comprehensive transcriptomic comparisons with embryonic tissues that those PSCs actually generate amnion, not trophectoderm. We further show that amnion induction is dependent on BMP signaling whereas trophectoderm induction from naïve cells is unaffected by BMP inhibition. Finally, we reveal that human ICMs isolated from expanded blastocyst by immunosurgery remain poised to produce trophectoderm with no requirement for BMP. We conclude that human naïve stem cells and ICM/epiblast retain developmental potential to produce trophectoderm. On the other hand, consistent with a post-implantation developmental program, primed PSCs have lost this potential and instead give rise to amniotic epithelium. These findings indicate that trophectoderm potency is an exclusive regulative feature of emergent pluripotency in primates. We speculate that lineage plasticity in the blastocyst may be significant for human reproductive efficiency.

Keywords: Human pluripotent stem cells, trophoblast, Erk signaling

CI138

INTRACELLULAR CALCIUM HOMEOSTASIS MEDIATES EXIT FROM NAIVE PLURIPOTENCY

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Progression through states of pluripotency is required for cells in early mammalian embryos to transition away from heightened self-renewal and toward competency for lineage specification. This transition is referred to as the exit from naïve pluripotency and coincides with embryo implantation, which can be studied in vitro using mouse embryonic stem cells (ESC). To identify novel genes required for exit out of naive pluripotency, we used a CRISPR knockout mutagenesis screen targeting the mouse genome with ~90k unique sgRNAs. The screen yielded 30 high confidence candidates (FDR<5%) in both expected and unexpected aspects of cell biology. Unexpectedly, we identified a role for intracellular Ca2+ homeostasis during exit out of the naïve state of pluripotency. Mutation of a plasma membrane Ca2+ pump encoded by Atp2b1 increased intracellular Ca2+ such that it overcame effects of intracellular Ca2+ reduction, which is required for naïve exit. Persistent self-renewal of ESC was supported in both Atp2b1-/- Tcf7l1-/- double knockout ESC passaged in defined media alone (no LIF or inhibitors) and in wildtype cells passaged in media containing only calcitonin and a GSK3 inhibitor. These new findings suggest a central role for intracellular Ca2+ in safeguarding naïve pluripotency, which may impact the derivation of therapeutic cellular products from pluripotent cells.

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Keywords: pluripotency, calcium, Atp2b1

CI145

MICROGRAVITY SUSTAIN SELF-RENEWAL AND ENHANCED PROLIFERATION OF HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESC) are a subject of great interest due to their potential applications in regenerative medicine. They are defined as pluripotent cells that have the capacity to self-renew and to generate all cell types of the body. Understanding the mechanisms that regulate the fate of hESCs will advance their use in biomedical applications. Gravity is a physical force that affects the physiological functions of cells, tissue and organs. There are several studies indicating that self-renewal of stem cells is enhanced in microgravity conditions. However, there is not report on the effects of simulated microgravity (SMG) on hESCs. In this study, we used SMG to

investigate its biological effect on hESCs. Undifferentiated H1 hESCs were cultured on PDMS-made microchannels coated with Matrigel and exposed to 0.02g SMG up to 96 h. The SMG was done replicating a model that uses modifications of a rotary cell culture system (RCCS) in which cells are located in the axes of rotation. Our results showed that SMG helps maintain cell viability, morphology, and stemness, and differentiation potentiality of hESCs, as indicated by the expression of hESC's markers: Oct4, Sox2, Nanog, Tra-1-60, Tra-1-81, SEEA4 and SSEA3. However, compared to cells cultured under 1g gravity (1G) condition, hESCs cultured under SMG condition had a significantly increased total number of cells. Analysis of cell cycle regulating Cyclin-dependent kinases (Cdks), their positive regulators (Cyclins) and negative regulators (Cdk Inhibitors), indicates that Cdk 2/4 expression is central in enhancing cell proliferation and maintaining self-renewal of hESCs in SMG. Future experiments will elucidate whether increased cell proliferation observed in SMG is due also to increase self-renewal capacity, and whether SMG could enhance the reprogramming efficiency of terminally differentiated cells towards induced pluripotent stem cells (iPSCs). In conclusion, SMG maintains self-renewal of hESCs with higher cell proliferation, which is modulated by increased activity of cell cycle regulating kinases.

Funding source: OU's CBR REF

Keywords: Human Embryonic Stem Cells, Microgravity, Proliferation

CI146

INTERPRETING THE ROLE OF INTEGRIN-LINKED KINASE (ILK) IN SELF-RENEWAL OF HUMAN PLURIPOTENT STEM CELLS

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Stem cells participate in the growth, preservation and repair of organs and tissues through an array of mechanisms, including the preservation of undifferentiated populations. Integrin-Linked Kinase (ILK) is a protein kinase regulated by interactions in the extracellular matrix via direct binding to integrins. ILK acts as a scaffold protein, while also conveying intracellular signals tied to gene transcription. Previous research indicates ILK's roles in sustaining self-renewal throughout a variety of stem cell populations, including cardiac stem cells, epidermal stem cells and breast cancer stem cells; however, ILK's role in human Embryonic Stem Cells (hESCs), is not yet explicated. ILK's presence is usually tied to the cytoplasm; yet, our data, obtained through Western Blot analysis, nuclear and cytoplasmic fractions and immunocytochemistry of undifferentiated hESCs, indicates ILK's presence in both the nucleus and cytoplasm of ESCs, clearly contrasting the expression of ILK in fibroblasts and cardiac cells derived from the differentiation of hESCs. It has been reported that nuclear ILK participates in Notch1-1C proteasomal degradation by interacting with the ubiquitinase Fbw7. Thus, we hypothesize that, in hESCs, nuclear ILK is involved in the degradation of Notch1 signaling, therefore leading to inhibition of its transcriptional activity, and ultimately differentiation prevention. Our analysis detected Fbw7 in the nuclei of fibroblasts and hESCs, with absence in cardiac cells differentiated from them. The specific nuclear co-localization of ILK and Fbw7, and the lack of transcriptional activity of Notch1 signaling in hESCs, support our hypothesis. Further studies will be performed to confirm that interactions between ILK and Fbw7 degrade Notch1 in hESCs, shedding light on novel proteasomal mechanisms involved in regulating the transcriptional activity of pathways that direct hESC differentiation.

Funding source: OU's CBR REF

Keywords: Human Pluripotent Stem Cells, Integrin-Linked Kinase, Self-Renewal

CI167

GENE REGULATORY LANDSCAPES IN HUMAN NAÏVE AND PRIMED PLURIPOTENT STEM CELLS

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During early human development, pluripotent cells transit from a naïve to a primed pluripotent stem cell state as they progress from pre- to post-implantation stages. Primed and naïve pluripotent stem cells (ESCs) can also be cultured in vitro, but the degree to which naïve ESCs share features of naïve cells in the embryo is not clear. Moreover, the gene regulatory networks underlying naïve pluripotency remain enigmatic. Here we combine single cell RNA sequencing data and computational tools to comprehensively define, with single cell resolution, the activity of gene regulatory networks in primed and naive human ESCs. Specifically, sequencing data from 431 primed and 474 naïve (t2iLGoY) single ESCs, were analysed using single-cell regulatory network inference and clustering (SCENIC). Here we will present our results on the activity of resultant gene regulatory networks, and highlight networks specific to primed and naïve ESCs. This analysis not only recovers expected transcriptional regulators but also identifies new potential regulators. We will also present our progress on experiments to validate the extent to which the identified transcriptional regulators are required for the induction and maintenance of naïve pluripotency.

Keywords: Naive pluripotency, Gene regulatory networks, Single cell-omics

CI169

THE PARENT-OF-ORIGIN DIRECTED GENE EXPRESSION STATUS OF MOUSE BLASTOCYST

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Parent-of-origin specific gene expression depends on epigenetic marks (imprints), which are asymmetrically distributed between maternal and paternal mammalian genomes. Genomic imprints correspond to DNA methylation or to recently reported allele specific H3K27me3. However, current maps of the imprinting landscape in early embryos are likely incomplete. We therefore set out to functionally and physically map the parent-of-origin-specific gene expression landscape in mouse preimplantation embryos. Transcriptome profiling of blastocysts from genetically distinct reciprocal crosses revealed novel uniparentally expressed genes. We validated some candidates in independent blastocysts, and observed that all tested genes lose their imprinted status upon implantation. To identify the epigenetic mechanisms underlying the monoallelic expression of those novel imprinted genes, we performed micro-whole-genome bisulfite sequencing on uniparental blastocysts, uncovering 859 differentially methylated regions (DMRs). Some novel uniparentally expressed genes lie near a subset of those DMRs. A further few candidates have previously been associated with the H3K27me3 mark. Among those, we confirmed the uniparental expression of the formative pluripotency marker Otx2, highlighting a link between genomic imprinting and pluripotent state transitions. Importantly, the vast majority of our candidates was associated neither with a DMR nor with H3K27me3, suggesting the existence of still unknown epigenetic mechanisms. Many known imprinted genes are located in clusters. We assigned novel imprinted genes to existing clusters, and further identified new clusters. Importantly, one of those is associated with a DMR and an imprinted lncRNA and contains the Wrap53 gene, a regulator of p53 activity. We propose a role for this blastocyst specific imprint for regulating cellular homeostasis. Our data suggest a complex programme involving several tiers of developmental stage specific imprinting.

Keywords: mouse preimplantation development, imprinting, DNA methylation

CI174

INO80-DEPENDENT H2A.Z OCCUPANCY PROMOTES THE ESTABLISHMENT OF BIVALENCY AND MAINTAINS DEVELOPMENTAL GENES IN A POISED STATE

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The INO80 chromatin remodeling complex is critically involved in transcription and gene regulation in development and disease. To fully understand its function in pluripotent stem cells, we derived mouse embryonic stem cells with conditional deletion of the core SWI/SNF ATPase Ino80, and we examined the impact of Ino80 deletion in the naïve and primed pluripotent states. We found that Ino80 deletion in the naïve state has minimal impact on cell morphology, growth, survival, and gene expression. In contrast, Ino80 deletion in the primed state resulted in significant cellular differentiation, increased cell death, and up-regulation of developmental genes. Mechanistically, we showed that INO80 occupies gene promoter regions in the primed state, especially those that are marked by the bivalent histone marks H3K4me3 and H3K27me3. In addition, INO80 is required for the histone variant H2A.Z occupancy at these bivalent loci, contradictory to its known involvement in H2A.Z eviction in yeast. In the absence of INO80, the reduced H2A.Z level impairs the recruitment of the polycomb repressive complex 2 (PRC2) and the installation of H3K27me3, resulting in the aberrant activation of downstream genes. Importantly, INO80 already occupies the developmental gene promoters in the naïve state. Upon the transition to the primed state, it promotes H2A.Z deposition and facilitates the formation of the H3K27me3 and bivalent domains. The INO80-bound bivalent genes are initially poised and only become fully activated during later development. Together, our results suggest that INO80-dependent H2A.Z occupancy plays a critical role in the establishment of the bivalent chromatin structure, and uncover a novel epigenetic mechanism by which INO80 regulates gene expression and developmental potential in stem cells.

Keywords: INO80, H2A.Z, Bivalent

CI329

LARGE-SCALE ANALYSIS OF LOSS OF IMPRINTING IN NAIVE HUMAN PLURIPOTENT STEM CELLS

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Naïve human pluripotent stem cells (hPSC) hold great potential for studying molecular processes and cell fate decisions that occur in the human pre-implantation embryo. Genomic imprinting is a parent-of-origin dependent monoallelic expression of a subset of genes and is required for normal growth and development. Previous studies showed that the conversion of primed hPSC into naive pluripotency is accompanied by genome-wide loss of methylation that includes imprinted loci. However, whether the loss of methylation in the imprinted loci causes the aberrant bi-allelic expression of imprinted genes and the extent of this imprint-loss are still unknown. Here we analyze loss of imprinting in over 100 RNA-seq samples of naive and primed hPSC from 14 different studies. Naive hPSC show much higher levels of loss of imprinting than their primed counterparts, and the extent of this loss is not different between embryonic stem cells and induced pluripotent stem cells. Interestingly, loss of imprinting is not random and shows a clear bias towards certain genes. Specifically, paternal methylated genes are more prone to lose

their imprinting than maternal methylated genes. Importantly, different protocols used for the primed-to-naive conversion exhibit different extents of loss of imprinting, with protocols that contain JNKi and p38i giving lower levels of loss of imprinting. Our analysis reveals recurring patterns of loss of imprinting that may point towards molecular mechanisms which are linked to the imprinting process. We suggest that specific conversion conditions may reduce the levels of loss of imprinting, which can in turn help to establish better models for the investigation of the early pre-implantation human embryo.

Keywords: Human pluripotent stem cells, Genomic imprinting, Epigenetics

CI338

THE PRESENCE AND ROLE OF NODAL-GDF3 HETERODIMER IN HUMAN PLURIPOTENT STEM CELLS

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Stem cells are defined by their ability to self-renew, but only a minority sub-population in human pluripotent stem cell (hPSC) cultures display this property. We have isolated and characterized this sub-population, which resembles the early post-implantation epiblast in its gene expression and metabolic activity. This self-renewing sub-population expresses high levels of transcripts for the TGF-beta superfamily members NODAL and GDF3, as well as receptors for these factors. Although Nodal is known to be essential for the maintenance of pluripotency in the mouse peri-implantation epiblast, Activin, another TGF-β factor, is used to maintain pluripotency of hESCs in vitro because recombinant Nodal has low biological activity. It is widely assumed, though not proven, that Nodal and Activin are bioequivalent. However, recent studies in zebrafish suggest that Nodal functions as a heterodimer with GDF3. In this study we report the presence and isolation of Nodal and GDF3 in the extracellular matrix of both genetically engineered HEK293 cells overexpressing NODAL/GDF3 cells, and in the extracellular matrix of hPSCs. In Nodal/GDF3 overexpressing HEK293 cells, immunoprecipitation studies show that NODAL and GDF3 exist as a heterodimer. Extracellular matrix preparations from HEK293 cells expressing the NODAL/GDF3 heterodimer support self-renewal of hPSC in vitro. The use of recombinant NODAL/GDF3 heterodimers to augment autocrine signaling could enable the propagation of pure cultures of self-renewing hPSC resembling the formative state of pluripotency, with possible enhancement of cloning efficiency and genetic and epigenetic stability.

Keywords: Human Pluripotent Stem Cell, Self-renewal, Nodal

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CI182

LINEAGE CONVERSION OF MOUSE MUSCLE CELLS TO AN EARLY PANCREATIC PROGENITOR REQUIRES INHIBITION OF MUSCLE MASTER REGULATOR MYOD

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Beta-cell dysfunction or loss leads to all forms of diabetes, resulting in hyperglycemia due to insufficient production of insulin. While beta-cell transplants have been successful, the shortage of donors requires finding new sources of replacement beta-cells. Although beta-like cells can be derived from stem cells, critical safety and efficacy obstacles leave this in vitro approach impractical. Tumorigenic risks inherent with using cultured pluripotent cells may be bypassed via an in vivo strategy. Direct in vivo lineage reprogramming has emerged as a potential alternative approach to replenish cell loss in degenerative diseases, such as beta-cells in diabetes. While in vivo reprograming to generate beta-cells have been achieved in mice, they have been sourced from the vital organs of the foregut endoderm. Therefore, we aim to increase the pool of cells amenable for in vivo lineage conversion. Our goal is to reprogram cells among distinct germ layers, such as skin (ectoderm), muscle, fat, and vasculature cells (mesoderm). These cells are abundant, regenerative, and non-vital, and therefore would be ideal sources of cells to be converted into beta-cells. Leveraging the zebrafish as a vertebrate platform for in vivo lineage conversion, we have identified two transcription factors, Oct4 and sox32, that can reprogram several differentiated cell types such as muscle cells and neurons (mesoderm and ectoderm) into endoderm lineages. Intriguingly, we observed that Oct4 and sox32 downregulate the expression of the muscle master regulator myod. Forcing myod expression to remain prevents induction of the endoderm program by Oct4 and sox32 in muscle cells but not in skin or neural cells, suggesting that myod loss is specifically required for in vivo reprogramming of muscle cells. Further, preliminary studies using the murine muscle cell line C2C12 shows that mis-expression of Oct4 and sox32 can also lead to an upregulation of several definitive endoderm markers such as Sox17, Foxa2 and Foxa3. With this finding, we plan to assess whether loss of myod will also be critical for reprogramming mammalian muscle cells. Fundamentally, our studies lead us to suggest that repressing the original lineage genetic program is necessary for and may enhance induced in vivo lineage conversion of muscle cells, and potentially other cell types.

Funding source: This work is supported by the Larry L. Hillblom Foundation, the Keck Foundation, and the California Institute for Regenerative Medicine.

Keywords: Transdifferentiation, Beta Cell, MyoD

CI185

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF TWO CONSERVED REGULATORY REGIONS IN THE SOX17 GENE LOCUS

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Sox17 plays an essential role in forming endoderm-derived organs, blood and lymphatic vasculature, and hematopoietic lineages. In mice, Sox17 mRNA originating from an upstream transcriptional start site (TSS) is expressed in vascular endothelium while a shorter form, initiating downstream, is expressed in definitive endoderm. A multispecies sequence alignment identified two evolutionarily conserved regions, termed CR1 and CR2, located near the upstream and downstream TSSs, respectively. To determine how each region contributes to the lineage-specific expression of Sox17, we generated mice in which CR1 (117bp) or CR2 (126bp) was deleted by CRISPR/Cas9 gene editing. CR1-null mice do not express Sox17 mRNA from the upstream TSS, are born in the expected Mendelian ratio, and transiently exhibit an increase at E9.5 of Pecam1, Tie2, Lyve1, Prox1 and Pdpn. Although these findings suggest dysregulation of lympho-vasculogenesis in early development, immunohistological analysis of highly vascularized adult organs shows grossly normal vasculature. In contrast, CR2-null embryos appear not to produce Sox17 from the downstream TSS, and are markedly growth retarded beginning at E9.5 with no embryos surviving past E11.5. CR2-null embryos exhibit pronounced abnormalities in the expression of pancreatic, hepatic, and biliary markers, and the structure of these tissues. Lung and thyroid markers, however, are normally expressed, suggesting that the loss of CR2 only impairs gut tube formation in a limited region. While both gut tube and vasculature are formed in the CR2-null mice, we have initial data showing nuclear Sox17 protein is not expressed in either tissue at E9.5, suggesting that the early cell fate decision and commitment to these lineages does not require Sox17. These findings are generally consistent with there being two alternative promoters in the Sox17 locus. The transient phenotype of CR1-null mice could result from functional redundancy with other Sox superfamily members during vasculogenesis, while the lethality of CR2-null mice clearly indicates an essential function for CR2. However, further analysis is required to fully define the spatio-temporal dynamics and complicated usage of the two conserved regions, how they regulate expression of Sox17, and whether they might also influence RNA processing.

Keywords: Sox17, Transcriptional regulation, Endoderm and Vascular Endothelium

CI186

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

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Experimental systems that enable a cell-type specific characterization of molecular phenotypes are necessary to identify and functionally annotate regulatory variants. Learning the proximate effects of these variants at multiple developmental stages (fetal and adult) is critical to fully understand the genetic contributions to health and disease. We used the iPSCORE collection of hundreds of human induced pluripotent cell (iPSC) lines derived from ethnically diverse individuals to derive "fetal-like" pancreatic progenitor cells (iPSC-PPCs). Here we present a robust standardized protocol for the derivation of iPSC-PPC lines based on the STEMdiff™ Pancreatic Progenitor kit (STEMCELL Technologies) that we have modified for scale and reproducibility. To date, we have used this protocol for 162 different differentiations from 152 individuals obtaining high-quality iPSC-PPCs. Using the optimized protocol, we obtain on average 8.06 x 107 iPSC-PPCs from one 6-well plate. The purity of the iPSC-PPCs measured by flow cytometry as PDX1+NKX6.1+ double positive cells is 52.56% (0.028 - 93.1%). To identify the cellular composition of the derived iPSC-PPCs, we selected seven iPSC-PPCs samples derived from six individuals at wide range of the PDX1+NKX6.1+ double positive cells (20.5% - 91.7%) and performed single cell RNA-seq (scRNA-seq), single nuclear ATAC-seq (snATAC-seq). We identified iPSC-PPCs, which account for >90% of cells, as well as more differentiated cell types, including mesenchymal, endothelial and endocrine cells. For all iPSC-PPCs samples we collected cell pellets for future molecular assays (RNA-seq, ATAC-seq, ChIP-seq for H3K27ac, H3K27me3, H3K4me1 and H3K4me3) as well as cryopreserved live iPSC-PPCs. To elucidate the proximate effects of regulatory variants in fetal-like pancreatic progenitor cells, we will perform integrative analysis of the proposed molecular phenotypes in conjunction with the whole-genome sequences that we have for all iPSCORE individuals. In addition, this study will result in the generation of a large-scale systematic data set that will serve as a model for the development of new statistical approaches for predicting genome function and will result in a better understanding of the molecular mechanisms by which genetic variation affects disease.

Funding source: California Institute of Regenerative Medicine (CIRM), NIDDK DK105541 and DK112155

Keywords: Human IPSC, genetics, differentiation, Pancreas, Diabetes

CI187

CHARACTERIZATION OF THE EPIGENETIC LANDSCAPE DURING DIFFERENTIATION FROM PLURIPOTENT STEM CELL TO MATURE HEPATOCYTE

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The adult liver is functioning as a detoxification organ, and thus is exposed to toxic stress numerous times during a life time. The pharmaceutical industry has a great need for human model systems for evaluation of putative toxic responses of drug metabolites and drug induced stress in the liver. In vitro cultured primary liver cells are currently used as model system, but these cells lose most of their metabolic functionality within a few days in culture rendering them a suboptimal test model. However, hepatocytes derived from human pluripotent stem cells (hPSCs) have shown promising results to be useful as an alternative test model. Therefore, further understanding of the epigenetic regulation during the differentiation is of critical importance. In this study, we compare the epigenetic landscape between human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) and their abilities to differentiate into mature hepatocytes. By applying genome-wide DNA methylation analysis using the Infinium MethylationEPIC bead chips we followed the epigenetic alterations through four defined differentiation stages; pluripotent, definitive endoderm (DE), hepatoblast and hPSC-derived hepatocyte (hPSC-HEP). Both methylation and hydroxy-methylation at these four stages were investigated to distinguish important time-dependent epigenetic differences throughout hepatic maturation. Advanced bioinformatic analysis such as multi-dimensional scaling was performed to identify the largest source of variation between the four investigated stages and results indicate that the altering of the epigenetic landscape is of great importance during the development and maturation of hepatocytes. Moreover, hierarchical clustering showed separation of hESC and hiPSC in early stages, which is of high interest to further investigate in the later differentiation stages. By studying the epigenetic landscape during the development from pluripotent cell to mature hepatocyte we gain important knowledge that can

guide further improvements of differentiation strategies towards more functional hPSC-derived hepatocytes. Our results could therefore contribute to such knowledge and provide a wider understanding, not only in the maturation of the cell, but also between different sources of pluripotent cells.

Funding source: This study was conducted under grants from the Knowledge Foundation (2016/0330) and (2018/125)

Keywords: Epigenome, Pluripotent Stem Cells, Hepatocyte

CI188

TRANSCRIPTOMIC AND EPIGENOMIC DATA INTEGRATION REVEALS THE DIFFERENTIATION TRAJECTORY FROM HUMAN PLURIPOTENT STEM CELLS TOWARDS HEPATOCYTES

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Drug development suffers from high attrition rates due to candidate drug toxicity, particular to the liver. While drug toxicity testing is possible with primary human hepatocytes in vitro, several limitations including donor availability and variation, and loss of functionality in culture impede their use. A way to circumvent these problems is to use human pluripotent stem cells (hPSC)-derived hepatocytes. To be useful for drug toxicity testing, hPSC-derived hepatocytes should recapitulate the functions of in vivo human hepatocytes, in particular the drug metabolizing machinery. In this work we performed high-throughput transcriptomic and epigenomic profiling of six hPSC lines differentiating towards hepatocytes to identify key regulatory drivers behind the differentiation process, sampled at several time points. Integrative omics analysis was performed with a matrix factorization technique called NOLAS (NOise-reduced LAtent Structures), which finds sets of features (e.g. genes and CpG sites) with statistically significant correlations of inferred latent variables (LVs). Features are selected from different omics modalities simultaneously and only significant features are used to build the LVs. The rationale behind this analysis is to filter out noisy features and identify the strongest intercorrelations within and across omics modalities. In contrast to existing approaches, which make use of all/the same set of features for every LV, NOLAS is free to select different subsets of multi-omic features when constructing its variables. This may make the models easier to interpret, given that different LVs can explain complementary biological processes. When applied to our data, NOLAS captured transcriptomic and epigenomic changes over time and clearly outlined the hPSC differentiation trajectory towards mature hepatocytes. By inspecting the strongest features selected by NOLAS, we could identify candidate genes and methylation signatures that may act as key regulators during the differentiation process. These finding provide important knowledge that will guide future improvements of hPSC differentiation protocols to develop highly functional hepatocytes for drug toxicity testing in vitro.

Keywords: hiPSC-derived hepatocytes, transcriptomic profiling, epigenetic profiling

CI191

HEPATOCYTE-LIKE CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS USING SMALL MOLECULES: A TRANSCRIPTOMIC AND FUNCTIONAL STUDY

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Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (iPSCs) hold great promise in toxicological applications as well as in regenerative medicine. Previous efforts on hepatocyte differentiation have mostly relied on the use of growth factors to recapitulate developmental signals under in vitro conditions. Recently, the use of small molecules (SMs) has emerged as an attractive tool to induce cell fate transition due to its superiority in terms of both quality and cost. In the current study, we differentiated HLCs from iPSCs using a protocol that only involves SMs and characterized gene expression changes during the course of the SM-driven differentiation using whole genome microarrays. Transcriptomic analysis of the SM-driven differentiation defined a hepatocyte differentiation track, and identified several key genes in major stages of hepatocyte differentiation. The final population of the HLCs derived using the SM protocol (SM-HLCs) not only displayed specific hepatic marker expression at both the transcriptional and the protein levels, but also demonstrated major hepatic functions, including serum protein (albumin, fibronectin, alpha-1-antitrypsin) secretion, urea synthesis, glycogen storage, and more importantly for toxicology applications, cytochrome P450 activity. In addition, SM-HLCs were scored with CellNet, a bioinformatics tool quantifying how closely engineered cell populations resemble their target cell type, and compared to primary human hepatocytes (PHHs), adult liver tissue, fetal liver tissue, HLCs differentiated using growth factors (GF-HLCs), and commercially available HLCs. Similar to GF-HLCs, SM-HLCs displayed a mixed phenotype of fetal and adult hepatocytes and had relatively low expression of metabolic enzymes, transporters and nuclear receptors compared to PHHs. Overall, the present study demonstrated the

usefulness of the SM-based hepatocyte differentiation method, offered new insights into the molecular basis of hepatogenesis and associated gene regulation, and suggested ways for further improvements in hepatocyte differentiation in order to obtain more mature HLCs that could be used in toxicological studies.

Keywords: hepatocyte differentiation, induced pluripotent stem cells (iPSCs), transcriptomics

CI195

IN VIVO MATURATION OF HUMAN PLURIPORTENT STEM CELL DERIVED BETA CELLS

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Stem cell-derived islet (SC-islet) organoids are a strong candidate for diabetes cell replacement therapy. Advances in differentiation protocols have allowed for generation of functional SC-islets from both human embryonic (hES) and induced pluripotent stem (hiPS) cells. The SC-islets consist of stem cell-derived β (SC-β), α, δ, and other pancreatic cell types. Differentiation of SC-islets in vitro generates dynamic-functioning SC-β cells similar to primary adult human islets, however, they remain transcriptionally immature. We transplanted hESC- and hiPSC-islets under the kidney capsule of mice with pre-existing diabetes and observed further maturation of the organoids. The SC-islets rapidly reversed diabetes in the mice, displaying glucose stimulated insulin secretion and euglycemic blood glucose within 2 weeks of transplantation. After six months in vivo, the SC-β cells maturate functionally demonstrated by greater insulin secretion. Furthermore, we performed single cell RNA sequencing on explanted SC-islets to uncover drastic transcriptional changes towards maturation. The grafted SC-β cells had elevated expression of key maturation genes including MAFA, SIX2, and G6PC2, which were previously underexpressed in their isogenic in vitro counterparts. We also detect reduction in misexpressed genes (MAFB, ALDH1A1) and other endocrine genes (GCG, SST) indicating further maturation and commitment to β cell fate. The grafted SC-β cells exhibit a mature transcriptome which strongly resembles 3 primary human islet donors. In addition, single cell sequencing revealed maturation of the SC-α cells to more closely resemble primary α cells. We also compared in vitro and grafted enterochromaffin and SC-δ cells. Similar results were obtained for both hESC- and hiPSC-islets. This study reveals transcriptional maturation of SC-islet organoids following transplantation and diabetes reversal in mice, providing greater understanding of endocrine cell maturation derived from hPSCs.

Funding source: NIH (5R01DK114233, T32DK108742), JDRF (5-CDA-2017-391-A-N), Washington University-Centene Personalized Medicine Initiative

Keywords: Beta Cell Maturation, Islet Organoids, Diabetes

CI323

CHROMATIN LANDSCAPE AND TRANSCRIPTION FACTOR NETWORKS DRIVING HUMAN HEPATOCYTE CELL FATE

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How the hepatocyte transcriptome is established during development, including how the underlying epigenetic dynamics control cell identity and function is unclear. This is especially important as changes in the epigenome and expression level of transcription factors rather than genomic mutations are implicated in liver diseases such as hepatocyte carcinoma. Here, we describe the changing epigenome during in vitro hepatocyte differentiation and identify missing links in the transcription factor network. By using ChIPseq against selected histone modifications at 4 timepoints during hepatocyte differentiation of iPSCs, we are able to identify genome-wide active, repressed and primed cis-regulatory regions. We found a highly dynamic epigenome with enhancers being activated and shutdown rapidly. Surprisingly, we identified only a small fraction of enhancers which are primed for activation in definitive endoderm and which are not shutdown during further differentiation towards hepatocytes. This small group of driver enhancers seems crucial for hepatic cell fate as it includes enhancer for master regulators of hepatocyte cell fate as HNF4a and CEBPa. Transcription factor motif enrichment analysis indicated TBX3 as potential regulator of the successively activated enhancers. We are currently working on a TBX3 knockdown model to test our hypothesis that TBX3 ultimately separates hepatic lineages from other definitive endoderm lineages such as the pancreas.

Funding source: This project has been funded by CIHR and Genome British Columbia.

Keywords: liver development, chromatin dynamics, transcription factors

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

CI322

TOWARDS THE DEVELOPMENT OF A HUMAN ENDOTHELIAL REPORTER TO TRACE DIFFERENTIATION IN REAL-TIME

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Effective vascularization of engineered tissues remains to be a challenge that limits longevity and functionality of these tissues. Endothelial progenitor cells (EPC) play a key role in angiogenesis through their recruitment, proliferation, and differentiation into mature endothelial cells (EC) to form functional vasculature. EPC and EC share many similar markers and primarily differ in their proliferative and clonogenic capacity. These qualities make it difficult to discern between the populations to study the differentiation dynamics. To study EPC differentiation, we propose the use of a reporter endothelial progenitor cell line that tracks the expression of a mature EC marker, endothelial nitric oxide synthase (eNOS), in real-time. To generate this cell line, several endothelial differentiation protocols were tested to produce EPC from human pluripotent stem cells (hPSC). Characterizations of the quality of the EPC are still ongoing. The genetic component of the reporter includes a constitutive promoter coupled with monomeric red fluorescent protein (mRFP) and the eNOS promoter coupled with enhanced green fluorescent protein (eGFP). To select an appropriate constitutive promoter, iPSC A18945 were transfected with plasmids containing CMV- or Ef1a-eGFP and analyzed through fluorescence microscopy and flow cytometry. iPSC transfected with Ef1α-eGFP showed higher eGFP+ populations and stronger signals compared to CMV-eGFP. Future work will focus on integrating the dual reporter system into hPSC and characterizing the differentiation into EC. This model EPC reporter system will be a valuable tool for studying vascularization strategies and it has a wide range of applications in drug screening and tissue engineering efforts.

Funding source: This research was funded by the Canadian Institutes of Health Research (CIHR). We would also like to acknowledge the networks ThéCell, PROTEO, and CQMF.

Keywords: endothelial progenitor cell, endothelial differentiation reporter, pluripotent stem cell differentiation

EPITHELIAL

CI201

INVESTIGATING THE ROLE OF MAF PROTEINS IN ENTEROCYTE FATE AND FUNCTION

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Intestinal enterocytes are the cells that absorb nutrients from our diet. Despite the fact that mice and man produce hundreds of millions of enterocytes every day, the molecular and genetic programs that lead to differentiation of this lineage are not well understood. Through transcriptomic analysis, we identified two transcription factors, cMaf and MafB that were highly enriched in intestinal villi at all developmental time points. Further, we found that these factors mark villus enterocytes, but not secretory cells or progenitor cells in the crypts. Maf family proteins play roles in differentiation of several other tissues by either promoting expression of differentiation factors and/or inhibiting cell proliferation. Misexpression of MafB in the intestinal crypt resulted in weight loss and severe morphological defects in mutant intestine. This was accompanied by crypt hypoproliferation. We are using additional loss and gain of function analysis to test whether Maf family proteins are necessary and sufficient for differentiation via promoting exit from the cell cycle, initiating expression of enterocyte specific genes, and/or promoting niche exit. This work will provide fundamental insights into the transcriptional pathways governing enterocyte fate and function.

Funding source: NIH NIDDK

Keywords: IntestinalStemCell, Differentiation, Enterocyte

HEMATOPOIETIC SYSTEM

CI214

INHIBITION OF P53-MEDIATED APOPTOSIS PROMOTES HEMATOPOIETIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED HEMOGENIC ENDOTHELIAL CELLS

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De novo generation of hematopoietic stem cells (HSCs) from human induced pluripotent stem cells (hiPSCs) could provide a virtually unlimited supply of autologous HSCs for clinical transplantation, and offer various approaches that enable gene therapy, drug discovery, disease modeling, and in vitro modeling of human hematopoietic development. However, the derivation of long-term self-renewing HSCs from hiPSCs in culture remains elusive. The tumor suppressor protein P53 plays important roles in normal and malignant hematopoiesis, and Trp53-deficient mice exhibit increased number of HSCs. However, its role in hematopoietic differentiation of hiPSCs has not been explored. In this study, we investigated the role of P53 in two stages of hematopoietic differentiation from hiPSCs: 1) differentiation of hiPSCs into hemogenic endothelial (HE) cells via embryoid body formation; 2) differentiation of HE cells into hematopoietic stem and progenitors cells (HSPCs). Although genetic deletion of TP53 in hiPSCs increased the growth of embryoid bodies, differentiation into HE cells was severely impaired and overall production of HSPCs was significantly reduced by TP53 deficiency. Thus, P53 is indispensable for differentiation of hiPSCs into HE cells. To study the role of P53 in endothelial-to-hematopoietic transition of hiPSC-derived HE cells, we utilized pifithrins, potent P53 inhibitors, to selectively inhibit the transcriptional or mitochondrial activity of P53. Inhibition of the transcriptional regulation by P53 using pifithrin-alpha and beta both impaired endothelial-to-hematopoietic transition of hiPSC-derived HE cells. In contrast, inhibition of mitochondrial-mediated regulation of apoptosis by P53 using pifithrin-mu significantly increased the number of HSPCs derived from hiPSC-derived HE cells. These results suggest that P53 positively regulates the development of HE cells, whereas the mitochondrial P53 pathway negatively regulates endothelial-to-hematopoietic transition of HE cells. Our

findings shed light on the importance of selecting hiPSC lines that retain normal P53 activity for HE differentiation, and provide an approach to promote hematopoietic differentiation of hiPSCs by pharmacological inhibition of P53-mediated apoptosis.

Keywords: Human pluripotent stem cells, Hematopoietic development, P53

IMMUNE SYSTEM

CI337

DEVELOPMENT OF HUMAN IPSC-DERIVED CD34+ CELLS, MONOCYTES, AND MESENCHYMAL STEM CELLS

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There is an unmet need for highly characterized and reproducible cell models for a wide range of applications including mechanisms of action, drug development, and toxicity testing. Human induced pluripotent stem cells (iPSCs) have the potential to differentiate into all somatic cell types and therefore hold great promise for development into cell models for variety of toxicity applications. Herein, we have developed processes for the scalable generation of iPSCs-derived CD34+ cells, monocytes, and mesenchymal stem cells (MSCs). It is well documented that starting cell types and donor background play an important role in the efficiency of iPSCs to terminally differentiated cells. We screened four iPSC lines, from different cell origin and evaluated the efficiency for differentiation into CD34+ cells and MSCs. To assess the differentiation potential of the iPSCs-derived cells, we demonstrated that CD34+ cells could be differentiated into erythroid cells, myeloid cells, and megakaryocytes while monocytes were capable of differentiating into dendritic cells and functionally active macrophages. Compared to primary MSCs, iPSCs-derived MSCs exhibited similar immunophenotypes and T-cell suppression properties as well as the ability to differentiate into adipocytes, osteoblasts, and chondrocytes. It can be problematic to obtain sufficient quantities of certain cell types, including CD34+ cells, for high throughput drug screenings. To address cell availability and donor variation issues, we have developed processes for the scalable and reproducible generation of highly pure, functionally active, assay-ready iPSCs-derived CD34+ cells, monocytes, and MSCs. Furthermore, we have validated iPSC-derived monocytes for conducting the monocyte activation assay, which can be used as an alternative to the rabbit pyrogen test for biologic drugs. Utilization of CFU-GM assays for myelotoxicity screening is hampered by the low throughput of the assay. We have explored the potential of using iPSCs-derived CD34+ for in vitro assessment of anticancer drug-induced myelotoxicity. This assay further enhances the wide variety of applications. These highly characterized iPSCs-derived cells provide as powerful tools for drug screening and toxicity testing.

Keywords: Drug screening and drug toxicity testing, iPSC derived Cell models, Assay ready cells

NEURAL

CI232

MODULATION OF HISTONE DEACETYLASE 2 (HDAC2) DRIVES NEURONAL GENE EXPRESSION, MITOCHONDRIAL DYNAMICS AND AD PATHOPHYSIOLOGY IN HUMAN STEM CELL DERIVED NEURONS.

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Alzheimer's Disease (AD) is a late onset neurodegenerative disease characterized by the presence of amyloid beta (Aβ) plaques and neurofibrillary tangles. The cellular processes that lead to this pathology are still not well understood. Human cellular models, such as those utilizing human induced pluripotent stem cells (hiPSCs), provide tools to gain mechanistic insight into the cell and molecular biology of AD pathogenesis. Epigenetic dysregulation of gene expression is implicated in AD, particularly affecting genes related to neuronal synaptic function that can affect cognition. Aberrant expression of the class I histone deacetylase HDAC2 and subsequent dysregulation of neuronal gene expression has been implicated in AD and brain aging. It has also been shown that HDAC2 influences mitochondrial dynamics and neuronal response to toxicity via modulating expression of Endophilin B1 (Endo B1), a multifunctional neuroprotective protein. In this study, we hypothesize that modulation of HDAC2 and Endo B1 in hiPSC-derived neurons will have measurable effects on neuronal gene expression, mitochondrial and AD phenotypes. We differentiated and purified neurons from hiPSCs derived from control and AD patients and characterized the effect of HDAC2 and Endo B1. Our data thus far demonstrates that knocking down HDAC2 increases the expression of genes involved in neuronal synaptic function, which corroborates work in animal models that demonstrates the role of HDAC2 in modulating cognitive phenotypes. We also observed that knock-down of HDAC2 promotes elongation of mitochondria and represses genes involved in mitochondrial fission. We performed targeted metabolomic analyses and observe a decrease in metabolites related to mitochondrial-mediated apoptosis in HDAC2 knock-down neurons. To further determine the role on HDAC2 on mitochondria bioenergetics, we are developing a Seahorse assay to measure neuronal mitochondrial metabolism, a CellROX assay to measure the effect on HDAC2 on the production of reactive oxygen species and qPCR for APP. Thus, lowering HDAC2 levels may be beneficial in neurons by simultaneously enhancing mitochondrial function

and decreasing amyloidogenic APP processing. Future work will determine if the neuroprotective effects of HDAC2 knock-down are mediated via Endo B1.

Funding source: Institute for Stem Cell and Regenerative Medicine Fellowship

Keywords: Histone deacetylase 2 (HDAC2), Metabolomics, Endophilin B1

CI240

HIGH-THROUGHPUT SCREENING OF IPSC-DERIVED NEURAL STEM CELLS FOR IDENTIFICATION OF SMALL MOLECULES THAT ENHANCE NEURONAL AND GLIAL DIFFERENTIATION

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Small molecules and recombinant proteins are currently applied to modulate relevant cell signaling pathways that can differentiate induced pluripotent stem cells (iPSCs) into specific cell types. Despite the development of several neural differentiation methods, current protocols are labor intensive, inefficient and result in immature cell populations. Improvements to the current differentiation protocols are necessary to facilitate the rapid, large-scale production of pure and functionally mature cell types. High-throughput screening combined with automated iPS cell differentiation offers a powerful platform for discovery of small molecules that can facilitate neuronal and glial differentiation. Because limited reproducibility, predictability and variance in manual cell culture can affect robustness of high-throughput screens, we developed an automated protocol for controlled differentiation of iPSCs into neural stem cells (NSCs). Using these NSCs, we then screened diverse small molecule libraries in combination with reporter cell lines in order to identify new compounds that can promote neuronal and glial differentiation. Here, we report on the identified lead compounds that induce expression of synaptic markers via epigenetic mechanisms of gene regulation.

Funding source: NIH Common Fund Regenerative Medicine Program

Keywords: neuronal, glial, screening

CI312

SPATIAL HETEROGENEITY OF MOUSE TELENCEPHALIC PROGENITORS AT SINGLE-CELL RESOLUTION

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The cellular complexity of mouse cerebral cortex has been intensively studied, yet a thorough characterization of distinct neurogenic regions of the developing telencephalon at single-cell resolution has not been described. Neurogenesis occurs within the embryonic ventricular zone (VZ) and subventricular zone (SVZ), and a detailed description of progenitors in these niches may give insight into gene pathways regulating brain patterning and initial cell fate decisions. Here, we use single-cell transcriptomics to study heterogeneity within VZ and SVZ in the dorsal telencephalon, source of glutamatergic projection neurons, and the three ganglionic eminences (GEs) that generate distinct populations of inhibitory GABAergic neurons. By harvesting Nestin-expressing cells, VZ progenitors were isolated from all four brain regions and compared to SVZ progenitors and postmitotic mantle zone cells. VZ progenitors show restricted spatio-temporal transcriptional patterns specific to their regions which subsequently lead to differentiation of heterogeneous secondary SVZ progenitors. Furthermore, early cellular response genes were differentially regulated between regions suggesting that highly dynamic morphogenic cues may be responsible for heterogeneity within the neurogenic niches and regions during development. Studies to investigate candidate genes that may regulate intra- or interregional cell fate specificity are underway. Our findings characterize the systematic landscape of transcriptional diversification in the developing telencephalon with the hope to uncover fate-determining genes that bring cellular diversification to multipotent telencephalic progenitors.

Keywords: Neural Stem Cells, Ganglionic Eminences, Single-cell RNA-sequencing

NEW TECHNOLOGIES

CI262

METABOLIC CHARACTERIZATION AND OPTIMIZATION OF PERIPHERAL SENSORY NEURON DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS

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Cellular metabolism is emerging as a critical factor in stem cell research suggesting that both metabolic status and cell culture conditions can alter the differentiation trajectory of stem cells. In-vitro live cell metabolic profiling facilitates the identification and optimization of key metabolic changes associated with stem cell differentiation, however, the measurement must be performed in a timely manner such that the entire differentiation process can be followed. Here we assess the feasibility of such a temporal workflow by following the cellular metabolic poise of induced pluripotent stem cells (iPSCs) as they differentiate to neural progenitor cells (NPCs) and on to peripheral neurons. Monitoring is initiated using singularized iPSCs cultured in 2D on XF culture plates, with real-time cellular metabolic insights achieved using the Agilent Seahorse XF analysis platform. Differentiation-associated metabolic observations were then related to differentiation markers measured using both fluorescence microscopy and PCR. These data reveal a slight reduction in glycolysis and a pronounced reduction in mitochondrial respiration as cells transition to NPCs. This overall reduction in metabolic activity continues through the second phase of differentiation from NPCs to functional neurons with a particularly significant reduction in glycolysis observed. To assess the impact of metabolic poise on the differentiation process, similar analyses were performed in the presence of galactose which, when used as a glucose replacement, increases cellular dependence on aerobically generated ATP. Interestingly, this increased aerobic poise was accompanied by an enhancement in differentiation efficiency to NPCs, providing a more homogeneous NPC population in a shorter time. These data support the central role played by metabolism in the differentiation process, and the utility of the Agilent XF platform in metabolic profiling through the differentiation processes. The optimized workflow developed for such temporal measurements now facilitates a detailed analysis of the interplay between metabolic poise and stem cell differentiation and has the capacity to reveal a deeper understanding of the differentiation process and inform more effective stem cell differentiation protocols.

Keywords: Metabolism, Mitochondria, Glycolysis

CI271

THE NOVEL DESIGNED PRC2 INHIBITOR, EEDBINDER-DCAS9, REVEALS PRECISE LOCATION OF FUNCTIONAL H3K27ME3 MARKS AND MEDIATES TROPHOBLAST TRANSDIFFERENTIATION.

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Key bifurcations in cell fates are controlled through epigenetic modifications. Particularly, broad H3K27me3 marks are known to repress developmental genes, however the precise location of functional H3K27me3 marks are not yet known. To identify these functional H3K27me3 loci in promoter regions we fused a computationally designed protein, EED binder (EB), that competes over EZH2 and thereby disrupts PRC2 function, to dCas9 to direct PRC2 inhibition at a precise locus using gRNA. Here we show that EBdCas9 identifies PRC2 requirement in single nucleosome to repress transcription of the downstream gene. In the case of downstream TBX18, we reveal the mechanism: distant, upstream TATAbox is normally silenced by PRC2 complex to repress gene expression. Local disruption of TBX18 at targeted PRC2 promoter region using gRNA results in gene activation, local and neighborhood spreading towards TSS of reduced H3K27me3, EZH2, and JARID2 marks as well as recruitment of RNA Pol II, H3K27ac and p300 marks. Using EBdCas9, we show iPSCs can be transdifferentiated to human trophoectoderm using gRNA specific to 100bp DNA regions of two transcription factors CDX2 and GATA3. Co-transfection of gRNA targeting CDX2 and GATA3 resulted in 40-80 fold increase of these transcripts. RNA seq revealed an overlay over the trophoectoderm (TE) dataset and the transdifferentiated samples. These transdifferentiated cells can develop to further trophoblast lineages, such as extravillous cytotrophoblast (EVT) and syntcytiotrophoblast (ST) based on the Chorionic Gonadotropin Beta (CGB) placental marker, showing that eliminating epigenetic PRC2 marks in specific locus in only two genes is sufficient for ICM-to-TE transdifferentiation. EBdCas9 can eliminate PRC2 specific epigenetic regulation in a single locus allowing the dissection of key functional nucleation sites for PRC2 activity.

Keywords: epigenetics, PRC2, novel design proteins

CI275

MAPPING GENETIC EFFECTS ON CELLULAR PHENOTYPES WITH 'CELL VILLAGES'

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A fundamental need in biomedical research is to understand how human allelic variation shapes cellular phenotypes. Our goal is to be able to genetically dissect any phenotype of interest to measure its heritability, and discover how genetic variation converges to affect biological processes, vulnerability to illness, and response to therapeutics. We developed population-in-a-dish experimental systems in which cells from hundreds of donors can be simultaneously phenotyped and subjected to genetic analyses. Co-culturing cell lines in this way provides orders-of-magnitude increases in scalability allowing statistically meaningful genotype-phenotype correlations, and minimizes aspects of variability that would otherwise plague comparisons of individual cell lines. As a model phenotype, we focused on discerning the effects of common variation on expression of Survival Motor Neuron (SMN), encoded by paralogues SMN1 and SMN2. We show that the power and scalability of our system affords the opportunity to rapidly and inexpensively correlate SMN phenotypic variation (as measured by flow cytometry) with underlying SMN copy number (CN), markedly surpassing genome-wide significance ($p = 8.72 \times 10\text{-}22$). We then asked whether our approach could illuminate the genetic foundation for patient variation in drug response and map the basis of this pharmacogenetic effect. We used flow cytometry to detect changes in SMN expression following treatment with the splicing enhancer LMI070. We show that LMI070 response correlates strongly with SMN2 CN (but not SMN1, $p = 6.55 \times 10\text{-}10$) confirming the hypothesis that LMI070 increases SMN levels by specifically modulating splicing of SMN2. We questioned why some donors with the same SMN2 CN showed variation in response to LMI070 and performed an in-depth re-analysis of the genomic architecture of the SMN locus. We discovered a novel drug-nonresponsive allele of SMN that lacks exons 7 and 8 (including drug binding site), rendering these donors unable to enhance SMN protein production in response to LMI070 treatment. These results provide a therapeutic guideline for genomics-informed stratification of patients with Spinal Muscle Atrophy, and illuminate the power of population-in-a-dish systems for connecting human genetic variation with disease-relevant biological processes.

Keywords: Human genetics, neuronal biology, spinal muscle atrophy

CI280

METABOLISM-REGULATING MICRORNA-BASED REPROGRAMMING METHOD FOR GENERATING HIGH-QUALITY HUMAN INDUCED PLURIPOTENT STEM CELLS

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Metabolic reprogramming (known as "Warburg effect") from OXPHOS toward glycolysis is a hallmark of cancer cells and pluripotent stem cells (PSCs). Despite its importance in determining PSCs' fate, our limited understanding of the molecular mechanisms underlying metabolic reprogramming likely impedes the successful generation of high-quality authentic human induced pluripotent stem cells (hiPSCs). We recently showed that SIRT2, which is directly regulated by miR-200c that is highly enriched in human PSCs, is critical for metabolic reprogramming and hiPSC generation, suggesting the possibility that pluripotency-associated microRNAs (miRNAs) influence the reprogramming process by facilitating metabolic reprogramming. Here, we identify 8 candidate miRNA clusters that are consistently enriched in hPSCs. To further understand the functional roles of these miRNA clusters as metabolic regulators, we explored how they can regulate metabolic reprogramming in human fibroblasts. In addition, we determined whether combining these metabolism-regulating miRNAs with the conventional reprogramming factors would facilitate generation of hiPSCs. We will discuss our novel findings regarding the potential functional roles of miRNAs as regulators of metabolic reprogramming and their application for better reprogramming methodology which will contribute to the generation of clinical grade hiPSCs.

Funding source: This work was supported by NIH grants (NS070577, NS084869, and OD024622) and the Parkinson's Cell Therapy Research Fund at McLean Hospital.

Keywords: Metabolic reprogramming, MicroRNA, Human induced pluripotent stem cells

Theme: Clinical Applications

ADIPOSE AND CONNECTIVE TISSUE

CA103

INTEGRATED ANALYSIS OF RNA AND SMALL RNA TRANSCRIPTOME FROM HUMAN ADIPOSE DERIVED BETA CELLS

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Diabetes is a pandemic manifested through glucose dysregulation mediated via inadequate insulin secretion by β-cells. A β-cells replacement strategy would transform the treatment paradigm from pharmacologic glucose modulation to a genuine cure. Stem cells have emerged as a potential source for β-cell engineering but the underlying genetic changes are uncertain. We differentiated functional β-cells from adipose tissue to determine if their transcriptome is comparable to native beta cells. We used next generation sequencing to explore gene expression changes prior to and after differentiation of patient matched samples (n=5) which revealed more than 5000 genes enriched. Adipose derived beta cells (adβ-cells) displayed comparable gene expression to native β-cells. Pathway analysis demonstrated relevance to stem cell differentiation and pancreatic developmental processes which are vital to cellular function, structural development and regulation. We additionally, explored the small RNA (sRNA) expression landscape in differentiated beta cells. We first annotated sRNA-seq to miRNA and found 149 microRNAs, of which 136 were upregulated (top five upregulated microRNAs were: miR-675, miR-328, miR-36.05, miR-125a and let-7i) and 13 downregulated (top five downregulated were: miR-138, miR-27a, miR-3529, miR-318 and miR-4677). We further analyzed data for piRNAs, we found 11 piRNAs, of which three were upregulated and remaining eight downregulated. Small RNA sequencing data from differentiated adβ-cells were analyzed for lncRNA expression, we found 313 lncRNAs, of which 75 were upregulated and 238 downregulated. We found 367 differentially expressed circRNAs, of which eight were upregulated and remaining 359 downregulated. We finally, aligned sequence data to GenCode annotation to find sn/snoRNA expression. We found two snRNAs (one upregulated and one downregulated) and 109 snoRNAs, of which 103 were upregulated and 6 were downregulated in differentiated adβ-cells. We conclude that the functionality associated with adipose derived beta cells is mediated through relevant changes in the transcriptome which resemble those seen in native β-cell morphogenesis and maturation. Therefore, they may represent a viable option for the clinical translation of stem cell-based therapies in diabetes.

Funding source: American Association of Plastic Surgeons Research Scholar Award Penn State Junior Faculty Research Scholar Award

Keywords: β-cells, ADSC, Adipose, mRNA, miRNA, Transcriptome

CA108

MSCS: A TRANSCRIPTOME MAP TO STANDARDISATION

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Mesenchymal stromal cells (MSCs) are a popular choice for advanced cell therapies, with hundreds of clinical trials ongoing worldwide. Despite the International Society Cell & Gene Therapy (ISCT) releasing their minimum defining criteria for MSCs in 2006, there is a lack of standardisation in the field and MSCs remain poorly defined. This has resulted in inconsistent clinical trials and hindered the progress of MSC-based therapies. We sought to address this problem by developing a candidate World Health Organization reference reagent for MSC identity. It can be used for flow cytometry assessment of the ISCT MSC cell surface markers. The reagent can be used to identify technical issues, perform batch to batch analysis and can add confidence to data. We have also conducted single cell RNA-sequencing to assess the cellular diversity between and within MSC populations from different tissue sources. We analysed MSCs from three adult tissues alongside MSCs derived from human pluripotent stem cells. We find that, while the different MSC populations express the established markers in the expected pattern, cells from different sources have distinct gene expression profiles. These results indicate that new source-specific markers are required to capture MSC diversity. The development of reference reagents, as well as clarification of the different cell types that are currently described as MSCs, will help standardise MSC-based therapies and improve their clinical efficacy.

Keywords: MSC, Standard, Single cell

CARDIAC

CA110

IMPROVED DIFFERENTIATION OF CARDIOMYOCYTES FROM INDUCED PLURIPOTENT STEM CELLS WITH SIMULTANEOUS GSK3 AND MTOR INHIBITION IN THREE-DIMENSIONAL SUSPENSION CULTURE

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Differentiation of cardiomyocytes from human pluripotent stem cells in three-dimensional (3D) culture will facilitate scale-up for clinical translation and may better reflect normal development compared with two-dimensional (2D) adherent culture. Protocols to differentiate cardiomyocytes from human pluripotent stem cells can result in significant batch-to-batch variability in both 2D and 3D. A prior study showed that combined treatment with the GSK3 inhibitor, CHIR99021, and the mechanistic target of rapamycin (mTOR) pathway inhibitor, rapamycin, enhances cardiomyocyte differentiation in 2D culture by reducing p53-dependent apoptosis. We treated undifferentiated induced pluripotent stem cells (iPSCs) with CHIR99021 (6 μM) to activate the Wnt pathway, and the mTOR inhibitor, Torin1 (10 nM), starting one day after seeding cells into suspension culture (day -3) in pluripotency media to two days after changing to RPMI/B27 basal media (day 2) (total of 5 days of treatment). IWP4 was added from days 2 to 4 to inhibit Wnt pathway signaling, and insulin was added on day 7. The percentage of cells positive for platelet derived growth factor receptor alpha (PDGFRa), a mesodermal marker previously shown to identify cardiac precursors, was significantly higher in cells treated with Torin1 ($p<0.05$), CHIR99021 ($p<0.05$), or both Torin1 and CHIR99021 ($p<0.01$) compared to DMSO control. We also observed a significant increase in the percentage of cells positive for cardiac troponin T (TNNT2) on day 14 of differentiation in cells treated with Torin1 (74% TNNT2+, $p<0.05$), CHIR99021 (90% TNNT2+, $p<0.05$), or both Torin1 + CHIR99021 (90% TNNT2+, $p<0.01$) compared to DMSO control (44% TNNT2+). The mean fluorescence intensity of TNNT2 was significantly increased in cardiomyocytes that received combined treatment with both CHIR99021 and Torin1 compared to control ($p<0.0001$). Cells that received combination treatment with both Torin1 and CHIR99021 had a significant increase in expression of NKX2.5 by western analysis on day 14 ($p<0.05$). We observed a trend toward decreased expression of Bax on day 0 of differentiation with Torin1 and CHIR99021 treatment, suggesting decreased apoptosis in 3D culture may facilitate cardiomyocyte differentiation, consistent with studies in 2D culture.

Funding source: NIH T32 Fellowship (T32HL007572) and the American Academy of Pediatrics Section Children's Heart Foundation Research Fellowship Award (J.C.G.)

Keywords: cardiomyocytes, 3D culture, differentiation

CA264

STEM CELLS: THE PROMISING THERAPEUTIC INTERVENTION FOR CONGENITAL HEART DISEASE: THE FACTS

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Congenital heart disease (CHD) is the most common congenital anomaly. Advancements in surgical techniques have increased newborn life expectancy, which remains the mainstay therapy. However, treatment in patients with single ventricle (SV) physiology remains palliative rather than curative. Interventions are not permanent, given that materials do not grow along with patients, and multiple surgeries are required. The most common complication of SV physiology is heart failure (HF), and there is a need for new therapies that achieve better long-term outcomes —such as stem cell therapy. We present to date reports in the literature on advancements for stem cell therapies in CHD and possible future applications; beginning with rat and pig models and concluding with human clinical trials. Animal models using multipotential cells aim to achieve myocardial regeneration and improve right ventricular function. Studies in rats demonstrated structural and functional improvement, including a reduction in left ventricle dilatation, myocardial fibrosis, and proliferation of endogenous cells after one year, with no tumor formation. In contrast, pig models have controversial results, such as the absence of functional benefits and differences in survival times of the intramyocardial injected cells. However, histological findings reveal perimyocytic fibrosis reduction. Most studies in humans are in patients with hypoplastic left heart syndrome undergoing stage II or III palliative surgery using cell therapy as a complementary treatment, employing intramyocardial or intracoronary injections to transplant cardiosphere-derived cells, umbilical cord, or bone marrow cells. Studies have found significant improvements in ventricular function and HF status. However, no change in overall patient survival has been observed with no improvement or prevention of late complications. Importantly, no study had attributable deaths to the intervention, adverse events, tumor or ectopic formations. Currently, there are six active clinical trials evaluating stem cell therapies in patients with SV physiology. Although results are promising, stem cell therapy for CHD is still at its beginning phase, and more studies are needed to elucidate the mechanisms of action and long-term outcomes before it can be safely used in the clinic.

Funding source: None

Keywords: Congenital heart defects, Stem cell transplantation, Pediatric

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CA257

OPTIMIZING EXPANSION AND DIFFERENTIATION OF HUMAN PSCS TO ISLET LIKE CLUSTERS IN CLOSED-SYSTEM BIOREACTORS, DEMONSTRATING PRECLINICAL THERAPEUTIC EFFECT.

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Establishing a high yield bioprocess for the differentiation and expansion of high-quality PSCs-derived Islet Like Clusters (ILCs), subject to GMP compliant standards, is essential for enabling future mass production allowing clinical use. Current production methods require extensive labor, substantial reagent handling, and numerous human interventions, all putting quality at risk and resulting in unsustainable costs. Optimizing conditions and materials for cell thawing, culturing, passaging, and differentiation of hPSCs in a closed continuously monitored bioreactor system, under automated conditions, will reduce human intervention and intra-batch variability while improving cost-effectiveness. Here we demonstrate a successful integration of multiple disciplines for the optimal scale-up of ILCs manufacturing, establishing a complete workflow, from a frozen PSC ampule to a final, purified, defined, ready to use, cellular product presenting preclinical therapeutic effect. Expansion and differentiation stages were optimized in DASbox® and DASGIP®, closed system stirred tank bioreactors, equipped with pitch blade impeller, adjusted for PSCs. Expansion was improved by continuous monitoring and control of several culture parameters, such as RPM, DO, and pH. Differentiation was enhanced by controlling feeding regimen (perfusion/fed batch) and DO levels. To minimize human handling, media change was performed using peristaltic pumps in both perfusion and fed batch methods. ILCs generated were then sorted and purified by MACS using a novel proprietary antibody combination (CD26- and CD49a+). Following sorting and purification, ILCs exhibited an improved GSIS and high gene expression levels of MAFA, Insulin, and NKX6.1. Upon implantation of the ILCs (microencapsulated in alginate) to STZ-treated diabetic immunocompetent mice, normoglycemia was achieved shortly post treatment and remained stable for the study duration. Integration of multiple disciplines led to a successful scale-up process achieving a comprehensive workflow for GMP-compliant mass production of ILCs. A closed system bioprocess substantially minimized human intervention, reduced labor and cost, generating high-quality ILCs with preclinical therapeutic effect, to be further assessed in clinical applications.

Keywords: PSCs-derived Islet Like Clusters (ILCs), Diabetes, scale-up

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

CA271

MANAGING THE CHANGING FACE OF ETHICAL AND LEGAL REQUIREMENTS: IMPLICATIONS FOR HUMAN PLURIPOTENT STEM CELL USAGE

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Ethical and legal requirements regulating the procurement of human biomaterials for the derivation of pluripotent stem cells (HPSC) lines are established to safeguard donors' interests. They also serve for delineating usage and access of such materials and their data, including intellectual property and other licensing conditions. From donors to researchers, to hPSC banks and finally, end users of HPSC lines or data, each stakeholder has expectations and obligations to fulfill. However, over time the changing face of stem cell regulation across jurisdictions, particularly pertaining to donor data, have rendered it challenging to reconcile present uses of previously collected materials, lines and data, which have been generated under standards that are -or might be – now considered incompatible or inconsistent with current guidelines (e.g. consent). In particular, the use of legacy collections of biomaterials, whether stored in public biobanks or distributed en masse commercially, may have unanticipated restrictions for end-users, depending on their desired target applications. Drawing on the experience of the European Human Pluripotent Stem Cell Registry (hPSCreg: https://hpscreg.eu/), we present a process for ethical and legal assessment of such legacy collections. We analyze the implications of evolving standards for all stakeholders with respect to downstream access and usage of the hPSC lines and their associated data.

Funding source: hPSCreg is funded by the European Union Grant agreement 726320

Keywords: stem cells, ethics, policy, law, regulation, banking, data, clinical

EYE AND RETINA

CA148

IPSC-INDUCED NEURAL CREST STEM CELL AS A SOURCE OF CELL-BASED THERAPY OF GLAUCOMA

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Glaucoma is one of the leading causes of worldwide blindness. Therapies based on reducing intraocular pressure (IOP), a major risk factor for glaucoma, slow down visual loss in most glaucoma patients. However, stem cell transplantation potentially can be a better way to prevent neurodegeneration as it can target the root of the blindness, the death of retinal ganglion cells (RGCs). Neural crest cells (NCCs) are a migrating and differentiating cell type which give rise to cartilage, bone, neuron, glial and mesenchymal cells, and many other cell types during development. NCCs can be a potential source for stem cell transplantation in glaucoma as defected development of NCCs leads to primary congenital glaucoma by affecting the formation of ocular and periocular structures such as the trabecular meshwork (TM). In this study, human iPSC-derived NCCs (hiNCCs) were transplanted into rat eyes using two different models of RGC death, an optic nerve crush (ONC) model and a TM laser model. hiNCCs started to extensively express an RGC

marker (RBPMS) 21 days after intravitreal injection in ONC rats. Implantation of hiNCCs slightly reduced the loss of axons in the optic nerve associated with ONC. In vitro studies showed that hiNCCs began to express RGC markers after co-cultured with human iPSC-induced RGCs for 14 days. Meanwhile, implantation of hiNCCs into the eye anterior chamber where the TM has been damaged by a laser treatment prevented IOP elevation induced by the laser action. The transplanted hiNCCs localized to the TM structure and began to express TM cell markers (AQP-1, SMA, and Collagen IV) within 21 days. In-vitro co-culture of hiNCCs with human primary cultured TM cells led to the enrichment of AQP-1+/Vimentin+ hiNCC derivatives from day 7 to day 21. Moreover, the mRNA expression level of TM cell markers was gradually increased, whereas NCC markers were dramatically decreased in co-cultures of hiNCCs and TM cells. Overall, these results demonstrate that NCCs have a strong adaptive ability depending upon the eye microenvironment, and they are a possible source for stem cell-based therapy for glaucoma and other eye diseases.

Funding source: Supported by the Intramural Research Programs of the National Eye Institute, NIH

Keywords: Glaucoma, induced pluripotent stem cells, neural crest stem cells

IMMUNE SYSTEM

CA158

MACROPHAGES M1 POLARIZATION AND MACROPHAGE-MEDIATED SUSTAINED DELIVERY OF THERAPEUTIC PROTEINS USING SELF-ASSEMBLING NANOSCALE PROTEIN COMPLEXES

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Circulating macrophages are recruited via a CCL2/CCR2 axis to the tumour micro environment where they typically polarize towards an immuno-suppressive M2 phenotype. Therapeutic strategies to (1) repolarize macrophages towards a tumor-hostile M1 phenotype and (2) utilize macrophages as “Trojan horses” to target drugs to cancer have been proposed. Here, we have explored the utility of a self-assembling polyhedrin protein from Bombyx mori cypovirus to provide a robust mechanism for both macrophage-targeting and subsequent sustained release of bioactive cargo over a period of weeks. This particular polyhedrin protein generates nanoscale, rigid, cubic co-crystals into which a second, bioactive cargo protein can be incorporated via an immobilization tag. Using GFP for visualization, we show that these co-crystals are efficiently ingested by different phagocytic cells. In addition, ingested crystals containing either CSF or SCF as the active cargo induce proliferation in monocytes. These results demonstrate the potential of co-crystals to polarize tumor infiltrating macrophages towards an M1 phenotype to modulate the overall architecture of tumour microenvironment over extended periods.

Keywords: Macrophage therapy, Trojan horse, Therapeutic protein delivery

NEURAL

CA170

IN VITRO AND IN VIVO CHARACTERIZATION OF CLINICAL GRADE HUMAN UNDIFFERENTIATED ALLOGENEIC PLURIPOTENT STEM CELLS FOR TREATMENT OF NEURODEGENERATIVE DISEASES

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Pre-differentiated stem cells of various origins are being tried for the treatment of neurodegenerative disorders. Pre-differentiation has two flaws: first, it is difficult if possible to determine the correct dose of the specialized neural cells for an individual patient and, second, the specialized cells can address only single factor of usually multifactorial neurodegenerative disorders. To address these problems, a suspension close system bioreactor was used to manufacture a line of NPCs that are able to tune their proliferation and vector of differentiation in response to micro-environmental cues. Immunofluorescence, flow cytometry, and teratoma formation assays were used to demonstrate that NPCs retained pluripotency, potency, genetic stability and sterility of the cell product. The NPCs express high levels of Oct-4, Sox-2 and low levels of MHC-I. Proteomic analysis revealed that the cells secrete a number of proteins that have neuroprotective qualities such as - Neuromodulin, 14-3-3 epsilon protein, Ceruloplasmin, Insulin Degrading enzyme, Insulin like Growth Factor II, and Activity dependent neuroprotective protein. Stereotactic injection of the NPCs into the cerebellum of spastic Han-Wistar (sHW) rat model of cerebellar ataxia, lead to repopulation of the depleted Purkinje cell layer with Calbindin-positive NPCs, and nearly complete amelioration of forelimb tremor, hind-leg rigidity, and gait abnormality, characteristic for sHW rats. Stereological analysis demonstrated significant increase in the number of the rat Purkinje neurons as compared to control animals. These data justify consideration of NPCs for development of regenerative treatments for neurological disorders.

Keywords: Human stem cells, allogeneic, Undifferentiated

CA174

INTRA-ARTERIAL HUMAN MESENCHYMAL STROMAL CELL DELIVERY THROUGH CARDIOPULMONARY BYPASS FOR NEUROPROTECTION IN A JUVENILE PORCINE MODEL

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Neurodevelopmental impairment is an important challenge for survivors after pediatric cardiac surgery. Cardiopulmonary bypass (CPB) can cause systemic and brain specific inflammation. Mesenchymal stromal cells (MSCs) have significant immunomodulatory properties. We hypothesize that MSC delivery through CPB is neuroprotective by modulating systemic and neuro-inflammatory responses. Two-week old piglets (n=16 total) were randomly assigned to one of 3 groups: (1) Control, (2) CPB + deep hypothermic circulatory arrest (DHCA), (3) DHCA followed by MSC administration. In group 3, 18F-FDG or superparamagnetic iron oxide (SPIO)-labeled MSCs (10x106 per kg) were delivered through CPB. Positron emission tomography (PET) was performed 1hr after MSC delivery to determine the whole body distribution of cells. Animals were sacrificed 3hrs after CPB for analysis with magnetic resonance imaging (MRI) and immunohistochemistry. Total cortical RNA was extracted and reverse transcribed followed by qPCR assessment of key target genes. Plasma cytokine/chemokines were determined by multiplex immunoassay. Clinically-relevant physiological biomarkers determined the effect of MSC delivery on multi-organ function. PET study showed that intra-arterial delivery through CPB uniformly distributed MSCs to most organs analyzed including the brain, heart, and kidney except that lungs and intestine showed lower uptake. T2* weighted brain MRI showed diffuse distribution of SPIO particles throughout the entire brain. MSCs were noted to have migrated into the extra-vascular space. qPCR data revealed increased expression of components of the STAT3 pathway after CPB, which was rescued after MSC treatment. MSC treatment also modulated plasma cytokine/chemokines expression following surgery. In the brain MSC treatment reduced microglia expansion and caspase activation resulting from CPB. Various biomarkers after MSC delivery did not differ compared with CPB group. No embolic events were observed by MRI. MSC delivery during CPB has the translational potential to minimize systemic inflammation and reduce microglial expansion and caspase activation in children undergoing CPB.

Keywords: Mesenchymal Stromal Cells, Cardiopulmonary Bypass, Neuroprotection

CA183

MICROCHIP ANALYSIS OF CELL COOPERATIVY OF NEURAL STEM CELLS TO BENEFIT CELL THERAPY CHOICES

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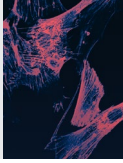
Multipotent neural stem cells (NSCs) are widely applied in pre-clinical and clinical trials as a cell source to promote tissue regeneration in neurodegenerative diseases. Frequently delivered as dissociated cells, partial aggregates or self-organized rosettes, it is unknown whether disruption of the NSC rosette morphology or method of formation affect signaling profiles of these cells that may impact uniformity of outcomes in cell therapies. Here we generate a neural cell-cell interaction microchip (NCCIM) to track an informed panel of cytokines simultaneously while also co-evaluating additional parameters of cell morphology and expression of biomarkers in a sandwich ELISA platform. We apply multiplex in situ tagging technology (MIST) to evaluate ten cytokines (PDGF-AA, GDNF, BDNF, IGF-1, FGF-2, IL-6, BMP-4, CNTF, β-NGF, NT-3) on microchips for EB-derived rosettes and dissociated rosettes to single cells or to partial aggregate spheres. Of the cytokines evaluated, EB-derived rosettes secrete three cytokines prominently, while



a wider range of cytokines is observed in partial aggregate spheres and cytokine signaling is temporarily disrupted upon dissociation to single cells. This study on NSC rosettes demonstrates the development, versatility and utility of the NCCIM as a sensitive multiplex detector of cytokine signaling in a high throughput and controlled microenvironment platform with potential use in neural cell, tissue or organoid applications. The NCCIM is expected to provide important new information to refine cell source choices in therapies as well as to support development of informative in vitro models including areas of neurodegeneration or neuroplasticity. This work is funded by New York State Stem Cell Science (NYSTEM), "Optimizing replacement cell therapies through microchip analysis of cell cooperativity".

Funding source: NYSTEM

Keywords: Multiplex, Cytokine, Human Stem Cells

CA192

HUMANIZED MOUSE MODEL TO TEST HOST IMMUNE RESPONSE TO IPSC-DERIVED DOPAMINERGIC CELLS FOR PERSONALIZED CELL THERAPY OF PARKINSON'S DISEASE

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Cell replacement therapy using human iPSC- or ESC-derived dopaminergic cells is a promising therapeutic strategy for the treatment of Parkinson's disease (PD). Because the brain has traditionally been considered an "immune-privileged" organ, the necessity for immunosuppression with allogeneic grafts has been controversial. More recent information has revealed the complex interactions between the brain and the immune system which raised the possibility of immune rejection of allogeneic cells over time. While autologous cell transplants would theoretically circumvent the requirement for immunosuppressive agents, it has been argued that the in vitro differentiation processing of autologous iPSC-derived cells could result in immunogenicity and therefore possible immune rejection by the host. This would negate the theoretical advantages of autologous cell replacement therapy. In order to investigate whether autologous iPSC-derived dopaminergic progenitor cells differentiated in vitro would be rejected or not, we sought to establish a NOD/SCID/IL2rγnull humanized mouse model. The model is reconstituted by injecting human PBMCs into the peritoneal cavity of the mouse followed by transplantation of either autologous or allogeneic dopaminergic progenitor cells into the striatum of the humanized mice. In this study, we demonstrate that transplantation of PD patient-derived midbrain dopaminergic progenitor cells (C4-mDAP), differentiated in vitro from autologous iPSCs (C4-iPSC), were not rejected by humanized mice reconstituted with the patient's PBMCs. In contrast, humanized mice reconstituted with allogeneic PBMCs rejected the C4-mDAP, demonstrating that they can be recognized by a foreign immune system. In addition, mDAPs differentiated from human ESCs were rejected by both mice humanized from either source. These data support the hypothesis that personalized cell transplantation therapy for PD using autologous iPSC-derived differentiated neurons without the use of immunosuppression is indeed a viable strategy (Schweitzer et al., 2020, NEJM, in press).

Funding source: This work was supported by NIH grants (NS070577, NS084869, and OD024622) and the Parkinson's Cell Therapy Research Fund at McLean Hospital.

Keywords: Personalized cell therapy, autologous iPSC-derived midbrain dopaminergic progenitor cells, Humanized mice

CA259

SINGLE CELL TRANSCRIPTOMICS IDENTIFIES HUMAN STEM CELL-DERIVED GRAFT COMPOSITION IN A MODEL OF PARKINSONS DISEASE

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Cell replacement is a long-standing and realistic goal for the treatment of Parkinson's disease (PD). Cells for transplantation can be obtained from fetal brain tissue or from stem cells. However, after transplantation dopamine (DA) neurons are a minor component of grafts and it has remained difficult to determine the identity of other cell types. Here, we report analysis by single cell RNA sequencing (scRNA-seq) combined with comprehensive histological analyses to characterize intracerebral grafts from human embryonic stem cells (hESCs)

and fetal tissue after functional maturation in a pre-clinical rat PD model. We show that neurons and astrocytes are major components in both fetal and stem cell-derived grafts. Additionally, we identify a cell type closely resembling a class of newly identified perivascular-like cells in stem cell-derived grafts. Thus, this study uncovers previously unknown cellular diversity in a clinically relevant cell replacement PD model.

Keywords: Parkinson's disease, human embryonic stem cell, single cell RNA sequencing

NEW TECHNOLOGIES

CA202

HIGH DENSITY BIOPROCESSING OF HUMAN PLURIPOTENT STEM CELLS BY METABOLIC CONTROL AND IN SILICO MODELING

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Human pluripotent stem cells (hPSCs) are a unique source for the production of functional human cell types, fueling the development of advanced in vitro disease models and future regenerative therapies. Most applications will require the constant supply of billions of cells generated by robust and economically viable bioprocesses. To harness the full potential of hPSCs instrumented stirred tank bioreactor (STBR) technology was combined with the power of in silico modeling. Perfusion feeding of matrix-free suspension culture enabled the feedback control of process-limiting parameters including pH stabilization, uninterrupted Glucose supply and obviation of osmolality peaks provoked by high density fermentation. The shear protectant polymer Pluronic decisively supported both single cell-based process inoculation and hydrodynamic aggregate size control as well. Wet-lab results laid the ground for establishing in silico process modeling and rational optimization strategies. Reiterative steps of combined in silico and in situ optimization enable the cell line-dependent long-term maintenance of exponential hPSC growth resulting into 70-fold expansion of the inoculated cells within 7 days yielding 3.5 x 107 cells/mL equivalent to 5.25 billion hPSCs in 150 mL process scale. Together, the method achieves a new level of matrix-free hPSC bioprocessing in three-dimensional suspension culture. The study provides a deeper understanding of stem cells' physiology, culture requirements and a straightforward strategy for the controlled, industry-compliant production of hPSCs and their progenies.

Keywords: In silico modeling, High-density hPSC bioprocessing, Advanced matrix-free hPSC culture

CA204

TACKLING THE HUMAN PLURIPOTENT STEM CELL (HPSC) SINGLE-CELL HURDLE: ROBUST AND EFFICIENT CLONING OF HIPSCS

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Human pluripotent stem cells (hPSCs) have potential to address a broad range of research and clinical applications, from disease modelling to cell therapies. However, the inability to robustly manipulate hPSCs as single cells remains a significant biological and technical hurdle. Current techniques rely on inefficient methods, such as limiting dilution (LD), which are time-consuming, expensive and incompatible with the sensitivity of hPSCs. To significantly enhance their application, it is critical to create a robust single cell cloning workflow amenable to hPSCs. We have previously demonstrated the benefits of the VIPS™ single cell seeding platform over manual LD for several mammalian cell lines. In addition to providing confidence in clonality, the VIPS resulted in higher seeding, thereby reducing the number of plates, reagents and time required for screening. Given the enhanced sensitivity of hPSCs compared to immortalized cell lines, optimization of the cell culture environment (including specialized surface coatings, media and culture techniques) is required to apply this technology to hPSCS. Furthermore, the complex culture environment highlights the importance of clonality and validation. Here we present a robust and efficient workflow for cloning hPSCs, with improved cloning efficiency and documentation of clonality. By optimizing growth conditions in combination with VIPS technology, multiple hPSC cell lines were successfully subcloned. Newly sub-cloned hPSC lines underwent extended culture prior to characterization for pluripotency. The VIPS approach resulted in an 3-4-fold improvement in average clonal outgrowth of singly seeded cells compared with manual LD. Pluripotency maker expression of Oct4 and Nanog were maintained within the sub-cloned lines over the course of 5 passages. Furthermore, sub-cloned lines were karyotyped via g-banding to assess for genomic stability and differentiated to examine germ layer potential. Importantly, this workflow significantly reduces the amount of reagents, time and scale, which enables a broader range of downstream applications, including therapeutic aims. This workflow may also allow for greater consistency between stem cell labs and future standardisation with the regulatory bodies for therapeutics.

Keywords: HIPSCS, Clonality, Therapeutic

CA214

LONG-TERM FUNCTIONAL SURVIVAL OF HUMAN STEM CELL-DERIVED ISLET LIKE CLUSTERS MICROENCAPSULATED IN ALGINATE WITH CXCL12 IN NON-HUMAN PRIMATES WITHOUT IMMUNOSUPPRESSION

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Human pluripotent stem cell-derived islet-like clusters (ILCs) represent a practical source for beta-cell replacement to achieve a functional cure for type 1 diabetes. Long-term survival and glycemic correction by ILCs encapsulated in various biomaterials to avoid systemic immunosuppression has only been demonstrated in rodent models. Unfortunately, translation of these approaches in large animals and humans is often problematic, in part, due to robust foreign body responses that lead to pericapsular fibrotic overgrowth and graft failure. Here, we report long-term functional survival of ILCs microencapsulated in alginate with the immunomodulatory and pro-survival chemokine CXCL12 in non-human primates without immunosuppression. Microencapsulated ILCs were transplanted into the greater omentum of a non-diabetic and a diabetic NHP, with a cohort of the microcapsules transplanted intraperitoneally in parallel in immunocompetent C57BL/6 diabetic mice. We tracked biochemical, immunologic/hematologic parameters, blood glucose (BG) and C-peptide levels over a six-month period. The microencapsulated ILCs recovered at 1, 3 and at 6 months post-transplant were analyzed for their survival, functionality and the local immune responses. The BG levels of the non-diabetic NHP remained within normal range throughout the study period without episodes of hypoglycemia, while exogenous insulin requirements of the diabetic NHP were reduced by ~60%. Normoglycemia was restored in all diabetic mice throughout the 6-month period. Non-fasting plasma C-peptide levels of the non-diabetic NHP remained relatively stable (~200 pM to 700 pM) while random and intravenous glucose-induced C-peptide could be detected in the diabetic NHP serum. Recovered ILCs from the non-diabetic NHP at 1, 3- and 6-months and those from the diabetic NHP at 6 months post-transplant were glucose-responsive. Majority of recovered microcapsules at all time points were free-floating without fibrotic overgrowth. Messenger RNA transcripts and protein of beta cell markers were detectable in recovered ILCs, albeit a significant decrease compared to pre-transplant levels at 6 months post-transplant. These preliminary findings provide the foundations for ongoing studies in larger numbers of diabetic NHPs.

Funding source: Juvenile Diabetes Research Foundation (JDRF)

Keywords: Type 1 diabetes, Beta-cell replacement therapy, Stem cell-derived islets

CA223

DESIGN OF ANIMAL COMPONENT FREE (ACF) AND CHEMICALLY DEFINED (CD) MEDIUM FOR HPSC AGGREGATES IN SUSPENSION CULTURE SYSTEM

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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 therapeutic potential. Production of hPSC in high quantities for clinical applications using standard 2D adherent culture is hardly achievable and process scalability is difficult. A promising approach to overcoming these hurdles is 3D suspension culture. Aggregate suspension culture enables reproducible production of high number of pluripotent cells and to proceed to differentiation procedure if desired. The quality of culture medium and its performance are particularly crucial with regard to therapeutic applications, since hPSC properties can be significantly affected by medium components and culture conditions. More than that, with the increased clinical interest and the stringent regulatory requirements the need to culture pluripotent stem cells or differentiated cells in an animal-components free (ACF) and chemically defined (CD) culture system is preferred to minimize the risk associated with disease transmission and immune rejection of the transplanted cells. To date, there is no efficient ACF and CD medium for 3D suspension culture of hPSC in the pluripotent state towards therapeutic differentiation and applications. This advanced culture system would greatly facilitate the development of a robust, clinically acceptable culture process for reproducibly generating quality-assured cells. The present study describes the development of ACF and CD medium suitable for hPSC expansion as aggregates in suspension. Results show that ACF and CD medium enables high proliferation rate of hPSC, while maintaining stable karyotype and high pluripotency marker expression.

Keywords: Suspension culture, Animal-component free, Chemically defined

CA225

UP-SCALING OF HIGH DENSITY HUMAN PLURIPOTENT STEM CELL BIOPROCESSING MAINTAINS CELLS DIRECTED DIFFERENTIATION POTENTIAL

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Human pluripotent stem cells (hPSCs) are a unique source for the production of functional human cell types, fueling the development of advanced in vitro disease models and future regenerative therapies. Most applications will require the constant supply of billions of cells generated by robust and economically viable bioprocesses. To harness the full potential of hPSCs, their novel high density cultivation was scaled up in instrumented stirred tank bioreactors applying rational, mathematical calculations. Commonly applied process upscaling strategies such as maintenance of constant impeller tip-speed – in our hands – performed inferior for upscaling highly complex process conditions that is matrix-free hPSC aggregates cultivation in stirred suspension. Therefore, we have developed a novel strategy that enables maintenance of constant aggregate size distribution patterns across different process scales. Consequently, when combined with feedback-based process control recently established by us, robust up-scaling from 150 mL towards 500 mL process scale was enabled including transition across independent bioreactor systems. Additionally, cells generated by this processes were efficiently differentiated into definitive endoderm, mesoderm derivative such as cardiomyocytes and ectodermal progenies as well, highlighting full maintenance of hPSCs' pluripotency and overall homogeneity of the cell quality resulting from this large-scale and high density hPSC production process. Together, the method achieves a new level of hPSC bioprocessing that is not only able to supply hPSCs in large quantities, but was also able to show their directed differentiation potential and thus fueling their production and differentiation for industry and therapies.

Keywords: Up-Scaling, High-density hPSC bioprocessing, Maintenance of pluripotency

CA250

EFFECT OF SURFACE PROPERTIES AND PROTEIN ADSORPTION ON HUMAN MESENCHYMAL STROMAL CELL ADHESION TO CULTURE SUBSTRATES

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Traditionally, the practice of culturing adherent cells to produce cell therapies has been conducted within tissue culture-treated polystyrene (TCPS) flasks to facilitate cell adhesion to the surface. In the clinical context, closed cell culture bag systems composed of fluoropolymers such as FEP provide an alternative to T-flasks, however the impact of culture vessel on cell adhesion and fate is poorly understood. We hypothesize that (1) cell-surface interactions are mediated through surface-adsorbed proteins from the culture media and secreted by the extracellular matrix and (2) protein adsorption and cell adhesion events can be controlled by modifying the FEP bag material, thereby tuning the surface properties. In our work, we generated plasma polymer coatings rich in amine (-NH2) and carboxylic (-COOH) groups on FEP to create more pro-adherent surfaces. We designed a custom enzyme-linked immunosorbent assay (ELISA) to assess the relative abundance of key proteins contained in typical GMP culture media on FEP surfaces, specifically insulin and albumin. We seeded mesenchymal stem cells onto treated FEP, non-treated FEP and TCPS in protein-free and protein-containing media and assessed cell adherence and spreading. We then probed mechanisms by which cells adhere to the culture vessel by adding a reagent known to abrogate integrin-mediated adhesion, specifically ethylenediaminetetraacetic acid (EDTA). Our findings indicate that our plasma polymer coatings enhance adhesion on FEP but the number of adherent cells remains lower than on TCPS. Additionally, we observed that mesenchymal stem cell adhesion was blocked in a concentration-dependent manner when inhibitory agent EDTA was added to the cell suspension in protein-containing media prior to seeding, potentially pointing to an integrin-mediated mechanism of adhesion. Conversely, adhesion was unaffected by EDTA in protein-free media. Further investigation of such cell-protein-surface interactions will inform the optimization of adherent cell therapy culture conditions.

Keywords: plasma coating, bioreactor bags, adhesion mechanisms

CA253

BIOFABRICATION OF THREE-DIMENSIONAL HUMAN SMOOTH MUSCLE CONSTRUCTS FOR INTESTINAL TISSUE ENGINEERING

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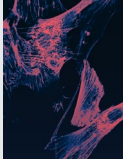
Recent advances in intestinal tissue engineering have led to improved biomimicry in terms of biological composition. Nevertheless, the complex architecture is still far from being



faithfully reproduced and, as it strictly relates to tissue function, is a necessary component that must be implemented into the engineered models to fully exploit their potential. Particularly challenging is recreating in vitro the orthogonal orientation of the smooth muscle (SM) layers as seen in vivo because important functions (i.e. peristalsis) cannot occur without such specific alignment. The aim of this project is biofabricating a three-dimensional (3D) highly organised SM structure resembling the longitudinal and circular architecture typical of the intestine with a view to create a biologically relevant gut model. Human pericytes derived from muscle and intestinal biopsies, namely mesoangioblasts (MABs), were used as SM precursors, given their established differentiation potential toward this lineage. The 3D shape and orientation were provided to the cells by a biocompatible photo-crosslinkable gelatine-based scaffold designed ad hoc and fabricated with two-photon bioprinting technology. MABs showed successful adhesion to the printed surfaces and morphological adaptation to linear and circular patterns. Remarkably, cells seeded onto hollow cylindrical 3D scaffolds, where the inner and outer surfaces are orthogonally orientated, effectively positioned cells according to the patterns and consequentially tubular hollow smooth muscle constructs were fabricated. Future work will focus on the generation of multicellular 3D tissue engineered models adding relevant intestinal cell types. Also, the gelatine-based scaffold will be substituted with an ECM-based material in order to have a truly biomimicking product, useful for in vitro studies of cell-cell and cell-ECM interactions. Lastly, in the view of creating a patient-specific 3D model, amniotic fluid stem cells will be used as muscle source given their ability of SM differentiation in vitro, thus paving the way for disease modelling and personalised pharmacological studies in pre-natal settings. On a final note, our SM construct is a valid 3D system for the tissue engineering of other hollow organs (oesophagus and ureter).

Funding source: GOSH Charity, INTENS, NIH

Keywords: Intestinal smooth muscle architecture, Two-photon bioprinting of 3D constructs, Human mesoangioblasts

CA254

CONTROL OF SHEAR STRESS DURING EXPANSION OF HUMAN EMBRYONIC STEM CELLS IN SUSPENSION CULTURE

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Widespread use of ESC in cell-based therapeutics will require reproducible production, and scalable culture of well-characterized cells under well-controlled conditions. Bioreactors will be used to produce successful commercialization of cell therapies in a large scale, and substantial progress has been made toward that; although, many challenges remain. For example, stirring and sparging of the suspension culture results in the formation of spatial differences in the pattern of liquid flow, leading to cell damage caused by hydrodynamic shear stress. Studies have shown that the addition of the non-ionic surfactant, Pluronic F-68 to serum-free cultures causes multi-functional effects that enhance cell yield in agitated cultures. They have shown that Pluronic provided effective protective to cells from shear by absorbing to the cell membrane. Some data suggest that differentiation and maturation of stem cells can be promoted by Pluronic F-68. In another study showed that using cytochalasin D may be effective in enhancing or inhibiting differentiation towards cells of the endodermal or mesodermal lineages. We have two unpublished data that one showed the cluster size of human embryonic stem cell (hESC) in suspension culture can be affected using Cytochalasin D (Cyto D). Second one showed that Pluronic treated- cells can maintain their stemness property and increase the fold of expansion that are bottlenecks characteristics in producing cells in pharmaceutical companies. In this study, we explore the impact of Pluronic F-68, and cytochalasin D -either alone or in combination- on an established GMP hESC cultured in suspension culture in serum-free, and xeno-free medium. Cells will be exposed to different sheer stress in shaker incubator in the present of Cyto D and Pluronic. Cells are serially passaged in the present or absence of Pluronic F-68 (0, 1%), and Cyto D (0,01 µM). Samples are taken every passage and analyze for for the impact of reagents on pluripotency markers (OCT4, NANOG, SSEA1, SSEA4, and TRA-1-60), aggregates size distribution, and cell yield.

Keywords: hESC, Stemness, Pluronic, and Cytochalasin D

CA272

FROM ALZHEIMER'S TO COVID-19: THE NECESSITY AND ATTAINABILITY OF TRULY XENOFREE STEM CELLS

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From complex neurological conditions like Alzheimer's to infectious diseases like COVID-19, human stem cell research has become an essential tool to understanding pathology, potential therapy and prevention. However, in order for human-based models to rapidly, precisely and effectively translate to therapeutics will require a shift to xenofree conditions. Another critical factor is the need to address diversity and disparities (e.g., ethnicity, age, environment). Although these aspects -- xenofree conditions, diversity and disparities -- may seem to be independent issues, they are in fact inexorably intertwined. To illustrate these points we analyzed: [1] non-human animal component contamination in purportedly xenofree protocols [2] perceived barriers to moving to truly xenofree research conditions and [3] diversity of samples in large publicly-available resources for induced pluripotent stem cells (IPS). [1] By examining widely used protocols for IPS reprogramming and differentiation, we demonstrate that researchers often continue to overlook the contamination of non-human animal components and remain generally unaware of xenofree alternatives. [2] To illustrate that conversion of protocols to truly xenofree conditions is within the reach of most labs, we surveyed commercially available xenofree options and show that these protocols can be addressed with minor costs and methodological adjustments. [3] To assess diversity and disparity factors we analyzed publicly available

resources. We found that these IPS collections were severely lacking in diversity and not representative of prevalence rates for diseases such as Alzheimer's. This deficiency in even our most significant open stem cell resources highlights how crucial shifts in sample collection, conceptual thinking and policy are needed to improve the scientific accuracy, clinical applicability and ethics of stem cell models. Conditions such as Alzheimer's and COVID-19 are both characterized by complexity of expression, differential effects on individuals and broad public impact. Together, our findings emphasize how human-relevance, xenofree conditions and diversity must be interlocked in the research design and the urgency of addressing these multifactorial aspects in accelerating pathways to therapy and prevention.

Keywords: xenofree, diversity and disparities, disease modeling

Theme: Modeling Development and Disease

ADIPOSE AND CONNECTIVE TISSUE

MDD103

HUMAN PLURIPOTENT STEM STELL DERIVED WHITE ADIPOCYTE ORGANOIDS WITH SECRETED FACTORS FOR ORGAN CROSSTALK APPLICATIONS

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Obesity presents a worldwide epidemic, often associated with the development of other diseases, such as type 2 diabetes, cardiovascular disease, and some forms of cancer, highlighting the need for measures to combat obesity and its comorbidities. Adipose tissue is not just a passive storage place for fatty acids, but plays a central role in lipid and glucose metabolism, as well producing adipokines which impact organ homeostasis. To study organ crosstalk, protocols for induced pluripotent stem cell (iPSC) derived organoids must be implemented. Since the majority of adipocytes in the human body are white, to study adipocyte/organ crosstalk will require a robust differentiation protocol for white adipocyte production, preferably in a 3D structure. A variety of approaches to generate adipocytes from iPSCs have been described, often as proof of concept to validate differentiated mesenchymal stem cells (MSCs). The field has been working with protocols that are either based on embryoid body formation or monolayers. Several groups report the limited capacity of iPSC derived MSCs to differentiate to adipocytes and it has been speculated that this is limited only to adipogenesis, but not to osteogenesis or chondrogenesis. The field has made an effort to increase the efficiency of adipogenesis, especially to create beige or brown adipocytes for obesity research. However to our knowledge there is no protocol that generates white adipocytes from iPSCS without embryoid body formation or transgene induction at a high efficiency. Here we describe a defined approach to generate white adipocyte organoids without additional ECM, expressing key adipocyte markers including PPARg, FABP4 and Perilipin, and importantly they accumulate lipids. The approach takes 40 days, however intermediates in the protocol can be cryopreserved and expanded, thus shortening the differentiation to just 19 days. This approach allows autologous tissue organoids to be interrogated easily.

Funding source: This work was partially supported by the Research Council of Norway through its Centres of Excellence scheme, project number 262613

Keywords: adipose, organoid, iPSC

CARDIAC

MDD108

HIPSC-DERIVED THREE-DIMENSIONAL CARDIAC MICROTISSUES INDUCE ELECTROPHYSIOLOGICAL MATURATION OF CARDIOMYOCYTES

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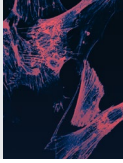
Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are used to study inherited arrhythmias and are emerging - and possibly shortly adopted - as in vitro preclinical model for assessing proarrhythmic risks of drugs. However, hiPSC-CMs applicability is still limited because they present immature electrical properties, unless when included into engineered tissues requiring costly equipment and large cell numbers. In this study, we generated 5000-cell scaffold-free three-dimensional microtissues (MTs) composed by defined proportions of cardiomyocytes (CMs), cardiac fibroblasts (CFs) and endothelial cells (ECs), all derived from hiPSCs. We showed that three-cell-types MTs produce significant maturation of hiPSC-CMs, by means of CMs-CFs physical interaction and paracrine signals from ECs. We observed more mature electrophysiological features, with increased upstroke velocity, longer action potential duration, and more hyperpolarized resting membrane potential, compared to MTs with only two cell types. The electrical maturation was accompanied by enhanced contractility and sarcomere organization. Importantly, these characteristics were retained in CMs even when dissociated from MTs. Single-cell RNA sequencing data supported functional results showing increased gene expression of ion channels involved in shaping CM action potential, in MTs compared to 2D



culture. This human stem cell-based MT system is a step forward for modelling those arrhythmias requiring postnatal CM function and will provide a simple but effective platform to accurately test drugs.

Keywords: hiPSC-derived cardiomyocytes, microtissues, cellular electrophysiology

MDD113

VERTICAL INTEGRATION OF MULTI-OMICS DATA TO INVESTIGATE THE EFFECTS OF DOXORUBICIN EXPOSURE IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Doxorubicin is a compound of the anthracycline family used as a chemotherapeutic agent due to its potent antitumor activity. However, doxorubicin has also severe side-effects including cardiotoxicity that may result in the development of heart failure. Interestingly, the mechanisms underlying the chemotherapeutic-associated cardiotoxicity remain poorly understood. Omics technologies allow researchers to collect information that provides insights into specific molecular mechanisms. Previously, we identified common differential expression patterns induced by doxorubicin in human pluripotent stem-cell derived cardiomyocytes at different molecular levels (mRNA, miRNA and protein) using the f-divergence cutoff index method. However, analysis of single-level omics data only provides limited information that fails to depict the complexity of regulatory mechanisms among the different molecular levels. Integrative analysis across the different layers of multi-omics data, so-called vertical integration, provides a more comprehensive view of the true heterogeneity and dynamics associated with biological processes. Here, we performed analysis and vertical integration across different multi-omics data modalities using the miodin R package to investigate the effect of doxorubicin on the interactions and regulatory associations between RNA and protein expression levels in human stem-cell derived cardiomyocytes. With an easy and streamlined workflow-based syntax, multi-omics data sets were integrated using miodin and fit into a Multi-Omics Factor Analysis (MOFA) model resulting in the inference of latent factors that account for the variation observed between and within the different multi-omics data modalities. The factors identified using MOFA analysis allowed us to capture a dose-dependent biological effect over time associated with doxorubicin response in stem-cell derived cardiomyocytes. We also identified panels of biomarkers from multiple-omics layers that will be candidates for further biological validation. Overall, this study highlights the functionality of integrative analysis of multi-omics data to identify interactions present across the multiple molecular layers, providing insights on the mechanisms associated with doxorubicin-associated cardiotoxicity.

Funding source: The study was supported by the Systems Biology Research Centre (University of Skövde, Sweden) under grants from Knowledge Foundation (2016/330) and Swedish Fund for Research without Animal Experiments (Project number N25/15)

Keywords: Integration multi-omics data, Human pluripotent stem cell-derived cardiomyocytes, Doxorubicin

MDD114

MODELING CARDIAC ISCHEMIA WITH HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Cardiovascular diseases are the leading cause of death worldwide. The most common cardiovascular disease is ischemic heart disease, where blood flow to myocardium is reduced or blocked leading to oxygen and nutrient deprivation as well as metabolic waste accumulation in the tissue. This causes damage and death of the cells in myocardium, including cardiomyocytes (CMs) that are responsible of the contraction of heart. Pathophysiology of ischemic heart disease is still not fully understood, thus new models for ischemia are needed alongside the currently mostly used animal cell models. Human induced pluripotent stem cells (hiPS) can be differentiated into CMs (hiPS-CMs) providing an unlimited source of human CMs and, furthermore, human model to study pathophysiology of cardiac diseases. Our aim is to develop a hiPS-based ischemia model to assess the detailed effects of low oxygen level to cardiomyocyte function, molecular biology, viability and metabolism. We have developed a cell culture platform that provides controlled environment for prolonged hiPS-CM studies outside a traditional incubator. The platform enables precise control of the cell culture environment, including temperature, oxygen and carbon dioxide. The concept is also compatible with video microscopy and microelectrode array (MEA) platform, allowing simultaneous analysis of the hiPS-CM contractility and electrophysiology. Hypoxia induced changes in cardiomyocyte metabolism and cell structure. Furthermore, western blot analysis supported the results by revealing alterations in the expression of sarcomeric proteins Analysis on the MEA data showed changes in the beating characteristics of hiPS-CMs, including beating rate, beat-to-beat interval and the amplitude of the signal. hiPS-CMs are considered immature compared to adult human CMs and have

been shown to be more resistant to ischemic injury. However, we observed characteristic changes seen also in adult CMs in the behavior of the hiPS-CMs as response to hypoxic treatment.

Funding source: Pirkanmaan Kulttuurirahasto Maud Kuistilan muistosäätiö Inkeri ja Mauri Vänskän säätiö

Keywords: human induced pluripotent stem cell, cardiomyocyte, ischemia

MDD116

WFS2-IPS CELL LINE WITH MUTATION IN THE CISD2 GENE DIFFERENTIATE IN CARDIOMYOCYTES BUT NOT IN PANCREATIC ISLETS

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Wolfram syndrome (WS) is an autosomal recessive monogenic and complex disorder characterized by diabetes insipidus, childhood-onset diabetes mellitus, gradual loss of vision due to optic atrophy, deafness, bladder, bowel and temperature regulation dysfunctions in addition to endocrinological, psychiatric, and neurological abnormalities [Urano F. 2016]. The majority of patients (WS1) harbor pathogenic bi-allelic mutations in the wolframin (WS1) gene (Inoue H 1998), and about 10% carry recessive mutations in the CIDS2 (CDGSH iron-sulfur domain-containing protein 2 also known as Miner 1 or ERIS) gene responsible for autosomal recessive Wolfram syndrome 2 (WS2), [Urano F 2016]. CISD2 is essential to mitochondrial integrity and intracellular Ca2+ homeostasis (Wiley SE 2013; Wang CH; 2014; Lu S 2014) and in knockout mice, its deficiency shortens life span and drives premature aging [Chen YF, 2009]. We reported on the generation and characterization of human induced pluripotent stem cells (hiPSCs) using primary fibroblasts of homozygous and heterozygous patients with the c.103+1G>A CISD2 mutation [La Spada, 2018]. This mutation causes the deletion of a functional protein probably due to nonsense mediating decay (NMD). Here we provide experimental evidence that shows the inability of the patient's derived iPSCs to differentiate into pancreatic progenitors but able to differentiate in beating cardiomyocytes with no differences in electrical properties between homozygous, heterozygous and unrelated controls. This cell model system is useful to study the mutation effects in the development of the reported multi-systemic clinical disorders described in this family and test eventual drug effects on the specific and associated clinical phenotype.

Keywords: Wolfram Syndrome type 2, CISD2 gene mutation, hiPSC

MDD126

MIMICKING FIBROSIS AND INDUCING STRESS INTO IPSC-DERIVED CARDIOMYOCYTES

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Muscle cells known as Cardiomyocytes are the contractile cells that make up the heart; they shorten during systole and lengthen during diastole to create the rhythmic cycle that pumps blood throughout the body. Typically, aging and genetic mutations are non-environmental factors that can contribute to heart dysfunction, e.g. Dilated Cardiomyopathy (DCM) where the heart enlarges and ventricle walls weaken. While many homozygous mutations can lead contribute to DCM, it is less clear with heterozygous mutations. However in a family with a history of DCM, we found enhanced rates of early onset DCM, which was linked to the presence of two heterozygous mutations in the genes VCL and TPM1. To better understand how this specific combination of mutations contributed to disease, induced pluripotent stem cells (iPSCs) were used to create patient-specific cardiomyocytes (CMs). While baseline performance of the iPSC-CMs was reduced, we also determined to what extend additional stress could induce disease in unaffected carriers of VCL and TPM1 mutations. Cells will be placed on tunable methacrylated hyaluronic acid (MeHA) gels to mimic fibrosis that occurs post heart attack. The hypothesis of this experiment is that the performance of the iPSC-CMs derived from unaffected carriers will decrease as the MeHA gels are stiffened whereas iPSC-CMs from affected carriers and genotypically normal patients will be reduced or unchanged independent of stiffening, respectively. We anticipate that this data will highlight the need to study gene-environment connections more closely.

Funding source: California Institute for Regenerative Medicine

Keywords: iPSC, Cardiomyocytes, Fibrosis

MDD130

HUMAN ENGINEERED HEART TISSUES AS A MODEL OF CARDIOMYOPATHY IN DUCHENNE MUSCULAR DYSTROPHY

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Cardiomyopathy is currently the leading cause of death for patients with Duchenne muscular dystrophy (DMD), a severe neuromuscular disorder affecting 1 in 3,500 to 5,000 males born in the US. Animal models have provided some insight into the mechanisms by which the absence of dystrophin protein causes cardiomyopathy, but there remains a need to develop human models of DMD to validate pathogenic mechanisms and identify therapeutic targets. Here, we have developed engineered heart tissues (EHTs) from genetically edited human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) expressing a truncated dystrophin protein. The 3D environment and uniaxial force provided sufficient maturational cues to expose the DMD phenotype in vitro. Compared to isogenic controls, DMD EHTs produced less contractile force, a phenotype not yet shown in

vitro. This is likely due to the cumulative effect of other DMD phenotypes exposed in EHTs, including increased cytosolic calcium content, delayed calcium reuptake, and increased beat rate variability, and delayed sarcomere development. In this study, we demonstrate that the DMD EHT platform promotes cardiac maturation to expose a variety of DMD phenotypes, ultimately providing a powerful platform for disease modeling.

Funding source: This material is based upon work supported by grants from the National Science Foundation Graduate Research Fellowship Program under Grant Number DGE-1762114 and the National Institutes of Health NIBIB under Award Number T32EB001650

Keywords: hiPSC-derived cardiomyocytes, Tissue engineering, Duchenne muscular dystrophy

EARLY EMBRYO

MDD138

IMPACT OF X CHROMOSOME MONOSOMY ON HUMAN PLURIPOTENCY AND NEURAL CREST DIFFERENTIATION

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X chromosome monosomy (45,X/XO) results in Turner's Syndrome (TS) in females. TS phenotypes include craniofacial abnormalities, cardiac defects, and increased risk for neuroblastoma. Additionally, it is estimated that 99% of XO embryos spontaneously terminate during pregnancy. Currently, nearly nothing is known about which genes cause these phenotypes. There are about 30 genes that escape X chromosome inactivation and have Y-linked homologs, and therefore are expressed from two copies in both males and females. We hypothesize that in TS, where only one sex chromosome is present, haploinsufficiency of these genes leads to transcriptional and cellular phenotypes that impact development. We initially examined these phenotypes in the pluripotent state by generating sets of isogenic iPSCs from mosaic male (XY/XO) and mosaic female (XX/XO) patients. RNA sequencing identified the X-Y gene pairs most likely to be candidates for haploinsufficiency, as well as dysregulated genes transcriptome wide. Across the transcriptome, many dysregulated genes were involved in the Wnt signaling pathway. Wnt signaling regulates many aspects of development, including specification of the neural crest lineage. The neural crest gives rise to a wide variety of tissue types, including several with connections to TS phenotypes, such as the craniofacial skeleton, smooth muscle cells of the aorta, and peripheral neurons. Using directed differentiation of our isogenic iPSCs, we have generated neural crest progenitor cells (NCPCs). We are using these NCPCs to investigate the impact of X monosomy on the neural crest lineage through RT-qPCR, immunocytochemistry, and flow cytometry, and transcriptional profiling by RNA-seq. Comparing XO iPSCs to their isogenic euploid control is a key advantage to our study because it precludes differences caused by inter-individual genetic variability and allows for the identification of changes truly induced by X monosomy.

Keywords: iPSCs, Aneuploidy, Neural Crest

MDD139

HIGH-RESOLUTION TRANSCRIPTOMIC ANALYSIS OF INDUCED PATIENT-DERIVED COLITIC ORGANOIDS RECAPITULATES AN INFLAMMATORY SIGNATURE

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Ulcerative colitis (UC), one of the two principal types of inflammatory bowel disease (IBD) affecting approximately one million Americans, is a chronic and debilitating inflammatory condition of the colonic mucosa that usually begins in young adulthood. Although the precise etiology is unknown, UC likely results from complex pathologic interactions that involve genetic predisposition, immune activity, and the colonic microenvironment. Lack of human-derived ex vivo models of UC is a major barrier to study how microenvironmental factors contribute to disease development. To address this problem, we have developed an induced human ulcerative colitis-derived organoid (iHUCO) with both epithelial and mesenchymal compartments as a superior model to study the role of microenvironmental interactions. We hypothesize that high-resolution transcriptomic analysis at the single cell level comparing iHUCOs to induced human normal colonic organoids (iHNO), improves our understanding of the mesenchymal and epithelial compartments in UC vs. normal colon. We reprogrammed the colonic fibroblasts isolated from UC patients to iPSCs (N=3) followed by directed differentiation to colon thus generating iHUCOs. In parallel, we used the same protocol to develop iHNOs (N=3) derived from the healthy part of the colon as a control. We established robust conditions to develop iHUCOs recapitulating histological and functional features of the primary colitic tissues, including the inflammatory secretome (elevated CXCL8 levels (7.167x NL; $p < 0.005$)), and the absence of neutral mucus secretion ($p < 0.0001$). Next, we performed single-nucleus RNA-seq (sn-RNA-Seq) using the 10x Genomics platform to conduct gene expression and cell population analyses within and across the iHN and iHUC organoids as well as in their parental fibroblasts. Consistent with the origin of iHUCOs, sn-RNA-seq revealed an inflammatory signature including cytokine-mediated signaling pathway (FDR = 0.00001), and immune response (FDR < 0.00004) biological processes (GO terms) in the mesenchymal compartment of iHUCOs. In summary, iHUCOs provide a superior model to study the complexity of UC including epithelial-mesenchymal interactions.

Keywords: iHUCOs, Ulcerative Colitis, iPSCs-derived organoids

MDD141

HYDROGEL 3D DEFINED CULTURE OF NAIVE HUMAN PLURIPOTENT STEM CELLS

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Lewis, Lara - *Cancer and Stem Cells, University of Nottingham, Nottingham, UK*
Merry, Cathy - *Cancer and Stem Cells, University of Nottingham, Nottingham, UK*

Naive human pluripotent stem cells (hNPSCs) have been a big revolution in human embryology, with recent reports showing that these cells have a higher differentiation potential than their primed counterparts, increasing their interest in regenerative medicine and tissue engineering. However, some human PSCs have shown resistance towards reversion to the naïve state, with the different protocols described requiring feeder cells and hypoxic conditions, and reportedly showing DNA instability over long-term culture. In order for these cells to be used at their full potential we need to improve their culture conditions to make them compatible with tissue engineering applications, by removing any undefined components; such as Matrigel or feeder cells. We have previously shown that a human serum-derived protein, Inter-a-inhibitor (IaI) can support the growth and long-term maintenance of hPSCs in vitro without inducing differentiation or genetic abnormalities. IaI can also support hNPSCs; by adding IaI to formulations for naïve resetting such as RSeT and 2iGöY media, we have reset and maintained hNPSCs in feeder-free, coating-free, defined culture conditions. The cells show transcriptional and protein expression of naïve markers, overall DNA hypomethylation and increased mitochondrial oxidative respiration. Protein expression analysis of the hNPSCs grown in naïve IaI conditions showed that they express less integrin receptors and lower integrin-related signalling. Therefore, we investigated the possibility of growing these hNPSCs in 3D synthetic hydrogels. We can now show that hNPSCs thrive in these synthetic, chemically-defined hydrogels. The cell colonies grow as 3D clusters in non-integrin dependent culture, expressing naïve markers such as DPPA3 and KLF17. In conclusion, we propose a novel platform to study early embryonic development in vitro using a defined, synthetic and xeno-free culture method. The hNPSCs adapt easily to this defined, naked hydrogel, without the need for integrin-related signalling. We show that this defined 3D method increases naïve pluripotency, generating purer culture populations and promoting naïve pluripotency without a hypoxic atmosphere. This is new platform can bring tissue engineering techniques toward modelling of the pre-implantation embryonic development.

Funding source: Wellcome Trust

Keywords: Hydrogel, 3D Culture, Naïve

MDD440

SYMMETRY BREAKING OF DIFFERENTIATING ADHERENT MOUSE EMBRYONIC STEM CELLS ON MICROPATTERNS

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Embryonic symmetry breaking precedes the formation of the body axes, an evolutionary and developmental hallmark giving rise to bilateral body plans with directed locomotion and cephalization. However, how symmetry is broken in the mammalian embryo is poorly understood, and quantitative in vitro assays capable of recapitulating these events are rare.

Previous work has demonstrated that asymmetrical cell fate patterning occurs in vitro in the absence of extraembryonic cells, suggesting that symmetry breaking is an inherent collective behavior of a pluripotent stem cell cluster. Hitherto, in vitro studies have focused on studying this phenomenon in three-dimensional cell aggregates, and there have not been any reports on symmetry breaking in adherent, largely planar, cell populations.

Our lab has established a two-dimensional adherent micropatterning platform to investigate symmetry breaking with precise control over the shape, size and position of each cell colony within a well. This assay allows for high throughput, both experimentally with multi-well plates, and analytically with specially developed quantification frameworks. Using this system, we observed a polarized expression of Brachyury (mesoderm marker) and Sox2 (ectoderm marker) at opposing ends of a colony when mouse pluripotent stem cells (mPSCs) are differentiated on small (diameter < 400um) micropatterns. We identified conditions wherein symmetry breaking robustly occurs and showed how colony size, colony density, input cell state, and the concentration of biochemical inducers including BMP4 and WNT would influence the extent of symmetry breaking in mPSC colony. Using continuous live-imaging, we further profiled the process of mPSC symmetry breaking on adherent micropatterns and tracked the migration trajectories of Brachyury-positive mesodermal cells. We are moving forward to using Fluorescent in situ sequencing to examine the complex cell-cell interactions during symmetry breaking. Together, we demonstrated that symmetry breaking is not dependent on a three-dimensional system and the platform we built allowed us to deconstruct the fundamental molecular and cellular events during symmetry breaking which yield new insight into the underlying developmental principles.

Keywords: Symmetry breaking, Mouse pluripotent stem cell, micropattern

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

MDD147

INVESTIGATING THE ROLE OF TBX2 AND TBX3 IN HUMAN ENDODERM DEVELOPMENT USING HUMAN PLURIPOTENT STEM CELLS

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TBX2 and TBX3 are highly related T-box transcription factors involved in the development of a number of mesodermal and endodermal tissues, with mouse models indicating that Tbx3 is critical for liver bud expansion. Interestingly, upon interrogation of recent publicly available gene expression datasets of purified pancreatic beta cells from type 1 diabetic (T1D) or type 2 diabetic (T2D) individuals, both TBX3 and TBX2 were upregulated in T2D beta cells while TBX2 was one of the most highly upregulated genes in T1D beta cells. Normal adult human pancreatic beta cells, in contrast to the mouse pancreas, do not express either TBX2 or TBX3. To explore the upregulation of T-box factors in diabetic patients and to study their role in the human pancreas and liver development, we used human embryonic stem cells (hESCs) to investigate the contribution that TBX2 and TBX3 have in committing cells to a pancreatic or a hepatic fate during development. Using a CRISPR/Cas9-mediated genome editing strategy, we generated TBX3 and TBX2 TBX3 null hESC lines, differentiating each using protocols that mimic pancreatic or hepatic developmental conditions. We found that mutant lines make pancreatic progenitors more efficiently, and differentiate less efficiently into hepatocytes when compared with wild type stem cell lines. Additionally, cells without TBX3 express the pancreas master regulator PDX1 during the hepatocyte differentiation protocol, while in WT cells PDX1 expression is undetectable during hepatocyte differentiation. These results suggest that TBX3 may be regulating liver development through the repression of PDX1, which is interesting considering previous studies have shown that PDX1 represses liver specific genes, including TBX3. We hypothesize that TBX3/2 and PDX1 may antagonize each other, helping to define a pancreas or a liver fate. These results provide insight into the aberrant expression of developmental genes like TBX2 and TBX3 in diabetic β-cells, with future studies aimed at gaining insights regarding the role of the dysregulation of β-cell identity in diabetes disease progression.

Keywords: diabetes, human embryonic stem cells, T-box transcription factors

MDD148

IN VIVO TRACKING OF STEM CELL-DERIVED ISLET ORGANOIDS USING MAGNETIC PARTICLE IMAGING

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Differentiating induced pluripotent stem cells (iPSCs) to insulin producing β cells is a promising approach for type 1 diabetes (T1D) cell replacement therapy. An issue with this therapy is the lack of in vivo monitoring technique that can determine graft outcome after transplantation. Magnetic particle imaging (MPI) is novel imaging modality that directly detects the superparamagnetic properties of iron oxide nanoparticles, and is specific, sensitive, and linearly quantitative. Here we demonstrate the feasibility of in vivo tracking of transplanted stem cell-derived islet organoids using MPI in a mouse model. Human iPSCs (hiPSC-L1) were differentiated to islet organoids according to Russ HA's protocol and labeled with magnetic nanoparticles for 48 hours. Cell labeling efficiency was examined by anti-dextran staining. Islet organoids phantoms comprising of different numbers of labeled islet organoids (25, 50, 100, 200 or 400, n=2) were imaged using an MPI system. The iron content of samples was calculated from the MPI images and verified using the inductively coupled plasma (ICP) mass spectrometry analysis. 800 labeled organoids were transplanted under the left kidney capsule of a NOD/scid mouse. Mice were imaged and 3D MPI images were acquired at 1, 7, 14, 28, and 42 days post transplantation. Quantitative assessment of the islet organoids was performed. Before euthanasia the left kidneys were collected for histological immunofluorescence staining. The results showed that 99% of the cells in islet organoids were labeled with nanoparticles as confirmed by anti-dextran staining. Image analysis of labeled organoids phantoms revealed a direct linear correlation between the iron content and the number of organoids (R^2=0.996, $P<0.0001$), and correlated with the results of the ICP analysis. Signals on 3D images representing labeled islet organoids were detected under the kidney capsule on the first day post Tx in all recipients. During the course of the study the signal from labeled islet organoids under the kidney capsule decreased, as expected. The kidney sections staining showed the presence of functional organoid grafts as confirmed by staining for insulin. In conclusion, longitudinal MPI can monitor transplanted islet organoids providing direct quantitative information of their presence in vivo.

Keywords: Magnetic particle imaging, Diabetes, Stem cell transplantation

MDD174

MODELING HEPATIC INSULIN RESISTANCE IN HUMAN WITH IPSC-DERIVED FATTY LIVER ORGANOIDS

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Nonalcoholic fatty liver disease (NAFLD) and its downstream diseases are growing rapidly, leading to increased morbidity and mortality. Hepatic insulin resistance is oftentimes accompanied by NAFLD. Contrasting systemic insulin resistance, NAFLD in particular causes hepatic insulin resistance through increased hepatic fat accumulation, wherein its association is highly variable depending on heritable and non-heritable factors. However, precise genetic and molecular mechanisms between hepatic fat and insulin resistance are not fully understood partly due to multiple confounding factors in clinics. The goal of this study is to investigate genotype specific association over hepatic fat accumulation and its association to hepatic insulin responsiveness by studying +20 genotyped human stem cell derived liver organoid library. We first generated an in vitro system that can enable population-scale phenotyping en masse by evaluating fat accumulation with human iPSC-derived liver organoids. Organoid level genotype-phenotype association studies validated the impacts of GWAS discovered key NAFLD risk alleles including PNPLA3 and GCKR. Next, we established assays to evaluate hepatic insulin resistance in normal vs fatty liver organoid, thereafter, insulin responsiveness was evaluated using scRNA, gene expression, and signal pathway analysis. In iPSC organoid carrying NAFLD risk allele, we confirmed phosphorylation of AKT with insulin stimulation in healthy conditions, whereas fatty liver organoids did not. Similarly, the FOXO1 and SREBF1C, which are induced by insulin, and the gluconeogenesis control gene PEPCK, were responded to insulin stimulation in healthy but not in fatty liver organoids. On the other hand, CREB that regulates gluconeogenesis showed minimal change, suggesting the FOXO1-PEPCK pathway plays an important role in the constitutive activation of gluconeogenesis in fatty liver conditions. Further analysis revealed that DGATs, an enzyme that converts diacylglycerol and fatty acyl-CoA to triacylglycerol, was increased by fat accumulation in risk carrier. In summary, human organoid based metabolic disease model informs key pathways to interrogate hepatic pathology and determine patient's precision by dissociating hepatic insulin resistance and NAFLD phenotypes.

Keywords: human iPSC derived liver organoid, insulin resistance, fatty liver disease

MDD178

IPSC-DERIVED HEPATOCYTES REQUIRE MACROPHAGES TO RESPOND TO EBOLA VIRUS INFECTION

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Ebola virus (EBOV) infection causes severe disease and liver damage in humans. High viral titers are produced in the liver, and increased viremia is associated with fatality. Therefore, the liver may be a critical target for therapeutic intervention. Patient data indicates that a pro-inflammatory response occurs in the liver during infection, but this requires further investigation. To understand the role of hepatocytes in human infection, we compared the response of immortalized (Huh7), iPSC-derived (iHep), and primary hepatocytes (PHHs) to EBOV. iHeps and PHHs were infected at similar but lower rates than Huh7 cells. Infected iHeps and PHHs formed classic viral inclusions, and EBOV-infected iHeps produced filamentous nascent particles by electron microscopy. All three hepatocyte platforms, however, were transcriptionally silent upon EBOV infection 1 day post-infection when compared to mock infected cells. This result was confirmed with functional tests and by fluorescent in situ hybridization (FISH). Since hepatocytes are not infected in isolation in vivo, we developed a co-culture of donor matched primary monocyte-derived macrophages (MDMs) and iHeps that mimics the transmission of EBOV to the liver from the blood. MDMs were infected with EBOV for 1 day and then either split onto uninfected iHeps or supernatant from infected MDMs was used as inoculum for iHeps. iHeps in co-culture with infected MDMs became infected and produced type I interferons in response to EBOV. We demonstrate the need for co-culture systems with multiple cell types to better understand the mechanisms by which EBOV induces host responses and disease.

Funding source: 1 F31 AI140656-01A1

Keywords: hepatocytes, Ebola virus, co-culture

MDD180

LNCRNA DIGIT AND BRD3 PROTEIN FORM PHASE-SEPARATED CONDENSATES TO REGULATE ENDODERM DIFFERENTIATION

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Cooperation between DNA, RNA and protein regulates gene expression and controls differentiation through highly structured interactions connecting regions of nucleic acids and protein domains and also through the assembly of biomolecular condensates. Here, we report that endoderm differentiation is regulated through the interaction of the long noncoding (lnc) RNA DIGIT and the bromodomain and extra-terminal (BET) domain protein BRD3. BRD3 forms phase-separated condensates, the formation of which are promoted by DIGIT, occupies enhancers of endoderm transcription factors, and is required for endoderm differentiation. Purified BRD3 binds acetylated histone H3 lysine 18 (H3K18ac) in vitro and co-occupies the genome with H3K18ac. DIGIT is also enriched in regions of H3K18ac, and depletion of DIGIT results in decreased recruitment of BRD3 to these regions. Our findings show that cooperation between DIGIT and BRD3 at regions of H3K18ac regulates the transcription factors that drive endoderm differentiation and suggest a broader role for protein-lncRNA phase-separated condensates as regulators of transcription.

Funding source: NIH/NICHD grant R01HD09277302 to A.C.M.

Keywords: BRD3, lncRNA DIGIT, Definitive endoderm

MDD181

HEPATIC EXPRESSION OF MUTANT TRANSTHYRETIN REMODELS PROTEOSTASIS MACHINERY IN HEREDITARY ATTR AMYLOIDOSIS

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Systemic amyloidosis represents a class of disorders in which misfolded proteins are secreted by effector organs and deposited as proteotoxic aggregates at downstream target tissues. Despite being well-described clinically, the contribution of effector organs such as the liver to the pathogenesis of these diseases is poorly understood. Here, we utilize a patient-specific induced pluripotent stem cell (iPSC)-based model of hereditary transthyretin (TTR) amyloid disease (ATTR amyloidosis) in order to define the contributions of hepatic cells to the distal proteotoxicity of secreted TTR. To this end, we employ a gene correction strategy to generate isogenic, ATTR amyloidosis patient-specific iPSCs expressing either amyloidogenic or wild-type TTR. We further utilize this gene editing strategy in combination with single cell RNAseq to identify multiple hepatic proteostasis factors, including many components of the adaptive unfolded protein response (UPR) signaling pathways, whose expression correlates with the production of destabilized TTR variants in iPSC-derived hepatic cells. We further demonstrate that enhancing ER proteostasis within ATTR amyloidosis iPSC-derived hepatic lineages via stress-independent activation of aforementioned adaptive UPR signaling preferentially reduces the secretion of destabilized amyloidogenic TTR. Together, these results suggest the potential of the liver to chaperone-at-a-distance and impact pathogenesis at downstream target cells in the context of systemic amyloid disease, and further highlight the promise of UPR modulating therapeutics for the treatment of TTR-mediated and other amyloid diseases.

Keywords: hereditary amyloidosis, hepatic disease, single cell transcriptomics

MDD184

HIPSC-DERIVED METABOLIC CELL TYPES REVEAL INDIVIDUAL-SPECIFIC GLUCOCORTICOID DRUG RESPONSE

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Glucocorticoids (GCs) are widely used and effective anti-inflammatory drugs, but their long-term causes many metabolic side effects including diabetes, central obesity, dyslipidemia, and hepatic steatosis. Thus, understanding the underlying mechanisms driving individual-specific response to GCs could inform personalized and precision medicine. GCs act via the glucocorticoid receptor (GR), a member of the nuclear hormone receptor superfamily which acts in chromatin to regulate genes in a cell-type specific manner. GRs recognize and bind to specific non-coding DNA motifs which function as enhancer regions to regulate gene expression. We hypothesized that natural genetic variation would influence individual-specific responses to GCs in liver and adipose tissue. To test this we generated hepatocytes and adipocytes from a cohort of induced pluripotent stem cells (iPSCs), and treated these individual-derived cells with dexamethasone (Dex), a potent synthetic GC. Treated cells were subjected to transcriptomic (RNA-seq) and cistromic (ChIP-seq) analyses, which demonstrated individual-specific effects of the glucocorticoid drug dexamethasone (Dex) on gene expression as well as genomic binding. Many single nucleotide polymorphism (SNPs) were identified as affecting GR binding and modulating nearby Dex-induced metabolic genes that could contribute to the side effects of Dex. These data endorse an iPSC-based experimental framework to discover genetic variants that impacts GR genomic occupancy and patient responses to glucocorticoid drugs, with implications for developing personalized therapies.

Keywords: Precision medicine, genetic variation, iPSC, hepatocytes, adipocytes, drug response

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

MDD188

IDENTIFICATION OF SMALL MOLECULE ACTIVATORS OF SLC2A1 GENE EXPRESSION IN BRAIN MICROVASCULATURE ENDOTHELIAL CELLS

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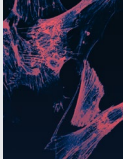
Glucose transporter type 1 (GLUT1) deficiency syndrome (G1DS) is a genetic disorder that impairs brain glucose metabolism. G1DS is caused by haploinsufficiency of SLC2A1 gene, which encodes GLUT1, a membrane-bound glycoprotein responsible for regulating glucose transport across the blood–brain barrier and glycolysis for brain development. Heterozygous deletion and various loss-of-function (LOF) missense mutations of SLC2A1 have been found associated with G1DS. G1DS manifests with a wide variety of neurological symptoms, including cognitive impairment, seizures, delayed brain development, intellectual disability and complex movement disorders that usually start in early childhood. Symptomatic treatments such as ketogenic diet management and anti-epileptic drugs (AEDs) for epilepsy/seizure have been used for G1DS with limited success, and there are no curative therapies targeting the genetic root cause currently. At Fulcrum, we sought to develop a small molecule therapy to stimulate the SLC2A1 gene expression in pathologically relevant cells. To achieve this goal, we first developed a G1DS relevant in vitro model using brain microvasculature endothelial cells (BMECs). We then generated the SLC2A1 haploinsufficiency model using antisense oligo (ASO) mediated knock-down (KD) and CRISPR/CAS9 mediated heterozygous knock-out (KO) approaches. Based on these genetic models, we established the genotype and phenotype relationship using glucose uptake and Agilent Seahorse glycolysis assays. Subsequently, we identified a set of small molecules that can transcriptionally upregulate SLC2A1 gene in this BMEC model. We are currently validating the potential targets using chemical and genetic approaches and evaluating the functional consequence of SLC2A1 upregulation in BMECs using a quantitative glucose uptake assay. In addition, efforts have been made to generate diseased BMECs from patient donor iPSCs for a “clinical trial in a dish” with validated small molecules. We expect that the identification of small



molecule modulators of SLC2A1 gene expression in the disease relevant BMEC model would likely accelerate the development of a root cause therapy for G1DS.

Keywords: GLUT1 deficiency Syndrome, SLC2A1 haploinsufficiency, Small molecule

EPITHELIAL

MDD203

HOW ABERANT NOTCH SIGNALING OF THE HUMAN PROXIMAL AIRWAY ALTERS SURFACTANT PROTEIN PRODUCTION OF CLUB CELLS

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The Notch signaling pathway promotes cell proliferation, differentiation, and patterning that is required for proper human lung development. Current research shows increased Notch1 signaling in alveolar type II (ATII) cells induces their proliferation and de-differentiation, resulting in defective surfactant protein B (SPB) processing, leading to abnormal lamellar body formation and faulty SPB production. However, little research has elucidated the role of SPB in the proximal airway, as well as how notch signaling regulates the production of SPB in secretory club cells. Club cells are known to generate ciliated, goblet and ATII cells but little is known about the impact of Notch inhibition on cell fate in the setting of SPB deficiency. Our study analyzes the influence of notch signaling on SPB expression in secretory club cells by inhibiting Notch signaling in human stem cell derived proximal lung epithelial cells. Using human stem cells, we performed directed differentiation using a cocktail of small molecules and growth factors, resulting in a heterogenous population of both 2D lung airway cells as well as 3D lung organoids. Immunofluorescence staining confirmed the presence of SCGB3A2, a marker of secretory club cells. DAPT, a Notch γ-secretase inhibitor, was added to the lung organoid cultures for 7 days. Grossly, the organoids exposed to Notch inhibition appeared stunted in growth as compared to the non-treated lung organoids. These were fixed and we aim to perform ICC to determine the population of SCGB3A2, FOXJ1, MUC5A and P63 proximal populations in the organoids. We then aim to use a SCGB3A2-mCHERRY reporter cell line to differentiate into 2D and 3D lung organoids, expose them to Notch inhibition, sort for SCGB3A2 and examine surfactant gene expression, protein expression and ultrastructures using transmission electron microscopy. In summary, these findings show that an in vitro stem cell derived lung organoid model is the optimal system to study the effects of deranged Notch signaling pathways during human lung development. We aim to show the importance of proper Notch signaling in protein expression in club cells. These findings may elucidate a novel mechanism of surfactant deficiency in neonatal and adult lung diseases, such as Respiratory Distress Syndrome, leading to the development of novel therapies.

Funding source: CIRM Bridges to Stem Cells Intern

Keywords: Pluripotent Stem Cell Differentiation, Epithelial Tissues, Notch

MDD204

IONIC REGULATORY EPITHELIA IN HUMAN PLURIPOTENT STEM CELL-DERIVED INNER EAR ORGANOIDS

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Genetic inner ear pathologies are one of the most common congenital disorders in humans. Over 110 genes have been linked to these hearing or balance disruptions. Recently, our group defined a method for generating inner ear sensory epithelia from human pluripotent stem cells that could be used as a model to evaluate the impact of gene mutations in human inner ear function. To date, we have focused on inner ear sensory cells, such as the hair cells. However, normal function of the inner ear is dependent on the electrical potential of the endolymph. This potential is maintained by the surrounding ionic regulatory epithelia (also known as non-sensory epithelia), such as the stria vascularis in the cochlea and the dark cell area in vestibular organs. Thus, the origin of inner ear pathologies is not limited to the sensory epithelia and in vitro models should, ideally, contain the functional combined unit of these epithelia to truly recapitulate pathologies. Here, we identify key features of ionic regulatory epithelia in human inner ear organoids. We generated inner ear organoids in a three-dimensional culture by modulating the FGF, TGF, BMP and WNT pathways and used immunohistochemistry to confirm production of inner ear hair cells and neurons. Vestibular transitional cells, dark cells, and associated melanocytes were identified surrounding the sensory epithelia, which indicates the presence of ionic regulatory epithelia. To compare these results with normal human inner ear development, human embryonic and fetal inner ears were harvested from healthy subjects following elective termination of pregnancy. Development of these sensory and ionic regulatory epithelia was followed over time and showed similar protein

expression patterns between early stage fetal specimens and inner ear organoids. Additionally, by using a Jervell and Lange-Nielsen syndrome patient-derived pluripotent stem cell line, we were able to generate similar inner ear organoids. This syndrome leads to deafness and vestibular dysfunction by a mutation in the KCNQ1 potassium channel that is normally expressed in dark cells and contributes to endolymph homeostasis. Our study shows the reconstitution of key cell types of the fetal inner ear's ionic regulatory system in vitro. Further research will focus on the ionic homeostasis of these inner ear organoids.

Keywords: Human Inner Ear Organoid, Ionic Regulatory System, Inner Ear Fetal Development

MDD205

MODELING GASTRIC POLYP FORMATION IN FAMILIAL ADENOMATOUS POLYPOSIS: REGIONAL SENSITIVITY TO WNT ACTIVATION IN GASTRIC STEM CELLS

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Familial adenomatous polyposis (FAP) is a condition characterized by excessive proliferation throughout the gastrointestinal tract, resulting in the formation of precancerous polyps that can progress to adenocarcinoma. Polyps form in response to increased Wnt signaling caused by mutations in the adenomatous polyposis coli (APC) gene. Studies in the colon suggest there is a 'just-right' level of Wnt signaling that is favorable for polyp growth, as indicated by correlations between site of APC mutation and polyp burden. However, although gastric polyps are common in FAP patients, there is little known about how APC mutations affect polyp burden in the stomach. To model gastric polyp formation in FAP patients, we used Sox2-CreERT2; APC580-flox/flox (APC -/-) and Sox2-CreERT2; APC580-flox/+ (APC +/-) mice and measured gastric stem cell responses after Wnt activation via tamoxifen treatment. In the distal stomach, as predicted, we observed hyperproliferation and tissue expansion in homozygous APC -/- mice with no change to proliferation in heterozygous APC +/- mice. In contrast, the body of the stomach did not show a proliferative effect of Wnt activation in APC -/- mice, suggesting differences in gastric stem cell responses to Wnt activation in the proximal and distal stomach. To measure gastric stem cell activity, we generated organoids from APC -/- and APC +/- mice. Notably, organoid forming efficiency of APC -/- mice was reduced in the proximal but enhanced in the distal stomach. These findings are consistent with the in vivo observations and suggest that there is a regional sensitivity to Wnt signaling activation required for stem cell expansion and polyp formation in the stomach. To further probe the requirements for polyp formation, we have established a biobank of nonpolyp and polyp FAP patient organoids containing a spectrum of APC mutations. FAP patient organoids vary in their growth properties and current studies are focused on associating APC mutation with organoid proliferation to further understand regional sensitivity to Wnt activation. Ongoing studies in mouse models and patient samples will help define how APC mutations drive polyp formation in the stomach and inform clinicians on FAP gastric disease by providing an understanding of human gastric stem cell responses to Wnt activation.

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Keywords: Organoids, Wnt Signaling, Gastric Stem Cells

MDD493

METHODS AND MEDIA FOR THE DIFFERENTIATION OF HUMAN INTESTINAL ORGANOIDS AND ORGANOID-DERIVED MONOLAYERS

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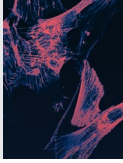
Organoids are a cutting-edge tool for regenerative medicine, disease modeling, and drug screening. IntestiCult™ Organoid Growth Medium (OGM Human) supports the stem cell niche and promotes efficient expansion of human intestinal organoids from human primary tissues. However, the low number of differentiated cells in these cultures can limit their utility for studies requiring mature cell types. The focus of this study is to develop media and protocols for differentiating both intestinal organoids and organoid-derived monolayer cultures to generate mature, differentiated cell types, including enterocytes, goblet cells, and enteroendocrine cells (EEC). The IntestiCult™ ODM (ODM Human) is designed to promote differentiation of both the absorptive and secretory cell lineages while reducing the supportive conditions for the stem cell niche, thus allowing the direct differentiation of established organoid cultures by simply replacing IntestiCult™ OGM with IntestiCult™ ODM after five days of growth. To establish a differentiated monolayer culture, cells from dissociated organoids were seeded onto Corning® Matrigel®-coated Transwell® plates in IntestiCult™ ODM. After 7 days, the monolayer cultures, with and without ALI differentiation conditions, were analyzed by immunocytochemistry, qPCR, transepithelial electrical resistance (TEER), and Ussing Chamber analysis. Differentiated organoid cultures, analyzed by qPCR, exhibited an 8.87 ± 0.86 (mean ± SEM; n=5)-fold decrease in



expression of the stem cell marker LGR5, a 1,599 ± 703 (n=5)-fold increase in expression of the goblet cell marker MUC2, and a 181 ± 76 (n=5)-fold increase in expression of the EEC marker CHGA. With ALI differentiation, there was increased expression of the enterocyte marker KRT20 353 ± 66-fold, MUC2 expression 28,012 ± 6,312-fold, and CHGA expression 16,256 ± 8,382-fold (n=3). Intestinal organoid-derived monolayers had TEER values averaging 1,101 ± 114 Ω*cm2 (mean ± SEM; n=10), compared to an average of 301 ± 22 Ω*cm2 (n=10) for Caco-2 cell line monolayers. Together, these data support the use of IntestiCult™ ODM for the differentiation of human intestinal organoids, or organoid-monolayer cultures, to better recapitulate the human intestinal epithelium for the study of human intestinal physiology, infection and disease.

Keywords: Organoids, Organoid-Monolayers, Differentiation

EYE AND RETINA

MDD222

HIGH THROUGHPUT SCREEN IDENTIFIES EPITHELIAL PHENOTYPE RESTORING DRUGS THAT SUPPRESS AMD PHENOTYPE IN COMPLEMENT INDUCED IPSC-MODEL

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Complement has been genetically linked as a major risk factor for Age-related macular degeneration (AMD). Still, no therapeutic drug has yet come out as a successful treatment for the dry form of AMD, likely because of insufficient knowledge of the mechanism of action of complement proteins (C5, C3) in causing disease pathogenesis. We developed AMD model using iPSC-RPE (Retinal Pigment Epithelium) to study the molecular events triggered by C5, C3 in disease pathogenesis. The study aims to discover drugs that restore the complement induced AMD phenotype in iPSC-RPE. Human iPSC lines of different AMD genetics were differentiated into RPE. Complement Competent-Human Serum (CC-HS) was used to induce AMD like phenotype in iPSC-RPE, Complement Incompetent-Human Serum (CI-HS) served as the control. Receptor blockers for C5aR1 and C3aR1, and depleted sera for C5 and C3 were used to confirm the role of the complement. Immunostaining was used to check APOE and lipid deposits, epithelial phenotype, and p65 localization for the NF-kB pathway. Western Blot was done to check for LC3-II levels, C5, and C3 pathway activation (ERK1/2, AKT). Phagocytosis assay assessed the functionality, and Trans Epithelial resistance (TER) measured the junctional integrity of iPSC-RPE. High throughput screen was done to discover potential drugs that can rescue RPE cell death. ACA and L-45870, two drugs discovered from high throughput screen, rescued the AMD phenotype caused by CC-HS treatment to iPSC-RPE. CC-HS treatment elicited APOE and lipid deposits, eroded the epithelial phenotype, compromised junctional integrity as measured by TER, and reduced the phagocytic ability of iPSC-RPE. Drugs reversed the molecular events triggered by CC-HS., LC3-II levels were recovered in drugs treated samples confirming the recovery of autophagy stress in iPSC-RPE. NF-kB pathway activity was also subdued in iPSC-RPE treated with drugs in presence of CC-HS. The cells treated with the drugs attained back the epithelial shape and hexagonality, polarization, and functionality lost with the CC-HS treatment. We developed AMD model using iPSC-RPE that recapitulated disease phenotype and dissected the cell-autonomous induction of sub-RPE deposits. The model helped us discover drugs that could potentially be used to treat dry AMD at an early phase of the disease.

Keywords: iPSC derived Retinal Pigment Epithelium, Complement, autophagy, AMD, drug screening

MDD224

IN VITRO MODELING OF THE GLAUCOMA RISK FACTOR POU6F2 USING HUMAN PLURIPOTENT STEM CELLS

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Retinal ganglion cells (RGCs) are the projection neurons of the visual system that connect the eye and the brain. Damage to, and subsequent degeneration of these cells is observed in optic neuropathies such as glaucoma. Among the factors associated with this degeneration, SNPs within the transcription factor Pou6f2 have recently been identified as a novel risk factor for glaucomatous neurodegeneration, yet the mechanism by which Pou6f2 regulates RGCs remains unclear. Human pluripotent stem cells (hPSCs) provide an attractive tool for translational applications including disease modeling, as they can be derived from patient sources and are capable of forming any cell type of the body, including RGCs. As previous studies have shown that Pou6f2 confers susceptibility to cell death in glaucomatous conditions, efforts were directed towards characterizing the expression patterns of Pou6f2 in hPSC-derived retinal organoids at early and later timepoints using cryosectioning and immunofluorescence. The onset of the expression of Pou6f2 was observed around D30 of retinal differentiation with a marked increase by D40 and a subsequent decrease at later timepoints. To further understand the role of Pou6f2 with respect to RGCs, a CRISPR/Cas9 engineered Pou6f2 knockout cell line was generated. There was no difference in the derivation of RGCs from the Pou6f2 knockout cells, however the impact on the RGC cell survival and on their morphology is yet to be investigated. The reported link between Pou6f2 expressing RGCs and glaucoma opens further avenues for exploration of this gene as a potential RGC subtype specific marker that predisposes the RGCs to die in glaucoma. The inclination of the Pou6f2 expressing RGCs to

apoptosis in glaucoma brings forth the question of whether the expression of this gene is detrimental to RGCs or has a role in neuroprotection, which is yet to be explored.

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Keywords: retinal ganglion cell, pluripotent stem cell, glaucoma

MDD226

ISOGENIC IPSC-DERIVED 3D-BIOPRINTING OF RPE/CHOROID TISSUE FOR PATIENT-SPECIFIC RETINAL DEGENERATION DISEASE MODELING

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Human iPSC can provide multiple retinal progenitors for regenerative therapy. Our recent advances in 3D-bioprinting allowed us to produce functional retinal pigment epithelium (RPE) and choroidal tissues. Here, we aim to combine multiple isogenic iPSC-derived cells to create an organized multilineage tissue for patient-specific ocular therapy and disease modeling. iPSC lines were generated from healthy and retinal degeneration patients (AMD and Best disease), and differentiated into RPE, endothelial cells (EC), pericytes, fibroblasts, and macrophages. Differentiated cells were purified, expanded and validated by immunophenotyping and functional tests. RPE monolayer maturation was confirmed using transwell membrane culture. Capillary formation was assessed by a hydrogel tubulogenesis assay. 3D-bioprinting was utilized to assemble iPSC-derived EC, pericytes and fibroblasts into choroid-like tissue on a biodegradable PLGA polymer, which was then co-cultured with an RPE monolayer. Healthy donor retinal EC, pericytes, and primary fibroblasts were used as a control. iPSC-RPE monolayers formed on transwell membranes exhibited physiological trans-epithelial resistance (TER) and phagocytic ability. RPE were polarized, as indicated by the apical microvilli formation. Endothelial cell identity was verified by acetylated-Dil-LDL uptake and expression of CD31, CD34, CD146 and CD144. Pericytes were differentiated from CD31- cells using TGFb3 and PDGF-bb. Pericytes expressed PDGFR-beta, NG2, and CD44. Fibroblasts were differentiated from pericytes and expressed FSP1, vimentin, connexin43, and collagen-I. The bioprinted choroid was allowed 7 days maturation period, after which RPE cells were seeded on the opposite side of the membrane and allowed to mature for 3-5 weeks. Studies in progress include i) vascular stabilization using activated macrophages in bio-printed choroid tissue and ii) comparisons of isogenic and non-isogenic combinations of RPE and choroid. Our results show that all five cell components (RPE, EC, pericytes, fibroblasts, and macrophages) can be successfully differentiated from isogenic human iPSC. This study provides the foundation for clinical application of iPSC-derived 3D-tissue, disease modeling, and study model of immune cell reaction in RPE/choroid.

Keywords: 3D-bioprint, iPSC differentiation, Retina/Choroid

MDD229

MODELING CONGENITAL BLINDNESS CAUSED BY MUTATIONS IN THE NPHP5 GENE USING HUMAN IPSC-DERIVED RETINAL ORGANOIDS

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Leber congenital amaurosis (LCA), a group of early-onset blinding diseases, is caused by mutations in over 25 genes. Mutations in ciliary gene NPHP5 cause ciliopathies that include retinal phenotypes including LCA. Using iPSC lines derived from patients with NPHP5 mutations and respective familial controls, we generated 3D cultured organoids with photoreceptors as well as retinal pigmented epithelium (RPE) in monolayer cultures. Patient and control iPSCs exhibited similar differentiation with consistent staining of retinal lineage specific markers. Morphology of cultured organoids revealed outer segment like structures on the apical side of both patient and control organoids. RNA-Seq analysis suggested dysregulation of rhodopsin mediated signaling pathway and photoreceptor homeostasis in patient organoids. Notably, we observed significant cilia structural abnormalities in patient fibroblast and RPE cells manifesting as longer and branched cilia. Moreover, immunostaining results showed accumulation of IFT88 in patient RPE cilia. These results suggest that the NPHP5 mutation induced cilia defects in photoreceptors and RPE in human organoid cultures by affecting the localization of key proteins in the outer segments. Our studies provide new insights into NPHP5-LCA pathogenesis using patient-derived cells and should help in designing therapeutic interventions.

Keywords: NPHP5, retinal organoids, Leber congenital amaurosis (LCA)

MDD235

A HUMAN ORGANOID-BASED MODEL FOR RETINOBLASTOMA

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Retinoblastoma is the most common eye cancer occurring in children under the age of five. It is caused by biallelic inactivation of the retinoblastoma gene RB1, presumably in cone precursor cells of the human retina. Efforts to model retinoblastoma in mouse are not satisfactory as the mutation of Rb1 alone is not sufficient for tumor formation. This indicates that the development of retinoblastoma in mouse and man follows different routes. In order to analyze especially early stages of retinoblastoma we have created a human organoid-based model using the CRISPR/Cas9 system. The human embryonic stem cell line H9 was modified to carry a random mutation in exon 3 (close to the splice donor site) either on one or both RB1 alleles. After characterization of 12 generated clonal lines, we started comparative differentiation into retinal organoids using the parental cell line H9 as well as a heterozygous (G12LS) and a homozygous (C7) clone. Both clones carry the RB1 variant c.374_380del (LRG_517t1) which results in a premature stop codon on protein level (p.Glu125Valfs*9). As analyzed by immunocytochemistry, generated retinal organoids contain all seven retinal cell types – mature rod (NRL) and cone (ARR3) photoreceptors are present in the outer layer and ganglion (BRN3), Müller glia (VIM), amacrine (TFAP2A), bipolar (PRKCA) and horizontal cells (PROX1) are located in the inner layer. Over time, the neural retina layer of the C7 RB1-/- organoids became loose and disorganized compared to RB1 wildtype and G12LS RB1+/- organoids. Immunostainings on day 152 indicated enhanced proliferation (Ki67) and a decrease in rod photoreceptors and amacrine cells in C7 RB1-/- organoids which is supported by analysis of mRNA expression of marker genes by qRT-PCR. Quantification of cell numbers in microscopy images revealed a significant increase of proliferating ARR3+ cone photoreceptors in C7 RB1-/- organoids. To identify and quantify cellular subgroups in retinal organoids, we performed single-cell RNA sequencing of RB1 wildtype, G12LS RB1+/- and C7 RB1-/- retinal organoids. Data analysis is in progress and results will be presented. We are convinced that our model will advance retinoblastoma research.

Keywords: CRISPR/Cas9, retinoblastoma, organoids

MDD236

MODELING RETINO-CORTICAL PROJECTIONS WITH HUMAN PLURIPOTENT STEM CELL-DERIVED ASSEMBLOIDS

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Human pluripotent stem cells (hPSCs) can differentiate into three dimensional organoids, which recapitulate the spatiotemporal organization of human tissue. Generated from the fusion of regionally-patterned organoids, assembloids model projections between distinct regions of the nervous system. As the projection neurons of the retina, retinal ganglion cells (RGCs) must extend axons over long distances in search of appropriate post-synaptic targets in the brain. The current study focused upon the generation of retinocortical assembloids in order to model the long-distance outgrowth characteristic of RGCs. Retinal and cortical organoids were differentiated from hPSCs using established protocols. Within the first two months of differentiation, retinal organoids expressed markers including BRN3B and Recoverin while cortical organoids expressed CTIP2 and SATB2. Retinocortical assembloids were generated by fusing retinal organoids with cortical organoids and analyzed at various time points, with results compared to age-matched retinal organoids grown alone. The effects of retinocortical assembloids upon retinal organoid proliferation and RGC viability was assessed histologically. Furthermore, RGC axonal outgrowth into cortical organoids was analyzed over time. Upon the generation of retinocortical assembloids, RGCs extended axons into cortical organoids within the first 3 days post fusion (dpf), correlated with a significant increase in retinal organoid area and proliferation by 7 dpf. Long-term assembloids allowed for significantly greater RGC survival compared to retinal organoids alone. Finally, RGCs displayed extensive axonal outgrowth into cortical organoids, with the ability to respond to environmental cues. Retinocortical assembloids served as an effective in vitro model of long-distance projections between the retina and the cortex, with RGC axons significantly extending into cortical organoids. Furthermore, by providing an appropriate environment for RGC axonal outgrowth, the growth of retinal organoids as a whole was enhanced, including a significant increase in RGC survival. As such, these results facilitate the use of retinocortical assembloids for disease modeling and therapeutic screening, as well as provide a model for studies of RGC axonal regeneration.

Keywords: assembloids, organoids, retinal ganglion cells

HEMATOPOIETIC SYSTEM

MDD245

MULTILINEAGE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS ENGINEERED TO EXPRESS TRUNCATED MECP2 PROTEIN

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Hematopoietic precursor cells (HPCs) derived from human induced pluripotent stem cells (iPSCs) are an excellent resource to study the onset of hematopoiesis in vitro and explore treatment options for hematological disorders and cancer. We have developed a defined in vitro differentiation protocol for the generation of iCell Hematopoietic Progenitor Cells 2.0 from episomally reprogrammed iPSCs from a healthy donor. Cryopreserved iCell HPC 2.0 are >90% CD34 positive, express CD43, CD31, CD45, CD41, CD235, generate multipotent/mixed colonies in serum free methylcellulose-based colony assay and megakaryocyte colonies in collagen-based colony assays. Cryopreserved iCell HPC 2.0 can be successfully differentiated to iCell Macrophages, iCell Microglia, erythroblasts, mast cells, and lymphoid cells. Cryopreserved isogenic HPCs were generated from engineered iPSCs harboring a frame shift mutation to create loss of function of Methyl-CpG-binding protein 2 (MECP2) to mimic disease modelling for Retts Syndrome. HPCs derived from MECP2 engineered iPSC matched phenotypic purity and functional requirements of iCell HPC 2.0. Further downstream differentiation of HPCs with impaired MECP2 function generated a relatively mature erythroid cell (CD71+, CD235+, beta globin+) than the parental iCell HPC 2.0 cells. These cells also revealed an increase in the efficiency of generating lymphoid (CD3+, CD8+ and CD56+) cells and myeloid cells (Macrophage and Microglia). The end stage myeloid cell lineages exhibited a lower inflammatory response compared to lineages derived from iCell HPC 2.0. These findings identify a novel role for MECP2 function in the onset of definitive hematopoiesis.

Keywords: Hematopoietic cells, Induced pluripotent stem cells (iPSC), MECP2

MDD252

MODELING FETAL B1 LYMPHOCYTE DEVELOPMENT USING PLURIPOTENT STEM CELL-DERIVED LIVER ORGANOIDS

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During development, the fetal liver is a major site of hematopoiesis in which hematopoietic stem cells (HSCs) give rise to all the different lineages of blood cells in the body. It is during this time that a unique population of B lymphocytes called B1 cells arise from specialized precursors and provide life-long “innate-like” immune responses that are lacking in adult (B2) B cells. Recent studies have shown that early B cell development relies on sonic hedgehog (SHH) signaling in the fetal liver stroma through a Gli3 dependent mechanism, yet the full cellular and molecular mechanism that governs B1 cell development remains unknown. Here we report a new B1 cell differentiation system originating from hematopoietic compartment co-differentiated in a pluripotent stem cell (PSC) derived fetal liver organoid system. Histological analysis showed formation of hepatic organoids expressing AFP, ALB, and EpCAM located adjacent to CD34 endothelial cells undergoing notch-dependent endothelial to hematopoietic transition (EHT). Colony forming cell (CFC) assays revealed that our organoid culture contains multipotent progenitors that can give rise to erythroblasts, macrophages, monocytes, and granulocytes as identified by Giemsa stain. Co-culture with MS-5 mouse stromal cells resulted in a unique CD45+/CD20+/CD27+/CD43+ B cell population that was not able to be produced from umbilical cord blood (UCB) or seen in adult spleen. Our results, for the first time, demonstrate the emergence of a unique B1 cell population from PSC in an organoid system that is unable to be obtained from cord blood progenitors. We anticipate this system to be a starting point for further characterization of early B cell development in the fetal liver, including examination of molecular pathways activated by the hepatic and hematopoietic cell interactions. Current studies are focused on the dependence of SHH protein Gli3 in the differentiation of early B cells by modulating levels of organoid Gli3 in vitro to increase B cell production. Given the unique response of B1 cells to vaccination and infections, future applications for this system include a novel ex vivo source for human antibody production as well as vaccine design.

Keywords: B1 cell, hematopoiesis, fetal liver

MDD437

PLASTICITY OF LEUKEMIA STEM/INITIATING CELLS IN MLL-REARRANGED B-ALL

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Leukemia stem/initiating cells (LICs) are the subset of leukemic cells that drive leukemia progression, resist therapy, and remain latent to spark disease relapse. Although LICs have been previously thought to be rare and phenotypically primitive among heterogeneous bulk leukemia cells, recent data suggest that the LIC state may actually be heterogeneous and dynamic. Here, we use single-cell transcriptomics combined with limiting dilution xenotransplantation assays to dissect the ontogeny of MLL-rearranged B-lymphoblastic leukemia (MLL-r B-ALL), an aggressive form of childhood leukemia. Compared to acute myeloid leukemia (AML), LICs are abundant in MLL-r B-ALL. Recapitulating their unique clinical behavior, MLL-r B-ALL cells can undergo a B-lymphoid to myeloid lineage switch, consistent with primitive, multipotent transcriptional programs in LICs. Although we identify rare, chemotherapy-resistant, transcriptionally and phenotypically primitive LICs, we also observe LICs emerging from more differentiated leukemic populations. These facultative LICs self-renew and possess the capability to replenish the full cellular diversity of MLL-r B-ALL, including primitive populations that bear multipotency programs. In mechanistic studies, we find that the LICs that drive this bottom-up reconstitution of the leukemic cellular ontogeny bear signatures of MYC activation and oxidative phosphorylation, implicating these pathways in this reconstitution process. We confirm recruitment of these pathways in actively reconstituting, phenotypically differentiated LICs, and define a linear pathway by which MYC rewires metabolism in MLL-r B-ALL. We find that MYC is required for this LIC plasticity in vitro and in vivo. Our findings define a novel mechanisms of LIC heterogeneity and interconversion. We conclude that the high LIC content and dual lineage and LIC plasticities of MLL-r B-ALL contribute to its chemotherapy resistance and persistently poor outcomes.

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Keywords: leukemia, stem cells, hematopoiesis

IMMUNE SYSTEM

MDD507

CARCINOGEN MEDIATED STEM CELL ALTRUISM LEADS TO MALIGNANT TRANSFORMATION OF CD271+ ORAL MUCOSA STEM CELLS IN MICE

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The initiating molecular event that deregulates self-renewal leading to malignant transformation is not yet clear, which limits our ability to develop chemo-preventive vaccines against stem cells undergoing malignant transformation. Previously, we identified altruistic stem cells (ASCs) in human embryonic stem cells that exhibit a transient state of low p53 in order to maintain stemness (self-renewal and undifferentiated state) during oxidative stress (PMID: 22689594). We speculated that ASCs may serve as defense mechanisms against cellular oxidative stress, but unfortunately some of these ASCs may be the target of malignant transformation. If so, immune system must have evolved to detect and eliminate ASCs to cancer stem cell (CSC) transition. To develop a mouse model of ASC to CSC transition where the potential immunesurveillance can be studied, C57BL/6 mice were fed the oral carcinogen 4-Niroquinoline-1-oxide (4NQO) for 10-weeks, and the CD271+/ABCG2+ oral mucosa stem cell population were immunomagnetically sorted from the tongue. These cells were then subjected to phenotypic analysis for ASC as described previously (PMID: 22689594), and then in vitro cultured in a methylcellulose media to identify clones exhibiting CSC phenotype. Next, a BCG-stem cell vaccine that can target cancer stem cells (Cancer Res 2017;77(13 Suppl):Abstract nr 3903) were injected to mice a week before the collection of CD271+/ABCG2+ cells. Results: We were able to isolate a transient cell population, the CD271+/ABCG2+ cells that exhibited ASC phenotype. By performing an in vitro clonogenic enrichment assay for CSC (PMID), we identified one CSC per 10^7 CD271+/ABCG2+ population. This CSC derived clone exhibited tumorigenic property when injected orthotopic to the tongue of congenic mice, confirming malignant transformation. Importantly, BCG-SC vaccinated mice led to complete elimination of the CSC within the ASC population. Notably, these mice then became resistant to 4 NQO mediated carcinogenesis. Conclusion: These

results suggest 4NQO carcinogen may activate ASC defense mechanism in the CD271+ oral mucosa stem cells, and some of these ASC may covert to CSCs, which can be targeted by BCG vaccine. Further studies are required to identify the putative immune surveillance mechanism against ASC to CSC transition.

Funding source: Department of Biotechnology, India; KaviKrishna Foundation, Assam,India, and KaviKrishna USA Foundation, Lincoln, MA

Keywords: Stem cell altruism, Carcinogenesis, Cancer Stem Cells

MUSCULOSKELETAL

MDD267

MODELING FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY IN ISOGENIC HUMAN INDUCED PLURIPOTENT STEM CELLS

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Facioscapulohumeral Muscular Dystrophy (FSHD) is a degenerative muscle disease without a cure or treatment. The genetic cause of FSHD lies in the contraction of D4Z4 repeats on 4q35 telomeric region encoding DUX4 homeobox protein, which is a potent cytotoxic transcription factor. Interestingly, individuals carrying deletions of the entire D4Z4 array on one allele are healthy. To assay the role of DUX4 in FSHD myopathy, we use a genome engineering approach mediated by site-specific nucleases to delete the 4q35 telomeric region and introduce an artificial telomere in its place. We targeted patient-derived induced pluripotent stem cells (iPSC) using our telomere editing approach in combination with zinc-finger nucleases to generate FSHD/control isogenic pairs. Southern blots show replacement of either the WT or the FSHD allele in different clones. Fluorescence in situ hybridization (FISH) is used to verify deletion of the FSHD permissive allele on chromosome 4. Corrected cell lines lacking the FSHD allele display a downregulation of DUX4 target genes. Corrected and FSHD isogenic pairs were differentiated into muscle and muscle associated cell types to assay FSHD permissive locus activity. Molecular analysis shows that DUX4 and target genes are expressed in iPSC-derived FSHD cells, while the corrected controls show a reduction or elimination of this expression.

Keywords: Facioscapulohumeral muscular dystrophy, Induced pluripotent stem cells, Disease modeling

MDD273

IDENTIFYING THE MOLECULAR MECHANISMS OF A MONOGENIC CHILDHOOD FORM OF PREMATURE JOINT DEGENERATION USING IPSCS

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Progressive Pseudorheumatoid Arthropathy of Childhood (PPAC) is rare but severe degenerative joint disease caused by loss of function (LOF) mutations in the gene encoding Wnt-inducible signaling pathway protein 3 (WISP3, also named CCN6). PPAC becomes symptomatic in children between the ages of 3 and 8, and then rapidly progresses to end-stage articular cartilage failure that is indistinguishable from end-stage osteoarthritis (OA). These patients also present with mild skeletal dysplasia, suggesting that growth plate cartilage is affected by CCN6 LOF mutations in addition to the articular cartilage lining the joints. We established an iPSC-based model to study the mechanism of PPAC pathogenesis because Ccn6-knockout mice failed to recapitulate the disease phenotype and pre-symptomatic cartilage tissue from PPAC children is unavailable. We generated iPSCs from 5 PPAC patients, and created isogenic iPSC lines by correcting their mutant CCN6 alleles. We also used CRISPR/Cas9 to induce CCN6 LOF mutations in wild-type hESCs. Using our established directed differentiation protocols, we generated both articular and growth plate-like cartilage tissues from these isogenic lines, both of which appeared phenotypically/histologically normal. In our initial transcriptomic analysis, we found that CCN6-defficient (PPAC) cells differentially activated other members of the connective tissue growth factor (CCN) family, specifically CCN2 and CCN3, suggesting a mechanism to compensate for the absence of CCN6 during cartilage development. Deregulation of CCN2 and CCN3 in previous studies was shown to modulate TGFβ signaling, which is important for proper joint and articular cartilage specification and an avenue we are pursuing in greater detail. Ongoing unbiased experimental approaches are expected to identify additional changes in gene regulation, cell signaling and/or extracellular matrix production during cartilage development, homeostasis, and following injury, which may be most relevant to disease pathogenesis as it presents after children start walking. We are optimistic that knowledge we gain about mechanisms that lead to cartilage failure in PPAC patients will point to new approaches for protecting cartilage from damage associated with more common forms of joint degeneration and OA.

Keywords: Disease modeling, Cartilage specification, CCN family

NEURAL

MDD279

IDENTIFYING CHANGES IN OLIGODENDROCYTE DIFFERENTIATION IN DOWN SYNDROME USING ISOGENIC PATIENT-DERIVED IPSCS

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Down syndrome (DS) is caused by triplication of chromosome 21 (HSA21) and is the most common genetic form of intellectual disability with a prevalence of 1 in 750 live births. Despite identifying the underlying cause more than 60 years ago, exactly how the presence of a third copy of HSA21 leads to the resultant intellectual disability is still unknown. Previous work has shown that one of the alterations in gene expression caused by Trisomy 21 is the downregulation of a network of genes regulating the oligodendrocyte (OL) lineage. This dysregulated gene expression identifies perturbed OL production as the potential underlying cellular mechanism of the decrease in white matter in the brains of people with DS. This white matter deficit could result in a slowing of neuronal communication in the brain and impact intellectual function in DS. In order to study the etiology of this deficit at the cellular and molecular level, we have established a culture system that allows us, for the first time, to study the developmental trajectory of the OL lineage in DS. We are able to differentiate isogenic iPSCs derived from people with DS into mature OLs using small molecules and growth factors and identify changes between the euploid and trisomic cells. This system enables study of the subtle differentiation differences that may be masked by a lentiviral induced differentiation system or by comparison of genetically distinct euploid and trisomic cell lines. Using this system, we have identified changes in differentiation capacity in the OL lineage in the trisomic cell line. Specifically, these changes occur in early OL development including the initial iPSC to neural progenitor cell (NPC) differentiation as well as the NPC to pre-OPCs transition in response to activation of the Sonic hedgehog pathway. With this ability to interrogate differentiation blockages at each developmental step in the transition from iPSCs to mature OLs, we aim to characterize the cellular etiology of the white matter deficit in DS. As white matter production is predominantly a postnatal process, a detailed understanding of the molecular mechanism of the deficit in DS opens avenues for potential therapeutics to improve the independence and quality of life of people with DS.

Funding source: NICHD R21 HD098542-01

Keywords: Down syndrome, oligodendrocyte, neural progenitors

MDD280

ISOLATION AND INVESTIGATION OF SUBTYPE-SPECIFIC HUMAN NEURONAL GROWTH CONES FROM FUSED BRAIN ORGANOIDS

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During development, distinct areas of the brain and subtypes of neurons form specific connections to construct functional circuitry. Growth cones (GC) of diverse projection neuron subtypes must navigate complex extracellular environments to reach distant, subtype-specific targets. These axon-terminal structures respond to substrate-bound and diffusible signals in a subtype- and context-specific. Recent studies strongly indicate that subcellular localization of specific molecular machinery to GCs likely underlies the precise behaviors of GCs during circuit “wiring.” While great progress has been made identifying signals that guide elements of axonal growth, it is becoming increasingly clear that intracellular, local growth cone biology critically controls precise circuit formation, their appropriate synapse formation, maintenance and function. Our laboratory has recently developed integrated experimental and analytical approaches that enable high-throughput, high-depth transcriptomic and proteomic investigation of purified GCs from fluorescently labeled, subtype- and stage- specific cortical projection neurons. This approach has already revealed unanticipated richness and complexity of GC molecular machinery and GC enrichment of transcripts and proteins in subtype specific mouse GCs, so highly GC- enriched that they are essentially undetectable above noise in parent somata. Due to the lack of material, human aspects of growth cone biology remain largely unstudied. Here, we used in vitro brain organoids, to model some elements of circuit development, during human brain development. We differentiated human pluripotent stem cells into brain organoids and fused them to recapitulate some aspects of cortical connectivity. Fused organoids developed strong reciprocal axonal connections with growth cones at their axon terminals, enabling isolation and purification of fluorescently labeled subtype specific human GCs. In the future, these approaches will enable direct investigation of human GC molecular machinery (RNA and protein), comparison to mouse and how GC machinery controls circuit formation and later synapse formation, maintenance and function in the human brain.

Keywords: brain organoid, growthcone, circuits development

MDD281

IMPROVED MODELING OF HUMAN ALZHEIMER'S DISEASE WITH AUTOMATED HUMAN IPSC NEURONS, ASTROCYTES AND MICROGLIA CULTURING PLATFORM

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The two key human brain pathological hallmarks of Alzheimer's disease (AD) are amyloid beta (Aβ) plaques and tau fibrillary tangles. Unfortunately, transgenic mouse models of AD do not recapitulate these two pathologies together, making it challenging for drug development. A humanized AD cellular model could potentially capture both pathologies. We generated a robust, consistent, and long term (3 months+) human iPSC neuronal culturing platform in 384 wells plates for experimentation. High content imaging was conducted to analyze cellular phenotypes. Thousands of neurons per well and 4 wells per condition. Our in vitro human iPSC AD model manifested several disease related phenotypes, including synapse loss, dendrite retraction, axon fragmentation, phospho tau translocation to the somatodendritic compartment, and finally neuronal cell death. Anti-Aβ antibody protected neurons from all these pathologies even late-stage AD pathology phenotypes such as tau phosphorylation and neuronal death. We further demonstrated that anti-Aβ antibody can provide benefit even after pathologies has emerged; when used within a window of 1/2 of disease timeline. These result supported the intimate link between Aβ and Tau pathology. Small molecules inhibitors to known kinases in the AD signaling pathways were tested and also conferred protection. This indicates that AD pathological signaling events are preserved in this system. Incorporating iPSC microglia, we also found the formation of Aβ plaque-like structures that are Aβ plaque dye positive. Furthermore, neurofilament and phosphor-tau staining revealed swollen and dystrophic neurites surrounded a subset of these Aβ plaques resembling human AD neuritic plaques. We found that microglia surrounded the Aβ plaques and reduced the number and size of the plaques. Human iPSC microglia co-culture conferred ~40% protection from synapse loss and p-tau induction. This protection is lost when cultured in an inflammatory state with the addition of IFNg, LPS, IL1b. Interestingly, we also found that microglia could work with anti-A^2 antibody to protect human neurons. This human iPSC AD model has recapitulated key AD pathologies and key cellular phenotypes between neurons, astrocytes and microglia. This model will facilitate target discovery and drug development efforts.

Keywords: Microglia, Alzheimer's, screen

MDD286

MODELING ALCOHOL-INDUCED NEUROTOXICITY USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED THREE-DIMENSIONAL CEREBRAL ORGANOIDS

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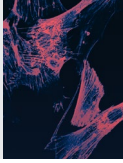
Maternal alcohol exposure during pregnancy often adversely affects the fetus, causing a range of symptoms known as Fetal Alcohol Spectrum Disorders (FASDs) such as cognitive dysfunction and psychiatric disorders, with the mechanisms and pathophysiology largely unknown. Recently developed human cerebral organoids from induced pluripotent stem cells are similar to fetal brains in the aspects of development and structure. These models allow more relevant in vitro systems to be developed for studying FASDs than animal models. In this study, we sought to quantify the downstream toxic effects of binge drinking on neural pathology phenotypes and signaling pathways using cerebral organoids. The results showed that alcohol induced apoptosis as well as ultrastructural changes (e.g., disruption of mitochondria cristae, decreased intensity of mitochondrial matrix, and disorganized cytoskeleton). This apoptotic effect of alcohol on the organoids exhibits concentration-dependent. Alcohol exposure also resulted in the mitochondrial dysfunction and metabolic stress in organoids as evidenced by 1) decreases of mitochondrial oxygen consumption rates linked to basal respiration, ATP production, proton leaking, maximal respiration, spare respiratory capacity, and 2) an increase of non-mitochondrial respiration. We also found that 199 genes out of 17195 genes analyzed were altered by alcohol. Bioinformatics analysis shows the association of these dysregulated genes with 36 notable pathways, such as psychiatric disorders, behavior, nervous system development and function, organismal injury and abnormalities, and cellular development. Additionally, 187 of these genes were previously reported in the literature as critical to neurodevelopment, and/or implicated in nervous system physiology and neurodegeneration. Furthermore, these genes form regulative networks in multiple pathways. Collectively, this study is the first to extend animal model studies on binge drinking-related FASDs to human models at the cellular, subcellular, and gene levels. Our novel studies provide valuable insight into alcohol-induced pathologic phenotypes, multiple signaling pathways, molecular networks involved, as well as a better understanding of the neurotoxic effects of binge drinking during pregnancy.



Keywords: cerebral organoids, alcohol, neurotoxicity

MDD290

LOSS OF POGZ ALTERS NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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POGZ is a pogo transposable element derived protein with multiple zinc finger domains. It is involved in mitosis and also influences chromatin regulation through its interaction with heterochromatin. POGZ is robustly expressed in human fetal brain. Although many de novo loss-of-function (LoF) POGZ variants are associated with autism and other neurodevelopmental disorders, the function of POGZ in brain development has not been extensively characterized. Our goal is to use genome editing and stem cell neural differentiation approaches to interrogate the function of POGZ in neuronal proliferation and migration. To this end, we generated multiple POGZ LoF mutations in H9 human embryonic stem cells (hESCs) using CRISPR/Cas9 genome editing and differentiated them into neural structures that preserve the neural architecture of early to mid-fetal human brain, a critical stage for studying disease mechanisms of neurodevelopmental disorders. We find that the complete loss of POGZ delays neuronal migration at the neural rosette stage that is analogous to the cortical ventricular zone in human fetal brain. Additionally, in comparison to isogenic control hESCs-derived cortical-like excitatory neurons, the neurons derived from multiple POGZ LoF hESC lines display simplified dendritic architecture. Further analyses, including gene expression changes, will provide additional insight into the molecular mechanisms by which POGZ regulates cortical development, as well as the pathophysiology of POGZ-associated neurodevelopmental disorders.

Keywords: POGZ, Stem cell, Neural differentiation

MDD304

IDENTIFYING CONVERGENT PROTECTIVE PATHWAYS IN HUMAN ALS MOTOR NEURONS USING PHOSPHOPROTEOMIC ANALYSES

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects upper and lower motor neurons (MNs). The pathological processes that drive MN degeneration in ALS are idiopathic and complex, confounding effective disease modeling and drug discovery. Here, we utilized MNs derived from ALS patients, and systematically tested pharmacological stressors for their ability to potentiate key ALS pathologies in a synchronous and biologically relevant manner. We found that several compounds causing dysregulated protein homeostasis, a universal feature of ALS, enhanced degeneration of ALS MNs compared to healthy control MNs. A subset of these compounds, particularly thapsigargin and tunicamycin, stimulated endoplasmic reticulum stress, neuritic degeneration and apoptosis preferentially in MNs. In contrast, treatment with a proteasome inhibitor, MG132, was less MN-selective, but did lead to the appearance of the insoluble TDP43+, ubiquitin+ aggregates that characterize the majority of ALS patients. To ensure the disease relevance of these assays, we next confirmed that two kinase inhibitors previously reported by our laboratory to be protective in a variety of assays using ALS MNs – kenpaullone, an inhibitor of CDKs, GSK3B, and, MAP4K4, as well as MAP4K4i-29, a specific MAP4K4 inhibitor – were also able to protect against the pharmacologically accelerated ALS degeneration. Lastly, we leveraged the scalability of these assays and performed global phosphoproteomic analyses on human MNs treated with kenpaullone or MAP4K4i-29, identifying convergent protective pathways that we validated with a selected test of small molecule inhibitors. Cumulatively, these studies add to the growing number of in vitro assays used to interrogate pathologies in ALS patient derived MNs and advance the efforts in finding effective drug targets for ALS treatments.

Funding source: Harvard Brain Science Association, gift from ALKAHEST, Inc.

Keywords: Amyotrophic Lateral Sclerosis (ALS), Motor Neuron, Phosphoproteomics

MDD310

MODELING FRAGILE X SYNDROME AND IDENTIFICATION OF HUMAN-SPECIFIC MRNA TARGETS OF FRAGILE X MENTAL RETARDATION PROTEIN WITH HUMAN FOREBRAIN ORGANOIDS

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Fragile X syndrome (FXS) is the most common inherited form of intellectual disability and a leading genetic cause of autism. FXS is caused by the loss of functional fragile X mental retardation protein (FMRP). FMRP is an RNA-binding protein forming a messenger ribonucleoprotein complex with polyribosomes in the regulation of protein synthesis. The recent failures of several clinical trials suggest a strong need for human-specific models of FXS to understand the unique factors that underlie human disease and to test the efficacy of candidate compounds. Three-dimensional (3D) organoid culture of human-induced pluripotent stem cells (iPSCs) provides a new platform to investigate human brain development in a dish, otherwise inaccessible to experimentation. To determine whether the loss of FMRP could alter the development of human brain organoids, we have generated forebrain organoids from three FXS male patients and three healthy male controls. We observed dysregulated proliferation of neural progenitor cells and neural differentiation as well as perturbed cell migration in FXS forebrain organoids. Interestingly these deficits were not observed with FXS mouse model. To identify the mRNAs bound by FMRP, we performed enhanced crosslinking and FMRP immunoprecipitation followed by high-throughput sequencing using human forebrain organoids and mouse embryonic forebrain from similar developmental stages. Our comparative analyses revealed the mRNAs bound by FMRP in both human forebrain organoids and mouse embryonic brains, and they were enriched in mRNAs that are critical for neurodevelopment and axonogenesis. Interestingly, we also identified a large number of mRNAs that were bound by FMRP only in human, and these are enriched in mRNAs that are involved in RNA metabolism and astrocyte differentiation. Furthermore, by overlapping the differentially expressed genes found using RNA-seq in the FXS organoids and human FXS fetal brain tissues, we were able to identify a subset of mRNAs that were bound by FMRP and displayed differential expression in the absence of FMRP specifically in human. Our study has identified human-specific mRNA targets of FMRP, which have the potential to serve as human-specific druggable targets for FXS and autism in general.

Funding source: National Institutes of Health, FRAXA Research Foundation, Emory Woodruff Health Sciences Center

Keywords: fragile X syndrome, FMRP, forebrain organoids

MDD322

MODELING BRAIN OVERGROWTH IN AUTISM USING HUMAN PLURIPOTENT STEM CELLS

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One of the most common genetic linkages associated with neuropsychiatric disorders, such as autism spectrum disorder (ASD) and schizophrenia, occurs at the 16p11.2 locus. Copy number variants (CNVs) of the 16p gene can manifest in opposing head sizes. 16p11.2 deletion carriers tend to have macrocephaly (or brain enlargement), while those with 16p11.2 duplication frequently have microcephaly. Increases in both gray and white matter volume have been observed in brain imaging studies in 16p11.2 deletion carriers with macrocephaly. Individuals with ASD and macrocephaly have more severe behavioral and cognitive problems and are less responsive to standard medical and therapeutic interventions, leading to very poor prognoses relative to individuals with ASD and normal head circumferences. Increases in brain size often precede clinical symptoms, suggesting that understanding the underlying mechanisms regulating brain overgrowth could provide a window of opportunity for intervention or mitigation of symptoms. Here, we use human induced pluripotent stem cells (hiPSCs) derived from controls and subjects with 16p11.2 deletion and 16p11.2 duplication to understand the underlying mechanisms regulating brain overgrowth. To model both gray and white matter, we differentiated patient-derived iPSCs into neural progenitor cells (NPCs) and oligodendrocyte progenitor cells (OPCs). In both NPCs and OPCs, we show that CD47 (a 'don't eat me' signal) is overexpressed in the 16p11.2 deletion carriers contributing to reduced phagocytosis both in vitro and in vivo. Treatment of 16p11.2 deletion NPCs and OPCs with an anti-CD47 antibody to block CD47 restores phagocytosis to control levels. Furthermore, 16p11.2 deletion NPCs and OPCs upregulate cell surface expression of calreticulin (a pro-phagocytic 'eat me' signal) and its binding sites, indicating that these cells should be phagocytosed but fail to be eliminated due to elevations in CD47. While the CD47 pathway is commonly implicated in cancer progression, we document a novel role for CD47 in regulating brain overgrowth in psychiatric disorders and identify new targets for therapeutic intervention. Furthermore, our study highlights that the balance between 'eat me' and 'don't eat me' signals may be more broadly playing critical roles during early neurodevelopment.

Keywords: iPSCs, autism, disease modeling

MDD325

LEVERAGING NGN2 NEURAL DIFFERENTIATION TO DEVELOP HUMAN IPSC-BASED GENETIC SCREENS FOR MODIFERS OF TDP-43 PROTEINOPATHY ASSOCIATED TOXICITY

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Tar DNA-Binding Protein 43 (TDP-43) is a ubiquitously expressed, essential protein whose disruption is known to be associated with several neurodegenerative conditions, including Amyotrophic Lateral Sclerosis (ALS). Although TDP-43 is normally localized to the nucleus, 97% of ALS patients present with insoluble, hyper-phosphorylated, hyper-ubiquitinated aggregates of TDP-43 in the cytoplasm of degenerating spinal cord motor neurons. Because these aggregates of TDP-43 manifest in patients regardless of the presence of a TDP-43 mutation, it is reasonable to consider that there are additional genetic modifiers that contribute to toxicity which are yet to be discovered. However, whole genome screening in in vitro neuronal systems is difficult due to the large number of cells needed and the historically low yield of neurons from most pluripotent stem cell differentiation protocols. In this study, we propose solutions to these challenges by using an inducible TDP-43 system in combination with NGN2-mediated differentiation to establish a platform that may be used to perform and validate large scale genetic positive selection screens for TDP-43 toxicity modifiers in human iPSC models. We hypothesize that we can

use cellular viability as a readout to discover additional modifiers that improve or worsen toxicity conferred by overexpression of TDP-43 or a cytoplasmically restricted variant because TDP-43 overexpression at the beginning of neuronal differentiation significantly reduces cell viability compared to non-toxic overexpression variants and negative controls. We believe that when these discovered modifiers are additionally examined with other assays for neuronal health metrics (i.e. axon complexity, axon regrowth capacity), we will be powered to generate a set of genetic candidates which may be further developed into therapeutic interventions for TDP-43 proteinopathy associated illnesses, including ALS.

Keywords: Screening, iPSC-Derived Neurons, ALS

MDD337

MODULATING SORL1 EXPRESSION AFFECTS ENDO-LYSOSOMAL TRAFFICKING FUNCTION IN HIPSC-DERIVED MODELS OF ALZHEIMER'S DISEASE

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The SORL1 gene encodes for the protein SorLA, a sorting receptor involved in retromer-related endosomal traffic. Many SORL1 genetic variants increase Alzheimer's disease (AD) risk, and rare loss-of-function truncation mutations have been found to be causal of AD. SORL1 is expressed in neurons and glia of the central nervous system and loss of SORL1 has been reported AD tissue. To model the causal loss-of-function mutations, we used CRISPR/Cas9 technology to deplete SORL1 in human induced pluripotent stem cells (hiPSCs) to test the hypothesis that loss of SORL1 (SORL1 KO) contributes to AD pathogenesis by leading to dysfunction in endo-lysosomal trafficking. In contrast, certain SORL1 variants may be acting in a protective capacity to help prevent AD pathogenesis. To model protective variants, we used a PiggyBac Transposon system to test the hypothesis that overexpression of SORL1 (SORL1 OE) protects against AD pathogenesis and endo-lysosomal trafficking dysfunction. We report that loss of SORL1 in hiPSC-derived neurons leads to early endosome enlargement, a cellular phenotype that is indicative of 'traffic jams' and is now considered a hallmark cytopathology AD. We validate defects in endo-lysosomal traffic in SORL1 KO neurons by showing decreased localization of amyloid-precursor protein (APP) to the degradative pathway. We confirm decreased function of the degradative pathway in SORL1 KO neurons through experiments using pH-sensitive pHrodo dyes. Conversely, in SORL1 OE neurons we show nominal early endosome morphology, reduced localization of APP to the degradative pathway, and increased functionality of the degradative pathway. Finally, we determined that defects in endo-lysosomal trafficking caused by loss of SORL1 affect synapse function by analyzing SORL1 KO neurons on multielectrode arrays. Collectively, these findings clarify where and how SORL1 links to AD. Moreover, our data, together with recent findings, underscores how sporadic AD pathways that regulate endosomal trafficking, and autosomal-dominant AD pathways that regulate APP cleavage, independently converge on AD's defining cytopathology.

Keywords: Alzheimer's disease, Endocytic network, Lysosome

MDD338

IMPROVED CELL SURVIVAL AND CYTOPROTECTION ENHANCE REPRODUCIBILITY AND PROPER DIFFERENTIATION OF CEREBRAL ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Self-organizing organoid models from human pluripotent stem cells recapitulate some aspects of brain development and function. However, current protocols are hampered by uncontrolled cell death, considerable organoid-to-organoid heterogeneity, and lack of standardization. We found that ROCK inhibitor Y-27632, the most widely used reagent to improve cell survival during embryoid body (EB) formation, is deficient in preventing cellular stress and apoptosis, leading to nonoptimal organoid formation. Here, we used a newly developed small molecule cocktail termed "CEPT", which greatly enhanced cell survival and cell quality during EB formation. The data demonstrated that improved cell survival during the early stages of EB formation has long-lasting consequences affecting not only total cell numbers and organoid size but also enhanced neuronal differentiation of organoids cultured up to 60 days. Single-cell RNA sequencing from two-month old organoids revealed that CEPT-generated organoids vs. Y-27632 treatment contained higher and more homogeneous expression levels of neuronal proteins and transcription factors representing different cortical cell types. Moreover, transcriptomic analysis of CEPT-generated organoids indicated significantly higher correlation to datasets from the developing human brain. Importantly, CEPT-generated organoids resulted in experimental reproducibility based on RNA sequencing analysis across individual organoids. In summary, our study identified uncontrolled cell death at the onset of organoid formation as a critical quality control determinant and demonstrated that the use of the CEPT cocktail dramatically improved morphogenesis, neuronal differentiation, and overall reproducibility of cerebral organoids.

Keywords: Organoid, Pluripotent stem cell, single-cell RNA sequencing

MDD341

HUMAN IPSCS-DERIVED DORSAL ROOT GANGLIA-LIKE ORGANOIDS FOR CRISPR/CAS9-MEDIATED RESCUE OF FRIEDREICH'S ATAXIA PATHOLOGICAL DEFICIT

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Friedreich's ataxia (FRDA) is an autosomal-recessive neurodegenerative and cardiac disorder occurring when transcription of the FXN (frataxin) gene is silenced due to an excessive expansion of GAA repeats into its first intron. FXN deficiency leads to impairments in mitochondria functions, iron metabolism and oxidative stress defense that primarily affect the dorsal root ganglia (DRG) sensory neurons. A major limitation in research on FRDA is the lack of experimental models to study this particular neuronal cell type. To fill this gap, we generated dorsal root ganglia organoids (DRGOs) by in vitro differentiation of human iPSCs. DRGOs have a strong transcriptional signature of native DRGs with the expression of a large set of peripheral neuronal markers and robust electrophysiological activity. Furthermore, when co-cultured with human intrafusal muscle fibers, DRGO sensory neurons contacted their peripheral targets and reconstitute the muscle spindle proprioceptive receptors. FRDA DRGOs recapitulated several aspects of the pathology including FXN silencing, diminished survival and defects in morphology, mitochondrial protein dysfunctions and impaired formation of muscle spindles. Remarkably, these pathological features were fully rescued only when the entire FXN intron-1 was removed and not with the excision of merely the expanded GAA tract. These results strongly suggest that removal of the repressed chromatin flanking the GAA tract is necessary to obtain a full rescue of FXN expression and extensively revert the pathological hallmarks of FRDA DRG neurons.

Keywords: Organoids, CRISPR/Cas9, Friedreich's ataxia

MDD343

HUMAN CORTICAL ORGANOIDS WITH FUNCTIONAL MICROGLIA-LIKE CELLS PROVIDE A TRACTABLE MODEL FOR ALZHEIMER'S DISEASE

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Microglia are the resident macrophages in the central nervous system (CNS). Their primary functions are to execute the immune surveillance in the brain and to facilitate brain network formation by regulating neuronal survival, synapse formation, elimination of apoptotic cells, and programmed cell death. Recent studies have demonstrated a critical link between impaired microglial function and neurodevelopmental (i.e., Autism, or Schizophrenia) and neurodegenerative (i.e., Alzheimer's disease, AD) disorders. However, investigating the function of microglia in health and disease state has been challenging due to the lack of easily accessible human models. Here, we develop a method to generate functional microglia inside human cortical organoids (hCOs) from human embryonic stem cells (hESCs), and apply this system to dissect the role of microglia in AD. Single-cell transcriptomics of mhCOs reveals the presence of a microglia cell cluster with an intact complement/chemokine system. Functionally, microglia in mhCOs protect parenchyma from cellular and molecular damage caused by inflammatory stress. We further determined the function of AD-associated genes highly expressed in microglia by using CRISPRi-mediated suppression in mhCOs. Together, mhCOs represent an innovative platform to investigate neurodegenerative disorders, and will serve to develop therapeutics in the future.

Keywords: MICROGLIA, BRAIN ORGANOID, ALZHEIMER'S DISEASE

MDD348

INVESTIGATING SIGNALING NETWORKS OF ALZHEIMER'S NEURONS TO CREATE FUNCTIONAL DIAGNOSTIC PROFILES

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Alzheimer's disease (AD) is the sixth leading cause of death nationally. As the current population continues to age, the incidence of AD is expected to rise, creating significant financial and emotional burden on patients, families, and healthcare systems. Although AD has been studied since the early 20th century, researchers have yet to find successful treatments targeting the hallmark pathologies of the disease, such as senile plaques and neurofibrillary tangles. Previous work supports the thought that AD progresses as aberrations in neuronal signaling to develop into excitotoxicity. These changes in neuronal signaling most likely begin before the onset of cognitive symptoms, as substantial neuronal loss has often already occurred when AD dementia is diagnosed. In this study we observe AD neuronal signaling in vitro via iPSC derived neurons to identify and characterize AD-associated neuronal network phenotypes. These signaling phenotypes may be used as early biomarkers and therapeutic targets in drug screens. We performed calcium imaging to assess the calcium kinetics of AD neurons and neuronal networks compared to a wildtype. Additionally, a machine learning model developed in the lab is used to assess disease phenotypes using calcium kinetics of individual neurons; we are currently training the model to accurately assess and predict AD. We also used micro-electrode arrays to analyze action potentials of neuronal networks because changes in action potential on a single cell level may not accurately depict the disease phenotype. In summary, by utilizing calcium imaging and micro-electrode arrays, we were able to study and characterize neuronal network phenotypes in AD. Future directions will include therapeutic drug screens to assess compounds' ability to rescue network function via calcium or action potentials. As opposed to traditional 'omics approaches, this work aims to combat AD by studying and targeting the functionality of neuronal networks affected by the disease. In order to meet and treat the growing demands presented by the aging population, multifaceted approaches will be necessary to provide improved therapeutics. A better understanding of how diseased cells have less functional networks may be a key part in the battle to successfully treat AD.

Keywords: Alzheimer's modeling, iPSC derived disease modeling, signaling kinetics

MDD351

HUMAN IPSC-ASTROCYTES RECAPITULATE A1 REACTIVE NEUROTOXICITY IN VITRO

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Astrocytes are the most abundant macroglial cell type in the central nervous system (CNS) and perform critical functions such as providing neuronal support, maintaining brain homeostasis, and modulating the immune response. Their increasing implication in neurodegenerative diseases makes them an appealing iPSC-based model system to investigate disease mechanisms. We generated human induced pluripotent stem cell-derived astrocytes and identified CD49f as a novel surface marker for astrocyte purification. hiPSC-derived CD49f+ astrocytes display highly heterogeneous morphology, express canonical markers, perform glutamate uptake, and transcriptionally resemble primary human astrocytes. Importantly, we show that these human astrocytes can be activated to exhibit the A1 neurotoxic phenotype recently described in rodents. Human iPSC-derived A1 astrocytes show reduced phagocytic capacity, impaired glutamate uptake, and are toxic to neurons. Bulk and single-cell RNA sequencing analysis reveals several dysfunctional pathways in A1 astrocytes vs. unstimulated, and suggests that A1 reactivity varies with astrocyte developmental stages. Taken together, this work establishes a novel, human, patient-specific in vitro model ideal for deciphering astrocyte-related pathogenic mechanisms of neurodegeneration, which we are now applying to the study of Alzheimer's disease and multiple sclerosis.

Keywords: astrocyte, iPSC, neurodegeneration

MDD357

IN VIVO PLATFORM FOR STUDYING PROPERTIES OF HUMAN NEURAL STEM CELLS

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An open question in developmental neuroscience is characterizing the anatomical maturation of human neural circuits. Although methods using in vitro monolayer cultures of human induced Pluripotent Stem Cells (hiPSCs) have provided insights at the cellular and molecular level, they have been limited in their ability to recapitulate the complex 3D architecture of neural circuits. Similarly, while animal models retain the complexity of neural circuits, neurons in model organism are anatomically and biophysically different than human cells, limiting the types of conclusions that can be drawn. To overcome these parallel challenges, we developed a novel approach to track the structural changes of GFP labeled neural precursor cells (NPCs) derived from hiPSCs using timelapse in vivo two-photon imaging. Cells at the NPC stage in vitro were labeled with a Lenti-CMV-GFP (MOI of 1), and subsequently transplanted into the superficial layers of primary visual cortex of SCID mice (N = 15) via stereotaxic injection. A 3 mm imaging window was implanted over the transplant site, enabling us to perform timelapse in vivo imaging (1-3 image sessions per week) in anesthetized animals for 2-4 months. We successfully tracked human NPC migration and proliferation, and revealed changes in the structure of neurites across a range of spatial (10-500 um) and temporal (2 hours to 4 months) scales. In order to analyze these large data sets, we developed custom image registration methods (employing mutual information calculations between datasets), particle tracking algorithms, and new statistical models for hypothesis testing. Upon completion of the final in vivo imaging session, animals were euthanized and immunohistochemistry was performed to reveal the molecular identity of transplanted cells. Post-mortem histology could then be registered to reference atlases, allowing us to relate the dynamics of human neurons characterized in vivo to the 3D organization of the mouse brain circuitry. Our approach thus offers a platform to integrate the strengths of both stem cell technologies and the rodent model to characterize the anatomical development of human neurons in vivo.

Funding source: NIH grant MH113924 (to KP)

Keywords: Neural stem cells, Transplantation, In vivo imaging

MDD360

HIGH-THROUGHPUT BRAIN ORGANOIDS REVEAL HUMAN NEUROGENESIS RELEVANT TRANSCRIPTIONAL PROFILES

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Modelling early stages of human brain development in vitro represent one of the most exciting areas of modern neuroscience. Until recently 2D tissue culture systems were the only method to model early neurogenesis in human. Recent breakthroughs in 3D brain organoid systems show that they can recapitulate aspects of human neurogenesis, such as cytoarchitecture, cell type heterogeneity, lineage progression and gene expression that are absent in 2D models. However, brain organoid research suffers from the lack of rigorous quality control for heterogeneity. To address this, we developed a high-throughput platform to generate organoids from multiple stem cell lines. We stringently controlled the quality of brain organoids using single cell RNA sequencing (scRNAseq) coupled with immunohistochemistry analysis. We show that early stage 3D brain organoids, as opposed to 2D neuronal cultures, contain a higher percentage of cells that acquire transcriptional signatures of fetal brain like cells. Those include apical progenitors (APs), basal progenitors (BPs) and neurons (N). Interestingly, upon application of RNA velocity analysis, we were able to capture dynamic transcriptional changes within the neuronal lineage of AP-BP-N. In particular, we were able to document the dynamic transcriptional signature of asymmetrically dividing BPs that give rise to basal progenitors and immature neurons. Finally, to test the robustness of our platform, we generated brain organoids from 21 patient induced pluripotent lines (iPSCs). We analyzed gene expression in over 80.000 cells dissociated from brain organoids derived from patient iPSCs lines. To improve the consistency of a large-scale experiment, we utilized methanol fixation of dissociated cells and employed data integration methods to correct batch effects. This data allows for rigorous testing of brain organoid reproducibility and line to line variability. After establishing this system, we are one step closer to employing human brain organoids for both disease modelling and drug discovery.

Keywords: brain organoids, neurodevelopmental disorders, high-throughput

MDD363

IDENTIFYING NOVEL DRUG CANDIDATE WITH A NEUROPROTECTIVE AND PRO-MYELINATING POTENTIAL FOR PROGRESSIVE MULTIPLE SCLEROSIS USING PRIMARY MURINE- AND HUMAN IPSC-BASED PHENOTYPIC SCREENING

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Progressive Multiple Sclerosis is a debilitating disease that remains untreatable, therefore the discovery of new agents promoting neuroprotection and supporting myelin regeneration is critically needed. Drug repurposing is a favorable alternative to the development of a new drug, with a significantly lower economic burden. The possibility of generating patient-specific human induced pluripotent stem cells (hiPSC) allows validation of prioritized compounds on disease-specific human cells. The project's aims are 1) to perform in silico-guided drug selection; 2) to perform drug toxicity test on primary murine cells; 3) to perform stressor-mediated assays on primary murine and hiPSC-derived neurons with functional and morphological outcome; 4) to evaluate pro-myelinating potential of hit compounds on primary murine- and hiPSC-derived oligodendrocytes (hiOL). Repurposed drugs have been pre-selected (n=270) using the bioinformatic tool SPOKE among more than 1500 compounds. Cytotoxicity was assessed on E17.5 mouse cortical neurons and mouse OLs cultured in 96-well plates exposed to 1/10 μM of the compounds for 24h. Rodent and human iPSC-derived OPCs were used to assess cytotoxicity and differentiation. Neuroprotective assays performed with metabolic (CCK-8), mitochondrial (JC1) and calcium signaling (FLUO4-AM) as functional outcomes. Toxicity of compounds has been evaluated: 160/270 were non-cytotoxic on primary mouse cortical neurons and OLs, and thus they have been further tested in neuroprotective assay settings with morphological (neurite length) and functional readouts: calcium signaling and mitochondrial assays are ongoing. A smaller set of hit compounds targeting multiple molecular pathways are being further tested in the neuroprotective regimen on hiPSC-derived neurons. In addition, screening identified compounds promoting both rodent and human OL differentiation into MBP+ cells. In conclusion, this platform based on primary murine and hiPSC-derived neuronal and oligodendroglial cell types provides a promising approach to identify potential therapeutic targets for progressive MS and may dissect novel molecular pathways regulating disease pathogenesis.

Funding source: International Progressive MS Alliance

Keywords: Progressive multiple sclerosis, Induced pluripotent stem cells, Drug discovery

MDD370

MODELING NEUROLOGICAL DISORDERS WITH HUMAN DENTAL STEM CELLS-DERIVED ASTROCYTES

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Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders which have increased in prevalence in the US from 1 in 120 in 2000 to 1 in 40 in 2018. There is currently no cure or treatment for ASD, and the cellular and molecular mechanisms driving ASD remain undefined. It has been shown that astrocyte mediated dysfunction and neuronal synapse development play key roles in neurodevelopmental disorders such as ASD. Although several genetic contributions to ASD have been discovered, it has been difficult to elucidate precise mechanism by solely relying on rodent models. Human pluripotent stem cells (iPSCs) have enabled the generation of patient-specific human neurons and glia from somatic cells, however the prolonged duration of iPSC differentiation and maturation toward astrocytes (24-40 weeks) has limited their research potential. Human dental pulp stem cells (DPSCs) are an ideal alternative stem cell source to generate neural lineages and astrocytes. DPSCs are of neuroectodermal (neural crest) origin and are competent to differentiate directly towards neuronal and glial lineages. Here we show a novel method to generate astrocytes from DPSCs, reducing the time to 3-4 weeks. This study is the first attempt to utilize DPSCs extracted from the third molar teeth of individuals diagnosed with ASD and control subjects to generate a unique resource for the ASD research community. Various parameters of astrocytes generated from DPSCs such as cellular, molecular and physiological mechanisms were evaluated. Immunofluorescent staining revealed the presence of biomarkers such as AQP4, S100B, and Kir-4. In addition, we have generated RNA-Seq data from DPSCs and DPSCs differentiated into astrocytes (DPSC-Astro) from both control and ASD patients to evaluate distinct gene expression. Our model captures DPSCs at the earliest stage of development, so we can follow their differentiation toward astrocyte formation. The DPSC-Astro model will advance our understanding of the causes of this neurological disorder and could directly lead towards developing future therapies for ASD.

Funding source: This work is supported by Tufts University, Tufts Collaborates Grants and BrightFocus Foundation.

Keywords: Neurological disorders, Dental Pulp stem cells, Astrocyte

MDD371

MOUSE ESC MODEL RECAPITULATES THE DEVELOPMENT OF THE DORSAL SPINAL CORD AND REVEALS WNT SIGNALING AS A REGULATOR OF SPINAL SENSORY NEURON DIVERSITY.

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Spinal cord injuries (SCIs) currently have no cure. Patients with SCI can lose the ability to move and respond to environmental cues, such as heat, touch, and pain. One potential therapy is to replace damaged tissue with embryonic stem cells (ESCs)-derived spinal neurons. However, while significant progress has been made generating spinal motor neurons from ESCs to restore movement, it has remained unresolved how to derive the six class of spinal dorsal interneurons (dI1-dI6) that mediate sensory perception. Towards this goal, we have developed the first directed differentiation protocols for dIs from mouse (m), human (h) ESCs and induced pluripotent stem cells (iPSCs). dIs are specified in vivo by the combinatorial activities of bone morphogenetic proteins (BMPs) and retinoic acid (RA) signaling. Using this paradigm, we were able to generate the full complement of spinal sensory interneurons by the timed addition of RA or RA+BMP4 in the ESC cultures. We find that RA directs ESCs to intermediate dI fates, i.e the dI4-6s, which mediate pain, itch, and thermo-sensation, while RA+BMP4 suppresses the dI4-6 fates and induces the dorsal most dI fates, i.e. the proprioceptive dI1s and mechanosensory dI2s and dI3s. The transcriptomic analysis shows that mESCs recapitulate the in vivo developmental program to make sensory spinal interneurons with a brachial/thoracic (neck/trunk) spinal cord identity. Moreover, the single-cell seq of differentiated dI populations showed the expected excitatory and inhibitory neurotransmitter signatures, indicating functional maturation. While these results are promising, the ESC-derived dIs arise as mixed populations, limiting their clinical usefulness. Using our in vitro system, we have sought to identify mechanisms by which BMP4 directs multiple dI populations from ESCs. Transcriptomic analysis of BMP4 treated mESCs reveals the rapid activation of Wnt signaling. Inhibiting Wnt activity in BMP4 treated progenitors suppresses the dI1-2 populations, resulting in the specification of dI3s alone. In summary, we have comprehensively characterized the conditions to derive the full complement of bona fide sensory spinal interneurons from ESCs and identified that the Wnt pathway can be modulated to obtain specific dI populations from hESCs to restore sensation after SCI.

Funding source: This work is supported by the BSCRC Innovation grant, Ablon scholarship and R01 (NS085097) to Samantha Butler. S.G is supported by BSCRC postdoctoral training fellowship.

Keywords: mouse embryonic stem cells, spinal sensory interneurons, differentiation

MDD380

MODELING CREUTZFELDT-JAKOB DISEASE USING HUMAN IPSC-DERIVED NEURONS

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Creutzfeldt-Jakob Disease (CJD) belongs to a family of human and animal diseases known as Transmissible Spongiform Encephalopathies (TSE) or prion diseases. CJD is a rapidly progressive, fatal neurodegenerative disease caused by aggregation of misfolded prion proteins. Approximately 10% of the cases of CJD are inherited in an autosomal dominant manner from several types of mutations on the PRNP gene encoding the prion protein, where the most common mutation is E200K. The process by which the infectious prion protein (PrPsc) actually causes neuronal damage is still under investigation. Here we report the establishment of the largest CJD E200K-specific iPSC library and their differentiation towards cortical neurons to characterize E200K PrP in terms of its expression levels, cellular localization, infectivity and neurotoxicity. Samples for reprogramming were obtained from carriers and non-carriers individuals of a large family, including samples from two clinically manifest individuals as well as two 96 y/o ”super-resilient” healthy carriers. In addition, we also used the CRISPR-Cas9 system to correct the E200K mutation in iPSC to establish a genetically matched isogenic pair. Upon neural differentiation of control and mutant lines, the cells presented normal neuronal morphology and physiology and expressed the characteristic cortical neuronal markers. Importantly, the cells also expressed both PrPc and PrPsc. By analyzing synaptic function and biochemical properties of PrP, we established a novel platform in which to study E200K prion pathogenesis, with the long-term goal of deciphering the mechanisms of neural injury mediated by abnormal protein folding with implications in many neurodegenerative diseases.

Funding source: CJD Foundation, NIH

Keywords: CJD/Prions, iPSC, Cortical Neuron Differentiation

MDD384

SCREENING IN IPSC DERIVED NEURONS FOR MODIFIERS OF ALZHEIMER DISEASE RELATED PHENOTYPES

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Alzheimer’s disease (AD) is a complex disorder with increasing prevalence and socio-economic burden. However, majority of strategies aimed at identifying therapies for AD have been focused on targeting Aβ or TAU, which make up the plaques and tangles commonly found in people with AD. Continued failure of the drug discovery process and the accompanying trials against these targets has necessitated more and better options for therapeutic intervention. Using a multi-parametric high content phenotypic readouts with neurons derived from human differentiated iPSCs with familial AD mutations, we aim

to optimize a platform for CRISPR based rescue screens for the various phenotypes associated with the mutations, such as endo-lysosomal transport, synaptic dysfunction and neuronal toxicity. The multiple-phenotypic rescue approach will enable identification novel key pathways and/or targets which could serve has drug candidates for the treatment of AD.

Keywords: CRISPR, NEURODEGENERATION, HIGH CONTENT PHENOTYPIC SCREENING

MDD448

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF α-SYNUCLEIN FUNCTION IN HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS USING HIGH-DENSITY MICROELECTRODE ARRAYS

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Parkinson's disease (PD) is a common neurodegenerative disease, characterized by a progressive loss of dopaminergic (DA) neurons, which currently affects about 1% of the population over 60. Excessive levels of α-synuclein have been implicated in both sporadic and familial forms of PD, hence, targeting the expression of the α-synuclein gene (SNCA) represents a potential therapeutic target to slow down the progression of the disease. In this study, we used human iPSC-derived DA neurons in combination with high-density microelectrode arrays to investigate the electrophysiological phenotypes of networks of healthy neurons and of neurons carrying a PD-associated mutation (SNCA A53T). In addition, we studied the impact of locked nucleic acid (LNA)-mediated down-regulation of SNCA expression on key electrophysiological properties in both cell lines. We found significant differences between healthy and A53T-mutant DA neuronal cultures: During the first week after plating, A53T cultures displayed higher levels of synchronized activity and an earlier onset of network bursts compared to control cultures. Over the course of development, network bursts remained a very distinctive feature, with control cultures displaying longer, yet less frequent bursts. Additionally, A53T neuron firing became more regular over time, while control neurons displayed the opposite behavior and developed a more irregular firing pattern. Features derived from the waveform of extracellularly recorded action potentials also differed between cell lines but showed considerable batch-to-batch variability. Nevertheless, classification of healthy and A53T-mutant cultures was possible with very high accuracy. LNA-mediated down-regulation of α-synuclein showed a prominent effect on network features, resulting in shorter but more frequent bursts for both healthy and A53T cultures. LNA-treated cultures also demonstrated overall reduced and less synchronous firing patterns. One potential explanation may be the role of endogenous α-synuclein in maintaining the presynaptic vesicular pool, which is required for prolonged periods of high-activity bursts. Our results provide further insights into the physiological role of α-synuclein in functional DA neuronal networks.

Funding source: This work was supported by the European Research Council Advanced Grant 694829 'neuroXscales' and a ETH Zurich / University of Basel Personalised Medicine project grant (PMB-01-18).

Keywords: Parkinson's disease, Electrophysiology, α-synuclein

MDD458

IDENTIFICATION OF PHENOTYPES SUITABLE FOR DRUG TESTING IN A GENETIC OR TOXIN-INDUCED MODEL OF PARKINSON'S DISEASE USING HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS.

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Parkinson's disease (PD) is a neurodegenerative disease caused by the progressive loss of midbrain dopaminergic (mDA) neurons. Genetic mutations affecting mDA neurons account for up to 15% of PD onsets, providing a valuable tool for studying basic pathophysiological mechanisms of the disease. One of the most relevant mutations is the A53T mutation in the SNCA gene, which causes the production of a misfolded form of alpha synuclein (αSyn), resulting in αSyn accumulation. The aim of this work is the identification of PD-relevant phenotypes for in vitro drug testing. We used human iPSCs-derived mDA neurons carrying the A53T mutation on SNCA gene and a healthy isogenic control (obtained from commercial source) differentiated in vitro for up to 35 days. Besides this genetic model we administered rotenone, a toxin widely used to create rat models of PD, to generate an acute or sub-chronic stress to healthy mDA neurons. The explored phenotypes include αSyn accumulation, calcium signalling and mitochondria functionality. To verify αSyn protein level we used Meso Scale Discovery® assays, recording progressive accumulation of αSyn in A53T cultures peaking at day 28 (1.61 ±0.18 folds, n=3). Moreover, spontaneous calcium oscillations were studied by FLIPR® technology. A 1.5-fold higher peak frequency was detected in

A53T cells, suggesting dysregulation of intracellular calcium homeostasis. Mitochondrial membrane potential (MMP) and Seahorse XF® assays were used as a read-out of mitochondrial functionality. MMP quantification by high content imaging showed a 10%-20% loss of mitochondria polarization in A53T cultures, while Seahorse XF® analysis revealed a higher basal respiration in A53T cells (up to 3 fold) compared to control with similar responses to Oligomycin and FCCP. This suggested qualitatively similar mitochondria in the two lines, but higher numbers of mitochondria in A53T cells, which we verified by high content imaging. To assess rotenone-induced damage to mDA neurons, we treated healthy cells with acute (10-50 nM for up to 72h) or sub-chronic (1-5 nM for 2 weeks) concentrations of rotenone. In acute treatment, rotenone reduced both MMP (IC50 of 9.5nM) and cell viability (RT-Glo assay, IC50 of 2.3nM). In sub-chronic treatment, a reduction of O2 consumption in the Seahorse XF® assay and of MMP levels was detected.

Keywords: Parkinson's disease model, iPSC-derived dopaminergic neurons, A53T mutation

MDD476

MECHANICS OF THE SUPPORTING HYDROGEL IMPACT HUMAN BRAIN ORGANOID DEVELOPMENT

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Brain organoids are tissue-engineered neural models that enable studies of realistic developmental and disease processes in culture. However, organoid failure and variability is common, likely due to the highly variable nature of Matrigel, a mouse tumour-derived material scaffold required to support organoid growth. As mechanics play a significant role in cell fate and function1, we evaluate the impact of external matrix mechanics on the development of brain organoids. Midbrain organoids were generated from human induced pluripotent stem cells2,3 and embedded in Matrigel-alginate interpenetrating hydrogel networks (IPNs) designed to support organoid growth. Alginate IPNs modulate gel mechanical properties to yield a range of stiffnesses, but do not present adhesive or cell-interactive ligands. Mechanical properties of the hydrogels were characterized by shear rheometry. Organoid tissue sections were stained to examine gross morphology, and cellular markers of developmental stage and mechanical activity. All hydrogel compositions yielded viable brain organoids, with characteristic neural rosettes. However, organoid growth was significantly restricted in the stiffest hydrogel, and staining revealed differences in developmental patterning in this hydrogel, including smaller rosettes. Current analyses are examining organization and quantity of neural progenitors, immature and mature neurons to further probe differences among hydrogel compositions. These results demonstrate that stiffness is an important parameter in brain organoid development, and suggest that organoid self-organizing capacity and developmental program may be influenced by external mechanics. These studies will elucidate the impact of culture environment mechanics on brain organoid development, ultimately informing design of a synthetic Matrigel substitute material for growing brain organoids.

Funding source: This work was supported by the Canada Research Chairs program in Advanced Cellular Microenvironments to CM, a HBHL platform grant and the Van Berkom-Saucier minibrain program to TMD, NSERC and CQDM.

Keywords: brain organoids, hydrogels, mechanics

MDD482

STANDARDIZED WORKFLOW FOR ANALYZING THE QUALITY OF HUMAN IPSC AND IPSC DERIVED FOREBRAIN CORTICAL NEURONS

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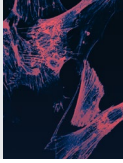
Human induced pluripotent stem cells (hiPSCs) derived from human somatic cell have given scientists new opportunities to model and investigate human diseases, as well as to develop new therapeutics. The routine application of hiPSCs and functional cells derived from hiPSCs in high throughput applications for drug discovery will require a constant supply of pluripotent, well characterized and quality controlled cell stocks. However, the absence of standardized quality control of IPSC in many groups and execution challenges laboratory efficiency and experimental reproducibility. We therefore established a workflow for comparing the quality of ten newly generated hiPSC lines derived from seven healthy individuals, relative to existing commercial lines. We monitored hiPSC morphology and proliferation in two different media (mTeSR1 and Essential 8), characterized them extensively for pluripotency, and evaluated their ability to differentiate into three germ layers. Genomic stability in the hiPSCs was further analyzed by G-banding and qPCR-based genetic screening. Using standardized dual SMAD inhibition procedures, all the iPSC lines gave rise to neural progenitors, and subsequently differentiated into cortical neurons. Neural differentiation was analysed at the protein level qualitatively by immunocytochemistry and quantitatively by q-PCR for NPC (SOX1, PAX6, NESTIN, ASCL1, SCL1A3), neuronal (MAP2, NCAM1, TUBB3), cortical layer (TBR1, Brn2, SATB2, FOXP1) and glial markers (SOX9, CD44).The efficiency of forebrain cortical neurons differentiation was variable from line-to-line which lead to careful considerate the cell lines that are selected for disease modeling and drug screening. Taken together, the workflow outlined here provides a simple,



standardized workflow for the routine quality control of hiPSC and differentiation potentiation to ensure that hiPSCs are of the highest quality for the basic research and their applications.

Keywords: HiPSC, quality control, cortical neurons

MDD491

THE SENESCENT ASTROCYTE SECRETOME IMPAIRS HUMAN IPSC-DERIVED NEURONS CARRYING SNCA DUPLICATION

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Old age is a major risk factor for Parkinson's disease (PD). One of the core mechanisms of aging is cellular senescence. In 2018, two studies linked senescence to neurodegeneration for the first time, based on PD and tauopathy disease in vivo models (Chinta et al., PMID: 29386135; Bussian et al., PMID: 30232451). Astrocytes were identified as the major cell type of the central nervous system to turn senescent (Bussian et al., PMID: 30232451). We could identify the PD-relevant stressor rotenone to induce astrocyte senescence based on a set of senescence markers. We further show that the senescent state in human astrocytes depends on mechanistic-target-of-rapamycin (mTOR) and DNA-damage-response signaling, both known to drive the senescent phenotype. It is yet unclear what features of senescent astrocytes contribute to the neurodegenerative pathology. This study could show that the astrocytic senescence associated secretory phenotype (SASP) impairs midbrain neurons differentiated from human induced pluripotent stem cells of PD patients carrying a duplication of the α-synuclein gene (SNCA) locus. The astrocytic SASP together with exposure to an environmental stressor amplifies dopaminergic cell death in SNCA duplication neurons.

Funding source: This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) 270949263/GRK2162 (to K.S., J.A. and B.W.).

Keywords: iPSC-derived neurons, astrocyte senescence, Parkinson's disease

MDD499

CULTURING HUMAN IPSC-DERIVED CORTICAL NEURONS ON COMPOSITE BIOSYNTHETIC SCAFFOLDS SIMULATING IN VIVO CIRCUITRY TO AID DRUG DISCOVERY FOR NEURODEGENERATIVE DISORDERS.

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Despite significant advances in studying psychiatric and neurodegenerative disorders, there is no absolute cure for most of these diseases. Drug discovery and development processes have particularly been challenging owing to lack of good models for studying human brain regions that mimic in vivo synaptic circuitry. There are several shortcomings to High Throughput Screening processes and having a secondary confirmation from more relevant physiological models would enhance the current screening process. Since the discovery of induced pluripotent stem cell, they have been actively used for differentiations and have also proven to be better than animal models owing to ease of manipulation in a dish and more similar to target human tissues. However, current differentiations protocols to cortical neurons do not take into account the naturalistic synaptic circuitry of human brain regions. In vitro neuronal cultures display random synaptic circuits and do not recapitulate in vivo neurophysiology. Our research utilizes composite 3-D photoreactive nanofiber scaffolds to provide guidance cues for the differentiation and growth of in vivo-like neural tissues from hiPSC or hNPC cultures. The scaffolds combine aligned nanofiber tracts for neurite guidance, random non-woven mats for somatic nesting, and photochemical surface-modification to drive tissue patterning. Our initial research is focused on recapitulation of the hippocampal Schaffer collateral circuit for field electrophysiology of human cortical cultures, which contain Glutamatergic, GABAergic, and glial cell phenotypes. Cellular phenotypes are detected immunohistochemically using neurotransmitter markers. Live imaging on scaffolds have successfully shown neurites growing along the aligned tracts. This technology is being developed to increase the accuracy and lower the cost of drug discovery processes against CNS and spinal cord targets by measuring the responses of human brain cells in an in vivo like context and avoiding the use of animals for brain slice preparation.

Funding source: NIH/NIMH

Keywords: Neural differentiation, Biosynthetic Scaffolds, Drug discovery

MDD506

INVESTIGATION OF ASD RISK GENES IN HUMAN BRAIN DEVELOPMENT

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Mechanistic studies of neurodevelopmental diseases are hampered by the complexity of these pathologies, the lack of single, ideal experimental models, and the need to assay across varied human genetic backgrounds. Despite the great medical and societal impact of neurodevelopmental disorders such as ASD, we have yet to answer key basic questions about cell types and developmental events affected in ASD. Answering these questions will be vital to understanding ASD etiology and to identify cellular and molecular targets for therapeutic progress. Here, we have leveraged ASD genetic information and the use of human brain organoids to investigate whether convergent mechanisms are at play across distinct ASD-associate genes. We have used CRISPR/Cas9 tools to generate heterozygous mutations in different de novo ASD risk genes (CHD8, SUV420H1, and PTEN) and grown brain organoids of advanced maturity from each line and isogenic controls. In order to gain information on specific cell types affected by each mutation, we have performed single-cell RNAseq analysis on organoids collected at 1, 3 or 6 months, using the 10X Genomics Chromium system. For some of the genes, experiments were repeated using multiple mutant iPSC lines (to include different genetic backgrounds) and using organoids derived with different protocols. We sequenced approximately 1,000,000 single cells and provide first evidence for convergent mechanisms and cell types affected across mutations. These data validate the use of human brain organoids to model cell type specific changes associated with neurodevelopmental disorders.

Keywords: neurodevelopment, 3D organoids, single-cell biology, disease modeling

NEW TECHNOLOGIES

MDD397

INTERNATIONAL SPACE STATION EXPERIMENT: EFFECTS OF MICROGRAVITY ON MICROGLIA-NEURON INTERACTION IN NEURAL ORGANOIDS FROM PARKINSON DISEASE AND MULTIPLE SCLEROSIS IPSCS

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Microgravity has been shown to have an impact on biological systems, including the immune system and the central nervous system (CNS). Neuroinflammation is a common feature of many neurodegenerative diseases, including Parkinson disease and multiple sclerosis. To investigate the effects of microgravity on neural survival and neuroinflammation, we designed an experiment to examine the interplay between neurons and microglia, the immune cells of the CNS on the International Space Station (ISS). Our strategy for studying neuron-microglial interactions over a month-long period on the ISS, was to generate neural organoids from iPSCs derived from donors affected by Parkinson disease (dopamine neurons) or progressive multiple sclerosis (cortical neurons), as well as the same cell types from unaffected controls. Microglia were derived from the same donor iPSCs and combined with a subset of the pre-formed organoids before the cells were delivered to the ISS. Cultures were placed in a temperature-controlled incubator (CubeLab, Space Tango) and launched onboard SpaceX's Falcon 9 rocket as part of the 19th SpaceX Commercial Resupply Services (SpX CRS-19) mission for NASA on December 5th, 2019. A parallel experiment

was replicated on the ground. The cultures were returned to Earth in the Dragon capsule that splashed down in the Pacific on January 7th 2020. Some of the organoids were placed into culture immediately after their return, and showed robust neural outgrowth, indicating that they had thrived during the month-long culture on the ISS. Post-flight analysis of gene expression and histology is currently in progress to determine the effects of spaceflight on the cells. The results of this research will provide valuable insight into the fundamental mechanisms underlying neurodegenerative diseases, with the potential for impact on discovery and development of biomarkers and therapeutics.

Funding source: Summit for Stem Cell Foundation, National Stem Cell Foundation, New York Stem Cell Foundation, Aspen Neuroscience, Inc.

Keywords: Neuroinflammation, International Space Station, Neural Organoids

MDD399

MONOQLO: AN AUTOMATED, DEEP-LEARNING BASED WORKFLOW FOR IDENTIFICATION OF MONOCLONAL CELL LINES

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Automated derivation of induced pluripotent stem cells (iPSCs) overcomes a critical restriction on scalability and technical variability that otherwise precludes the use of iPSCs in large-scale population studies. When using virus-based methods for iPSC reprogramming of adult cells, a critical step in ensuring cell line performance is the isolation and expansion of cells derived from a single starting cell, a step referred to as monoclonalization. Monoclonal iPSCs are typically derived from plating via serial dilution, or seeding at low density, followed by manual colony picking. Due to the possibility of technical complications during cell culturing or sorting, it cannot be assumed that any given line is truly monoclonal, or has retained the morphological markers associated with pluripotency, necessitating post hoc verification. This is typically achieved via manual analysis of microscopy images, which is a highly time-consuming process reliant upon human judgement. As a result of this lack of standardization, such methods cannot be reliably upscaled without exacerbating the technical variability of cell lines. To overcome these issues, we report the design of a deep learning workflow that reliably identifies monoclonality and classifies colony quality from imaging at regular intervals. The workflow, termed Monoqlo, integrates multiple convolutional neural networks and, critically, leverages the chronological directionality of the cell culturing process. Monoqlo provides a highly scalable framework, capable of analyzing datasets numbering in the tens of thousands of images in under an hour using commodity hardware. Through the combination of automated stem cell culture and deep learning, this work demonstrates a novel use case for machine learning in cell culture and biotechnology-driven applications. By removing the dependence on manual inspection and human judgement, Monoqlo fully standardizes the monoclonalization process, allowing colony selection protocols to be infinitely upscaled while minimizing technical variability.

Keywords: Deep-learning, monoclonal, colony identification

MDD400

MELANOCYTE DIFFERENTIATION STATE DETERMINES ONCOGENIC COMPETENCE IN MELANOMA

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Cellular lineage plays a crucial role in determining the transcriptional response to oncogenic mutations. However, the mechanisms linking developmental lineage programs to oncogenic competence remain poorly understood. Here, we established a human pluripotent stem cell (hPSC)–derived cancer model to investigate the mechanisms that master melanoma competence depending on the state of differentiation. Together with a zebrafish cancer model, we showed that melanocytes (MCs) were refractory to melanoma formation, while their progenitors, neural crest (NC) cells and melanoblasts (MBs),

were highly responsive to the same tumorigenic insults. We revealed that the NC/MB states and the MC state had a different chromatin landscape reflected by differential expression of epigenetic–related factors. Re–expression of development–specific chromatin modifiers in mature MCs allowed cellular rewiring and dedifferentiation and it was necessary for tumor initiation. This study shows that oncogenic competence within a given lineage is regulated by expression of stage-specific chromatin factors that control the transcriptional response to a specific mutation.

Keywords: Cancer competence, Melanoma, Neural Crest

MDD416

INSULIN RESPONSE IN HUMAN STEM CELL-DERIVED METABOLIC TISSUES

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Type 2 diabetes (T2D) has a long-term progressive pathogenesis, beginning with insulin resistance in peripheral tissues such as muscle, fat and liver. Some molecular changes leading to insulin resistance are known, but the primary cause(s) remains unclear. Model organisms lack many of the disease pathologies associated with human T2D, and mechanistic human in vivo study is intractable. Human stem cell-derived tissues are the ideal model to recapitulate the human aspect of the disease. However, most differentiation protocols are not optimized for metabolic research, and common culture conditions and additives suppress metabolic signaling such as insulin response. We generated human stem cell-derived tissues, and optimized their culture conditions to maximize insulin response, while retaining cellular viability and differentiation efficiency. As readouts we looked at signaling events such as phosphorylation of AKT2, but also at functional responses such as insulin-stimulated glucose uptake and production. Through a design of experiments approach we systematically tested various media additives for their effect on insulin signaling. This approach resulted in the generation of culture conditions where human physiological levels of insulin lead to downstream signaling activation, which to our knowledge is a first in human stem cell-derived tissues. So far this was successful for fat, liver, and skeletal muscle. With these insulin-responsive human cells, we can now study novel biology in insulin response and resistance. We have already shown a transcriptional response as early as 5 minutes after insulin stimulation, experiments which are impossible to conduct in humans. Several crucial transcription factors, and known lipid and glucose metabolism regulators, such as MLXIPL and SREBF1, have a conserved insulin-regulated response in multiple tissues. In this setting we then induce insulin resistance, through genome editing or chemical means, and comprehensively survey the changes in insulin response. Ultimately, we aim to illuminate novel and druggable targets in the insulin signaling pathway that would be of value in understanding and treating insulin resistance in the setting of human metabolic disease.

Keywords: Insulin, Diabetes, Metabolism

MDD420

HUMAN STEM CELL-DERIVED NGN2-ACCELERATED PROGENITOR CELLS ENABLE INTERROGATION OF NATURAL VARIATION IN GENE EXPRESSION AND ZIKA VIRUS SUSCEPTIBILITY

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Variation in the human genome influences neural progenitor cells (NPCs) and their role in fetal brain development and disease. Modeling this diversity is challenging given that current in vitro stem cell-based culture systems rely on prolonged differentiation protocols that show variability in efficacy across cell lines. Here, we report on a rapid and reliable method for producing stable cultures of human stem cell-derived NPCs through a simple 48-hour induction protocol. These cells, which we have dubbed Stem cell-derived NGN2-accelerated Progenitor cells (SNaPs), satisfy the biochemical, transcriptomic, and functional requirements for dorsal telencephalic NPC classification, and could be derived from dozens of hESC and hiPSC donors. Like their in vivo counterparts, SNaPs are susceptible to Zika virus (ZIKV) infection, which is a neurotropic pathogen associated with microcephaly and other neurodevelopmental disorders. Interestingly, we found this viral vulnerability to vary dramatically across cell lines. To explain this differential NPC response to ZIKV, we performed genome-wide CRISPR-Cas9 survival screens to identify genetic host factors in SNaPs, and coupled

these findings to expression quantitative trait loci (eQTL) that arose from single-cell RNA sequencing (scRNA-seq) analysis of 48 SNaP lines cultured in a shared environment known as a "village-in-a-dish." Our efforts succeeded in uncovering a single nucleotide polymorphism in the antiviral gene IFITM3 that strongly correlated with ZIKV infectivity. Intriguingly, risk allele frequencies at this locus are highly variable across different global ancestries with proportions as high as 46% in Europe and as low as 0.6% in regions in which flaviviruses are endemic, thus hinting at a potential evolutionary selection against this genotype in the face of high rates of RNA virus infections. Together, this work demonstrates how genetic variation can impact cellular phenotypes, while also highlighting the broad potential of the SNaP method to accelerate future large-scale in vitro investigations of the developing human brain.

Funding source: NIH/NIMH grants U01MH105669 and U01MH115727. M.F.W. is supported by the Burroughs Wellcome Fund Postdoctoral Enrichment Program award (1018707) and a K99/R00 Pathway to Independence Award (NIH/NIMH 1K99MH119327-01).

Keywords: neural progenitor cells, Expression quantitative trait loci (eQTL), Zika virus

Theme: Tissue Stem Cells and Regeneration

ADIPOSE AND CONNECTIVE TISSUE

TSC103

MOLECULAR SYNERGISM TO COUNTERACT HUMAN STEM CELL FATE BY DEFINED FACTORS

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Stem cells can be defined as undifferentiated elements, capable of self-renewal and differentiation following exposure to specific environmental stimuli. Fat tissue represent an important source of stem cells, the so called "adipose derived stem cells" (ADSCs), which under external stimuli can differentiate towards different lineages. We previously identified specific conditioned culturing media, able to commit stem cells towards targeted cellular phenotypes. In particular, defined compound as Vitamin D, were able to influence stem cell fate, acting as epigenetic modulators on the expression of specific genes. Here, we aimed at evaluating the role of another important molecule, metformin, on ADSC cell fate and metabolic activity. In particular ADSCs were cultured in a specific adipogenic conditioned medium (ADM), in the presence of Metformin, alone or in combination with Vitamin D. Our results show that the combination of the two compounds is able to counteract the appearance of an adipogenic phenotype, indicating a feedforward regulation capability of Metformin on Vitamin D metabolism, by acting on CYP27B1 and CYP3A4. Moreover, within the ADSCs committed toward adipogenesis a beige-like phenotype, together with autophagy was detected. We can conclude that the combination of the molecules tested is able to influence stem cell decisions, through the modulation of adipogenesis, suggesting their possible application in clinical practice in the near future.

Keywords: Stem cell fate, Adipogenesis, Gene expression

TSC105

LOW OXYGEN GRAPHENE AS A PLATFORM FOR OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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The effects of low oxygen graphene can be utilized as both a cell-adhesion substrate and a delivery platform for the osteogenic differentiation of human mesenchymal stem cells (MSCs). Graphene, a 2D high surface area lattice monolayer is composed of hexagonally arranged carbon atoms, and can be functionalized by chemical modifications to generate nanocomposites with specific physicochemical properties. In this study, we first identified the mass concentration of graphene that showed a significant increase in osteogenesis, by Alizarin red staining and quantitation, and subsequently, the expression and cellular localization of extracellular matrix (ECM) glycoproteins was assessed in vitro by immunofluorescence. The expression of ECM proteins laid down by human MSCs was evaluated in osteogenic cells. ECM is a network structure composed of various biomolecules, controls the cellular behavior and provides a favorable microenvironment for the growth and differentiation of cells. The results show that graphene is cytocompatible towards MSCs and the amount of graphene coated on a surface modulates cell adhesion, spreading, growth, and osteogenic differentiation. We also observed that human MSCs express various integrin subunits and lay down the matrix i.e. rich in fibronectin, suggesting the interaction between the two, to play a major role in adhesion and osteogenic differentiation of human MSCs on graphene surfaces. In this study, we will discuss (1) bone ECM composition and key integrin proteins implicated in osteogenic differentiation, (2) the use of graphene alone and graphene modified with ECM–mimetic peptides/protein

fragments that will promote the mechanical fixation of implants to bone and to enhance bone healing within large defects. This study suggests that a layer of graphene as a potential bone scaffold will be beneficial for osteoblast attachment, proliferation, and differentiation.

Funding source: Supported by NIH R15 National Institute of Arthritis and Musculoskeletal and Skin Diseases

Keywords: Osteogenic, Graphene, ECM

CARDIAC

TSC118

HUMAN CELL-DERIVED EXTRACELLULAR MATRIX PROMOTES RAPID STRUCTURAL AND FUNCTIONAL MATURATION OF HIPSC-DERIVED CARDIOMYOCTYES IN 2-D CULTURE

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Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) offer the opportunity for cardiotoxicity screening of drugs in development using functionally beating human cardiac cells. 2D hiPSC-CM monolayers are a very attractive preparation for high throughput screening cardiotoxicity assays. However, obtaining mature phenotypes of hiPSC-CMs remains an elusive goal. Recent evidence has shown the importance of the extracellular matrix (ECM) stiffness and composition to promote aspects of hiPSC-CM maturation and for drug responsiveness. ECM from murine sources has been shown to be effective for maturation, however the use of animal cell products may have unknown incompatibilities with human cells. Here we tested the hypothesis that human cell derived ECM promotes more complete maturation of hiPSC-CMs relative to murine ECM. For human ECM, perinatal stem cells were seeded onto tissue culture plates and allowed to grow to confluence. At confluence cells were induced to secrete ECM, and then a non-ionizing detergent was utilized to decellularize the matrix. The resulting ECM was washed thoroughly and air-dried. As much as possible, the microenvironment elaborated by the cells was left intact, so that an intact matrix with structural, mechanical, and biochemical cues were undisturbed. iCell2 Cardiomyocytes (Cellular Dynamics International, CDI) were thawed and plated as confluent monolayers on each ECM. hiPSC-CM structural maturation was quantified using confocal microscopy and function was determined using mitochondrial and voltage/calcium sensitive dyes. hiPSC-CMs cultured on human ECM rapidly (7d) matured as evidenced by rod shape morphology, anisotropic alignment, multi-nucleation, myofilament compaction, cardiac troponin I expression, rapid impulse propagation velocity (~45cm/s) and response to isoproterenol (positive inotrope). Mature hiPSC-CMs also had greater mitochondrial staining using MitoTracker-RedCMXRos. The ability to produce mature hiPSC-CMs represents an important step forward for the field and demonstrates the importance of providing an appropriate extracellular matrix for cells in vitro. This approach may be valuable for drug screening/toxicity testing, basic and translational research, and the development of cell-based therapies.

Funding source: Research reported in this publication was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number R44ES027703.

Keywords: Cardiotoxicity, Stem Cell Derived Cardiomyocytes, hiPSC-CM Maturation

TSC127

IMPROVED VENTRICULAR FUNCTION THROUGH IMPLANTED HUMAN IPSC DERIVED CARDIOMYOCYTE GRAFT

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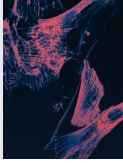
Over 500,000 new cases of Chronic Heart Failure (CHF) occur annually in the United States alone, with mechanical assist devices and heart transplantation as the only options for late-stage CHF. The predominate cause of CHF is the loss of cardiomyocytes from myocardial infarction resulting in maladaptive left ventricular (LV) remodeling. This remodeling includes the formation of fibrotic scar tissue not conducive for the renewal and regeneration of lost cardiomyocytes. Therapeutics that mediate the toxic microenvironment and stimulate cardiomyocyte renewal are being pursued but have not been achieved. We tested the hypothesis that administration of human induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) and human neonatal fibroblasts (NFB) to the epicardium following permanent left coronary artery ligation could improving cardiac function and reversing the maladaptive LV remodeling. Hemodynamic data shows that graft-treated rats (n=11) present an decreased ($P<0.05$) LV end diastolic pressure (14±3 mmHg) and Tau (time constant of LV relaxation, 30.7±2.5 msec) when compared with the CHF rats, (n=14, 26±2 mmHg and 37.9±2.4 msec respectively). Additionally, graft-treated rats had lower peaked developed pressure (124±4 mmHg versus 154±11), and LV±dP/dt (4554±209 versus 5686±556 mmHg/sec and -2737±102 versus -3855±492 mmHg/sec) than CHF rats. The graft-treated rats have increased wall thickness, smaller ventricular cavities, and increased myocyte density in LV scar tissue. These data support the therapeutic benefit of the cardiac graft in terms of improving LV function. In addition we show that the transplanted cells are cleared within 21 days post-implantation with IgG donor-specific antibody (DSA) highest on days 21 and 49. This suggests the cells of the engineered graft likely do not integrate into the host heart tissue but its therapeutic benefit derives from stimulating resident cardiac cells. While the mechanism of action is unknown, the current theories consist of complex immune interactions, and paracrine factors. Additional



investigations are underway to determine the grafts mechanism of action with specific focus on the microenvironment. We hope to reveal this mechanism to improve efficacy of the therapy.

Funding source: This work was supported by the NHLBI T32 HL007249-43, WARMER Research Foundation, Sarver Heart Center, University of Arizona, and The Martin and Carol Reid Charitable Remainder Trust.

Keywords: Chronic Heart Failure, Induced Pluripotent Stem Cell Derived Cardiomyocytes, Implanted Tissue Graft

EARLY EMBRYO

TSC129

METABOLIC CONTROL OVER MTOR DEPENDENT DIAPAUSE-LIKE STATE

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Future treatments manipulating quiescence in aging stem cells, or cancer stem cells should significantly improve modern medicine. However, the regulation of quiescence in any stem cell stage or type is poorly understood. We have identified regulators of an in vivo quiescent stage, embryonic diapause, the environmentally inducible suspended stage in development and have generated an in vitro diapause-like model for further dissecting the process. With these new tools, we have shown that downregulation of mTOR by starvation induced LKB1-AMPK activation is necessary for the induction of a diapause-like state and that glutamine transporter SLC38A1 primes for mTOR-dependent exit from the quiescence. Forced expression of a non-diapause Lkb1 splice variant results in a constitutive diapause-like state due to a phospho-AMPK dependent increase in glucose transporters and decrease in mTOR activation. These data also explain why amino acid composition in uterine fluid is critical for diapause regulation. The glutamine transporter Slc38a1 is essential for diapause state. These data show that amino acid composition and Lkb1 splice variant regulate mTOR-dependent diapause metabolic and epigenetic state. Since metabolism has recently been shown to be a critical determinant of cellular fate, it is likely to play a role in quiescence and may also contribute to the epigenetic state of diapause stage. These studies set the stage to better understand how cells communicate to synchronize this enigmatic, reversibly paused embryonic diapause stage.

Keywords: Diapause; Pre-implantation; Mouse ESCs; Metabolism; Lipolysis., mTOR; Glutamine transporter; LKB1; Amino Acids., Pluripotent Stem Cells; Epigenetic; H4K16Ac.

TSC419

IN VITRO INTERACTION OF HUMAN EMBRYONIC STEM CELLS WITH HUMAN AMNIOTIC EPITHELIUM REPRESENTS AN ALTERNATIVE STATE OF PLURIPOTENCY

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Conventional conditions to maintain human embryonic stem cells (HESC) imply the use of inactive mouse embryonic fibroblast (iMEF) as a feeder layer. However, it has suggested that the culture of hESC on iMEF could be an artifact; since it does not correspond to the in vitro counterpart of the epiblast during human embryonic development. Our previous studies demonstrated the human amniotic epithelial cells (HAEC) as an alternative feeder layer to derive and maintain HESC. Here, we showed that the culture of HESC on HAEC could represent a different pluripotent stage, a similar lapse when the amniotic epithelium derives from the epiblast during peri-implantation. The HESC on HAEC shown comparable 'pluripotency core' expression levels as conventional primed HESC on iMEF and did not express markers of naïve pluripotency. However, differences found in their cytokines and phospho-kinases proteomes suggested that HESC on HAEC exhibits an alternative molecular state as compared with conventional feeder condition. Interestingly, HESC on HAEC showed no gene expression to endoderm and mesoderm induction (GATA6, SOX17 and BRACHYURY), whereas exhibited an upregulation in ectoderm lineage markers (OTX2/OTX1); which suggest a preference in their differentiation potential toward anterior cytoarchitecture (forebrain). Also, RNA-seq differential expression analysis determined a unique

neural induction and forebrain development genes signature in HESC on HAEC as compared with conventional primed and naïve HESC. Neural differentiation assay was carried out (dual SMAD inhibition protocol) to confirm whether these differences in the transcriptome reflected a distinctive functional potential. The results demonstrated an increase in the detection of neural markers of mature neurons (MAP2), deep cortical layers (FOXP2, CTIP2) and interneurons (GAD67, CALRETININ) when HESC were induced to differentiate at neural lineage under HAEC as a feeder layer, as compared with the iMEF layer. These data suggested that the interaction of HESC with HAEC is an in vitro condition that resembles the anteriorized epiblast when acquiring a neural identity.

Funding source: CONACYT (272968 and 252756) and INPER (212250-3230-21214-01-16).

Keywords: human amniotic epithelium, spectrum of pluripotency, anterior epiblast

TSC428

DIRECTIONAL FLUID FLOW DICTATES THE FORMATION OF THE PROAMNIOTIC CAVITY AND ESTABLISHMENT OF THE DISTAL-PROXIMAL AXIS

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During the peri-implantation stages, the mouse embryo radically changes its appearance transforming from a hollow-shaped blastocyst into an egg cylinder. At the same time the epiblast gets reorganized from a simple ball of cells into a cup-shaped epithelial monolayer enclosing the proamniotic cavity. The function and mechanism of formation of this cavity have been so far unknown. Here, we aimed to decipher the mechanism of lumenogenesis and the role of the proamniotic cavity using 3D in vitro model of epiblast development, in combination with direct analyses in early mouse embryos. We found that the process of lumenogenesis in the epiblast is driven by reorganization of intercellular adhesion, vectoral fluid transport and mitotic paracellular water influx from the blastocoel into the emerging proamniotic cavity. By experimentally blocking lumenogenesis, we found that the proamniotic cavity acts as a microenvironment for ligands and associated factors of critical developmental pathways, such as the Nodal signalling cascade. Accordingly, embryos where the formation of proamniotic cavity was experimentally blocked displayed features of impeded Nodal signalling, such as failure in proper induction of signalling centers and establishment of the early body axes. Therefore, we determined that the proamniotic cavity functions as a hub for communication between the early lineages, enabling proper growth and patterning of the post-implantation embryo.

Funding source: This work was supported by the German Research Foundation (DFG) Emmy Noether grant.

Keywords: Lumen formation, Distal-proximal axis, Embryonic development

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

TSC373

HUMANIZED ENDOTHELIUM IN GENE EDITED ETV2 NULL PIG EMBRYOS AS A PLATFORM FOR EXOGENIC ORGAN PRODUCTION AND XENOTRANSPLANTATION

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Organ transplantation remains limited due to the shortage of donor organs. This may be addressed in the future by xenotransplantation, using pigs to grow unlimited humanized transplantable organs for patients having chronic end-stage diseases. However, organ rejection initiated by donor endothelial cells remains a challenge associated with xenotransplantation. Therefore, we pursued a novel strategy, to generate pigs with humanized endothelial cells with the goal of providing a universal platform for exogenic organ production by reducing immunological rejection. We used gene editing and somatic cell nuclear transfer (SCNT) technologies to engineer porcine embryos deficient in ETV2, a master regulator of hematoendothelial lineages. ETV2-null pig embryos lacked endothelial and hematopoietic lineages and were embryonically lethal. Blastocyst complementation using GFP-labeled wildtype porcine blastomeres rescued these ETV2 porcine mutants resulting in viable chimeric embryos with entirely donor-derived hematoendothelial cells. Using embryo complementation strategies together with human induced pluripotent stem cells (hiPSCs), or hiPSCs overexpressing the antiapoptotic factor BCL2, we demonstrate survival and proliferation of the chimeric embryos in vitro and the engraftment of hiPSCs into the ETV2 mutant embryos in vivo at embryonic day 17-18. In these embryos, all endothelial cells were of human origin. Our data demonstrate the successful use of complementation strategies and the feasibility of engineering human-porcine chimeric embryos. Future studies will be needed to further increase the efficiency of chimerism.

EPITHELIAL

TSC158

METABOLIC STATE IS A KEY FACTOR TO MAINTAIN STEMNESS IN THE INTESTINAL STEM CELLS

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The plasticity of intestinal epithelial cells to function as stem/progenitor cells when the canonical intestinal stem cells that highly express Lgr5 gene (Lgr5-ISCs) are ablated or damaged is well documented and accepted. Our group is the first to have demonstrated that this plasticity of other epithelial cells can also be marshalled by altering the nutritional environment. Specifically, feeding mice a purified diet (NWD1) formulated to mimic intake of several common nutrients each at its level tightly linked to substantially increased incidence of sporadic colorectal cancer in western/developed countries suppresses Lgr5-ISCs lineage tracing and recruits an alternate cell population. This takes place through extensive alterations in gene expression in each population. Even though the diet suppresses stem cell functions of the Lgr5-ISCs, the diet is highly protumorigenic, accelerating and increasing tumor development in mouse genetic models, and provides the only model of sporadic intestinal tumor development when fed to wild-type mice. We have investigated the physiological changes of the intestinal epithelium induced by feeding NWD1. We have found that NWD1, in suppressing stem cell functions of Lgr5-ISCs, maintained mucosal homeostasis by recruiting other stem cell compartments. Gene expression analysis of Lgr5-ISCs under different diet conditions revealed that western-style diet significantly shifted metabolic pathways in Lgr5-ISCs. Moreover, Ppargc1a, a prime regulator of mitochondrial biogenesis, was remarkably downregulated in Lgr5-ISCs fed with western-style diet, and targeting genetic inactivation of Ppragc1a to the Lgr5-ISCs was sufficient to inhibit their stem cell function. In addition, we found that the nutritional environment had an impact on the function and morphology of mitochondria. Collectively, our data suggest that the nutritional environment to which mice are exposed has a major impact on the function of stem cells by altering their metabolic state, which results in mobilization of alternative stem cell population under homeostasis.

Keywords: Intestinal stem cells, Western-style diet, Intestinal epithelial homeostasis

TSC168

LINKING SIGNALING DYNAMICS WITH CELL FATES IN LIVE MICE

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Many tissues are composed by multiple cell types, which are derived from and maintained by tissue resident stem cells. How stem cells coordinately differentiate into multiple cell fates is largely unclear. Recent studies underline the heterogeneity among stem cells or common progenitors, suggesting cell fates are diversified at the stem cell/progenitor level. By tracking and manipulating the same stem cells and their progeny at the single-cell level in live mice, our recent work uncovered an unanticipated flexibility of homeostatic stem cell differentiation in the hair follicle. Though stem cells have been shown to acquire plasticity upon injury, we demonstrated that stem cells at the single-cell level can flexibly establish all the differentiation lineages even in uninjured conditions. Furthermore, stem cell derived hair progenitors in the structure called matrix, previously thought to be unipotent, dynamically change differentiation outcomes. These findings suggest important roles of dynamic extrinsic signaling in regulating cell fates. Additionally, our work suggests that tissue architecture can also influence cell signaling, as evidenced by finding that the organization of the differentiating cells in the hair follicle can buffer ectopic signaling such as excessive Wnt activation. In order to probe deeper the connection between signaling dynamics and cell fates, we are examining the ERK/MAPK signaling, which integrates diverse signal transduction pathways and regulates various cell fates both in vitro and in vivo, including in the hair follicle. By using a novel ERK signal reporter, we can capture the real-time signaling dynamics with respect to different cell fates in the hair follicle in live mice. Time-lapse analysis uncovered an architecture of different signaling dynamics, in which distinct cell fates show specific frequency and activation state of the ERK signal. Preliminary data from pharmacologic manipulation suggest that EGFR and FGFR signaling pathways contribute to the distinct types of ERK activation in the architecture. By combining live imaging with drug treatment and genetic manipulations, we start to understand the impact of the tissue architecture on signaling dynamics as well as the roles of differential signaling dynamics in regulating cell fates.

Funding source: This research is supported by The New York Stem Cell Foundation. This research is supported by NIH/NIAMS.

Keywords: Stem cell differentiation, Hair follicle, ERK/MAPK signaling

TSC378

BMP5-REGULATED STEM CELL SELF-RENEWAL MAINTAINS PROSTATE TISSUE HOMEOSTASIS AND PROMOTES CANCER INITIATION

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Tissue homeostasis relies on the fine regulation between stem and progenitor cell maintenance and lineage commitment. In the adult prostate, stem cells have been identified in both basal and luminal cell compartments and can both act as the cells-of-origin for prostate cancer. Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer. We found that the transcription factor GATA3 is progressively lost in Pten-deficient mouse prostate tumors. Moreover, 75% of the more aggressive hormone-resistant human prostate tumors show loss of active GATA3. We show that deleting Gata3 enhances normal adult prostate stem/progenitor cells self-renewal capacity in both organoid and allograft assays. This phenotype results from a local increase in BMP5 activity in basal cells as shown by the impaired self-renewal capacity of Bmp5-deficient stem/progenitor cells. Like enforced expression of GATA3, Bmp5 gene inactivation or BMP signaling inhibition with a small molecule inhibitor are sufficient to delay cancer initiation of Pten-deficient mice. Our results show that basal stem/progenitor cell maintenance is regulated by a balance between BMP5 self-renewal signal and GATA3 dampening activity and establish BMP5 as a key regulator of basal prostate stem cell homeostasis. In addition, it identifies a potential therapeutic approach against Pten-deficient prostate as well as skin cancer.

Keywords: Prostate stem cell, GATA3, BMP5

TSC412

THE ROLE OF SPINDLE ORIENTATION IN MOUSE PROSTATE STEM/PROGENITOR CELLS AND DEVELOPMENT

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During prostate development, basal and luminal cell lineages are generated through symmetric and asymmetric divisions of bipotent basal stem cells. However, the extent to which spindle orientation controls the symmetry of divisions and tissue architecture, and the upstream factors regulating this process, are still elusive. Using mouse genetics and microscopy, we show that loss of Gata3 in the developing prostate leads to a mis-localization of PRKCZ, which results in mitotic spindle randomization during progenitor cell division. Inherently proliferative intermediate progenitor cells accumulate, leading to an expansion of the luminal compartment. These defects ultimately result in a loss of tissue polarity and defective branching morphogenesis. Those observations led us to further investigate the role of spindle orientation in the developing prostate. We use a genetic approach to specifically target mitotic spindle regulators identified in other tissues. Analysis of these mutants leads us to the identification of spindle regulators in the developing prostate stem/progenitor cells. It also suggests that spindle orientation affects tissue architecture. Additional techniques, such as ex-vivo live-imaging of tissue sections, lineage tracing and sphere-forming assays, will be used to explore the role of spindle orientation on cell fate and stem cell potential. Together, these results advance our understanding of spindle orientation regulation in the stem and progenitor cells of the developing prostate. This project will also improve our comprehension of the connection between oriented cell division and lineage specification, cell fate and tissue architecture.

Funding source: Research funded by CIHR, FRQS, the Charlotte and Leo Karassik foundation and the Dr Victor KS Lui Foundation.

Keywords: Prostate progenitor cells, Spindle orientation, Cell fate determination

HEMATOPOIETIC SYSTEM

TSC211

IDENTIFICATION AND CHARACTERIZATION OF A LARGE SOURCE OF PRIMARY MESENCHYMAL STEM CELLS TIGHTLY ADHERED TO BONE SURFACES OF HUMAN VERTEBRAL BODY MARROW CAVITIES

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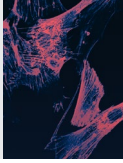
Therapeutic allogeneic mesenchymal stem/stromal cells (MSC) are currently in clinical trials for evaluating their effectiveness in treating many different disease indications. Eventual commercialization for broad distribution will require further improvements in manufacturing processes to economically manufacture MSC at sufficient scales required to satisfy projected demands. A key contributor to the present high cost of goods (COG) for MSC manufacturing is the need to create master cell



banks (MCBs) from multiple donors, which leads to variability in large-scale manufacturing runs. Therefore, the availability of large single donor depots of primary MSC would greatly benefit the cell therapy market by reducing costs associated with manufacturing. We have discovered that an abundant population of cells possessing all the hallmarks of MSC is tightly associated with the vertebral body (VB) bone matrix and are only liberated by proteolytic digestion. Here we demonstrate that these vertebral bone-adherent (vBA) MSC possess standard characteristics (e.g., plastic adherence, surface marker expression, clonogenic growth, and trilineage differentiation) of MSC and, therefore, have termed them vBA-MSC, to distinguish this population from loosely associated MSC recovered through aspiration of the bone marrow (BM) compartment. Direct comparisons of vBA-MSC to deceased donor vertebral body and living donor iliac crest BM-MSC further established equivalency. Pilot banking and expansion was performed with vBA-MSC obtained from 3 deceased donors and it was demonstrated that bank sizes averaging 2.9x10^8 +/-1.35x10^8 vBA-MSC at passage one were obtainable from only 5 g of digested VB bone fragments. Each bank of cells demonstrated robust proliferation through a total of 9 passages without significant reduction in population doubling times (18.1+/-2.6 days). The theoretical total cell yield from the entire amount of bone fragments (~300g) from each donor with limited expansion through only 4 passages is 100 trillion (1x10^14) vBA-MSC, equating to over 10^5 doses at 10x10^6 cells/kg for an average 70 kg recipient. Thus, we have established a new and plentiful source of MSC which will benefit the cell therapy market by overcoming manufacturing and regulatory inefficiencies due to donor-to-donor variability.

Funding source: National Institutes of Health grants AI138334, AI129444, and HL142418 to EJW

Keywords: Human mesenchymal stromal/stem cells, Bone marrow, Large-scale manufacturing

TSC397

THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR C IN ZEBRAFISH HEMOGENIC ENDOTHELIUM FATE DECISIONS

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Hematopoietic stem and progenitor cells (HSPCs) are multipotent cells that self-renew or differentiate to establish and replenish the entire blood hierarchy. A balance between self-renewal and differentiation is essential for the maintenance of HSPCs and production of progenitors. To achieve the goal of unlimited in vitro generation of HSPCs for clinical bone marrow transplants, we will need a better understanding of in vivo HSPC development. HSPCs arise from the hemogenic endothelium of the dorsal aorta (DA) in the embryo. We use zebrafish to study endothelial-to-hematopoietic transition (EHT) because the embryos are transparent and develop externally, allowing direct observation of in vivo cellular events. My long-term goal is to identify factors that impact HSPC differentiation during EHT. One candidate, VEGFC, is expressed in the DA of both mouse and zebrafish. VEGFC has been shown to play a role in fetal liver erythropoiesis, but its developmental role in definitive hematopoiesis has not been explored. RNA sequencing of mouse and zebrafish hematopoietic cell lineages shows high expression of VEGFC specifically in long-term HSCs, and diminished expression in differentiated populations. I hypothesize that VEGFC maintains HSPC identity by negatively regulating alternative hemogenic endothelium fates. Preliminarily, we have shown in zebrafish that morpholino knock-down of vegfc results in decreased HSPC emergence and altered cell behavior during EHT. During the peak of HSPC emergence from the DA, vegfc knock-down embryos show decreased HSPC emergence, this lack of HSPCs persists into the thymus. HSPCs in vegfc knock-down embryos also display altered cell behavior, they crawl similar to myeloid cells, instead of the typical and well described budding from the DA. vegfc knock-down embryos and genetic mutants also show an increase in total mpx:gfp+ neutrophils in the caudal hematopoietic tissue (CHT), the zebrafish equivalent of the mammalian fetal liver. We plan to further investigate the role of VEGFC in a mammalian system using human pluripotent stem cell differentiation protocols and aorta-gonad mesonephros explants. We hope to use the results of this research to improve our ability to create HSPCs in vitro to be used in clinical bone marrow transplants.

Keywords: VEGFC, Hematopoiesis, Zebrafish

MUSCULOSKELETAL

TSC239

INTRINSIC MOUSE SKELETAL STEM CELL AGING DRIVES ABERRANT NICHE DYNAMICS

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Skeletal aging and disease are associated with a disruption of the delicate balance between the opposing actions of osteoblasts and osteoclasts that are responsible for maintaining the integrity of bone tissues. Adult stem cells hold great promise for regenerative therapies, however, progress in harnessing that potential for skeletal tissues has been hampered by the lack of studies on highly purified skeletal stem cells. We recently described bona fide mouse and human skeletal stem cells and their defined downstream progenitor populations. Here we show, through detailed functional as well as single-cell genomic studies, that intrinsic aging of mouse skeletal stem cells (mSSCs) alters niche signaling and skews lineage output thereby generating an aged bone marrow niche characterized by increased osteoclastogenesis and simultaneous attrition of osteochondrogenic capacity. Strikingly, exposure of the SSC lineage to a youthful circulation by transplantation into a young host or heterochronic parabiosis, and reconstitution with young hematopoietic stem cells fail to improve bone parameters in aged mice. We have identified Csf1-Csf1r (Colony stimulating

factor-1 – Colony stimulating factor-1 receptor) signaling as the main mediator of skeletal and hematopoietic lineage interaction up-regulated during aging. High levels of recombinant Csf1 impaired fracture healing in young mice while genetic ablation of Csf1 reduced bone loss with age. Bone regeneration experiments further showed that local application of a finely tuned dose of a small molecule blocker for Csf1 in combination with Bmp2 is necessary for successful rejuvenation through the skeletal stem cell pool. Importantly, the analysis of analogous cell populations from human tissue suggests that this signaling axis is conserved in aged individuals. These findings provide mechanistic insight into the complex, multifactorial mechanisms underlying stem cell aging and offer new prospects for rejuvenating the aged skeletal system by targeting the bone marrow niche.

Keywords: skeletal stem cell, Bone aging, Rejuvenation

TSC242

HUMAN MENSTRUAL BLOOD-DERIVED MESENCHYMAL STEM CELL POTENTIAL TO DIFFERENTIATE INTO CHONDROGENIC LINEAGE USING DIFFERENT GROWTH FACTORS OF TGF-ß3 SUPERFAMILY

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Cell-based therapies such as multipotent mesenchymal stem cells (MSCs) seem promising therapeutic approach for cartilage engineering and regeneration. Menstrual blood has been proposed as one of the perspective MSCs sources (MenSCs), due to their ease of access, as compared to the classical bone marrow MSCs (BMMSCs). However, chondrogenic differentiation potential of MenSCs in vivo models remains unclear. Transforming growth factor β (TGF-β) has been considered as major factor for induction of chondrogenic response in BMMSCs, however other members of TGF-β superfamily also seems to contribute in the early stages of MSC chondrogenesis. The aim of this study is to evaluate chondrogenic differentiation capacity of MenSCs and BMMSCs using TGF-β3 superfamily members in vivo and in vitro. Human MenSCs (n=9) and BMMSCs (n=5) were isolated and characterized according to stem cell surface marker expression (flow cytometry) and adipogenic/osteogenic differentiation capacity (Oil-Red/Alizarin). The cells were embedded into atelocollagen/PLLA gels and stimulated to differentiate into chondrogenic lineage using TGF-β3 and Activin A for 21 day. Those cell constructs were inserted subcutaneously into nude mice (BALB-c) for 9 weeks and analyzed by cartilage protein (collagen type II, aggrecan) expression (histology, biochemical analysis). In addition, the cells were differentiated into chondrogenic lineage in pellets, using different growth factors (TGF-β3, BMP-2, IGF-1 and Activin A) and analyzed by chondrogenic gene expression (SOX9, Collagen II, Aggrecan) (RT-PCR). MenSCs showed similar to BMMSCs expression of surface markers (CD44; CD73; CD90; CD105) and the ability to differentiate into adipogenic and osteogenic lineages. Chondrogenic differentiation of MenSCs was remarkably more pronounced with Activin A than with TGF-β3, according to SOX9 and collagen type II expression, however differentiation in BMMSCs was stronger with TGF-β3. BMP-2 and IGF-1 has also differentially modulated chondrogenic gene expression in both cell types. In conclusion, menstrual blood might appear a promising stem cell source for cartilage repair, however, different growth factors are required for induction of their chondrogenic differentiation, as compared to BMMSCs.

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Keywords: Menstrual blood-derived mesenchymal stem cells, Chondrogenic differentiation, Growth factors

TSC243

HINDLIMB UNLOADING IN MICE DRIVES SKELETAL DEFECTS VIA CELL AUTONOMOUS EFFECTS ON SKELETAL STEM CELL FUNCTION

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Adult bone is highly responsive to changes in metabolism, hormone levels, and mechanical load. In the latter case, prolonged mechanical unloading (disuse) that occurs with paralysis, bed rest, or spaceflight has profound negative effects on bone that lead to increased fracture risk and impaired fracture healing. This condition, referred to as disuse osteopenia (DO), is further complicated by slow and incomplete bone regeneration after weight bearing recovery (WBR). Emerging data indicate that DO bone loss is accompanied by increased marrow adipose tissue (MAT). Recent studies identified a population of LepR+ skeletal stem cells (SSCs) that are the main precursor of adult bone and MAT but whether disuse alters LepR+ SSC function and if this correlates with skeletal pathology is unknown. Therefore, we subjected mice to 8 or 14 weeks of hindlimb unloading

(HU) followed by 8 weeks of WBR and correlated metabolic status, hormone level, and bone density/microarchitecture with changes in the SSC transcriptome. MicroCT analysis confirmed that HU induced DO and MAT accumulation, which only partially reversed with WBR. Moreover, serum profiling revealed that MAT accumulation was uncoupled from serum leptin levels and metabolism was unaffected by HU. Transcriptional profiling and gene set enrichment showed HU SSC gene expression was widely downregulated with numerous metabolic, cell signaling, and differentiation pathways affected while stem cell maintenance genes were induced by HU. Pathways involved in neuronal signaling were also altered in HU, which correlated with increased marrow innervation. After WBR, SSCs upregulated nucleic acid metabolism, ribosome biogenesis, and growth factor response genes, and K-means clustering of HU and WBR datasets revealed that Notch, Wnt, and Hedgehog pathways remained downregulated in WBR. Together, these data indicate SSCs enter a 'quiescent-like' stasis due to HU and, while they are responsive to WBR, key pathways of skeletal homeostasis do not recover. These data may explain the poor regeneration of bone following DO and illuminate DO prevention strategies for future study.

Keywords: Skeletal Stem Cell, Bone, Metabolism

TSC247

IDENTIFICATION OF A LATENT RESIDENT PROGENITOR POPULATION IN THE ADULT TENDON

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Tendons connect and transfer force between the muscles and bones of the body, making them highly prone to injury. These injuries have slow and imperfect healing with limited treatment options, resulting in scarring and reduced mobility. Currently, we have limited knowledge about the identity and molecular mechanisms that regulate the tendon cells following injury. Progress in the field has been limited because of a lack of specific markers to identify tendon progenitor cells in vivo. In order to impact regenerative biology approaches to tendon injury and age-related tissue degeneration, it is essential to identify the cell types and pathways responsible for tendon repair mechanisms. In our study, we first sought to determine cell cycle activity in adult tendons, we analyzed cell turnover rates from postnatal stages to adulthood and identified a unique cell population that proliferated and responded to tendon injury. To determine the cell turnover rates during growth, homeostasis and injury we used the doxycycline (Dox) inducible TetO-H2B-GFP (Col1a1:tetO-H2B-GFP; ROSA:rtTA) together with EdU, BrdU labeling, RTqPCR and RNA-seq. We found higher proliferation rates during early postnatal stages prior to 1 month of age, and significantly lower turnover rates after 1 month of age and detected a distinct transition of the limbs' tendons from cell growth to physiological homeostasis. By using the inducible TetO-H2B-GFP pulse-chase method in Axin2CreErt2 lineage, we found a distinct latent subpopulation of Axin2+ cells in the adult tendon during homeostasis. Remarkably, in cell cultures, the Axin2+ cells proliferated and expressed tendon marker genes in higher rates than the Axin2- cells and displayed higher progenitor activity. In-vivo Lineage tracing of the Axin2+ cells following Achilles tendon injuries showed higher proliferation and demonstrated that Axin2+ cells were the predominant cell population at the healed site. Deletion of Porcupine, a factor necessary for Wnt secretion, in Axin2+ and Scleraxis+ lineage resulted in a severely impaired healing response. Therefore, we believe that the Axin2+ cells that reside in the tendon serve as a unique latent progenitor cell population, that responds to tendon injuries, and populates the tendon injured site in a cell autonomous manner.

Funding source: HFSP Long Term Fellow

Keywords: Regeneration, musculoskeletal, Progenitor cells

TSC253

HYPOXIA STIMULATES CELLULAR REPROGRAMMING IN MUSCLE CELLS

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Cellular reprogramming is a phenomenon in which fully differentiated cells are reprogrammed into pluripotent stem cells. This occurs through the activation of critical transcription factors Oct4, Sox2, and Nanog. Recent studies have indicated that the addition of hypoxia to culturing conditions increased the efficiency of cellular reprogramming by increasing the expression of Oct4, Sox2, and Nanog. Furthermore, cells exposed to hypoxia displayed enhanced stem cell behavior such as increased cell migration, cell proliferation, and the ability to multiply differentiate. Therefore, we hypothesize that hypoxia can impart the same effect on muscle cells and reprogram them back into multipotent stem cells. To prove this concept, we cultured muscle cells, such as myoblasts (C2C12) and primary (Pax7 positive) muscle satellite cells under hypoxia to investigate changes in the level of transcription factors (Oct4, Sox2, and Nanog) and changes in the expression of muscle development gene (Msx1). We discovered that hypoxia increased the expression levels of Oct4, Sox2, and Nanog after 24 hours of hypoxia. Additionally, after 4 hours of hypoxia, Msx1 increased in conjunction with hypoxia-inducible factor (HIF)-1a suggesting that Msx1 plays a pivotal role during cellular reprogramming. We also investigated changes in cellular behavior. After hypoxia, cells showed increased capacity for differentiation compared to the control. When placed in a neural media, hypoxic muscle cells showed the capability to form neurospheres while the control

cells did not, suggesting that hypoxia conferred multipotent properties onto the treated cells. Overall, our results suggest that hypoxia stimulated the reprogramming of muscle cells into progenitor-like cells, which has implications for its role in tissue regeneration and muscle healing after injury or disease.

Funding source: NIH

Keywords: Hypoxia, Cellular reprogramming, Skeletal muscle

TSC263

INVESTIGATING EXTRAVASATION POTENTIAL OF HUMAN SKELETAL MUSCLE PROGENITOR CELLS IN DMD MOUSE MODELS

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Human pluripotent stem cells (hPSCs) hold great promise in modeling diseases such as Duchenne Muscular Dystrophy (DMD), a genetic muscle wasting disease. hPSCs can be directed to differentiate to skeletal muscle progenitor cells (SMPCs) and delivered to DMD mouse model to regenerate the muscle. However, current delivery strategies do not result in large scale engraftment or ability to reside in the niche cross multiple muscles. Therefore, we have developed an approach to systemically deliver SMPCs to multiple muscles through intra-arterial cell delivery. The prevailing view of systemic delivery holds that cells should cross the endothelial barrier from bloodstream into the muscle to promote regeneration, in a process called extravasation. Here we show that human SMPCs are capable of residing in multiple muscles after their systemic delivery, but their extravasation potential is limited. We have evaluated extravasation potential in two DMD mouse models mdx and mdxD2, a more severe model, and found that cells are routinely found remaining in blood vessels and capillaries. To understand differences in each model we performed single cell RNA sequencing (scRNA-seq) and identified genes that have potential to modulate dynamics of extravasation in each mouse model. Understanding the regulation of SMPCs extravasation will provide insights into approaches to enhance SMPCs regenerative potential across multiple muscles and improve translational potential of stem cell derived therapies to muscle.

Funding source: Qatar National Research Fund-QRLP CIRM (DISC2-08824)

Keywords: Human Skeletal Muscle progenitor Cells, Extravasation, Muscular Dystrophy

TSC268

HIPSC GENERATION AND REDIFFERENTIATION INTO INDUCED MYOGENIC CELLS - DEPENDENT ON THE SOMATIC CELL TYPE OF ORIGIN?

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Human induced pluripotent stem cells (hiPSCs) are a keystone to unrestricted cell numbers which are necessary for gene correction and repopulation of large organs such as skeletal muscle in genetic muscular dystrophies. hiPSCs have been generated from many different cell types and several protocols have been established to differentiate them into muscle cells or dedicated muscle stem cells. However, the biotechnological and therapeutic capabilities of these induced myogenic cells remain unclear. In addition, whether the epigenetic memory passed on to the hiPSCs from the somatic cell type they originated from has an influence on their myogenic differentiation capacity has not yet been examined. Myoblasts and peripheral blood mononuclear cells (PBMCs) represent the most relevant cell types for the generation of autologous hiPSCs for the treatment of muscular disorders. Thus, we compared PBMCs and myoblasts, derived from the same donors (n = 3-5), in regard to their ability to reprogramme into hiPSCs, their transcriptomic characteristics, their ability to redifferentiate into the myogenic lineage and their potential to contribute to myofibre regeneration in an immunocompromised mouse model. We found considerable differences in the ability of myoblasts and PBMCs to reprogramme into hiPSCs with a higher efficiency for myogenic cells. RNA-Sequencing of myoblast and PBMC-derived hiPSCs revealed 122 significantly differentially expressed transcripts that are involved in signalling pathways potentially influencing the differentiation capacities of pluripotent stem cells. However, the in vitro differentiation experiments revealed no significant differences in the number of myogenic cells obtained after transgene-free differentiation of myoblast- and PBMC-derived hiPSCs from the same donor. Intramuscular transplantation of induced myogenic progenitor cells into immunocompromised mice resulted in human myofibre formation for both cell types, but quantification is pending. We conclude that myoblasts are a better source to generate autologous hiPSCs for patients with muscular dystrophies due to their significant higher reprogramming efficiency as compared to PBMCs.

Keywords: Reprogramming from Myoblasts and PBMCs, Transgene-free myogenic differentiation, In vitro quantification + transplantation

TSC272

IS CELL SORTING FOR PURITY NECESSARY IN THE GENERATION OF MESENCHYMAL CELLS FROM HUMAN IPSC FOR CLINICAL APPLICATIONS?

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Human induced pluripotent stem cell (iPSC) derived smooth muscle progenitor cells (pSMCs) display potential for treatment of stress urinary incontinence (SUI), but safety concerns remain. The well-known tumorigenic potential of stem cells requires derivation of a pure lineage-committed cell population, conventionally accomplished by cell sorting. However, cell sorting methods significantly reduce the viable cell yield necessary for clinical therapy. Thus, we sought to compare the purity of unsorted to sorted iPSC-derived pSMCs. Two human embryonic stem cell (ESC) lines and four iPSC lines derived from female patients (ages 18-94) were differentiated on Matrigel or iMatrix-511 with a standardized protocol to induce the desired intermediate CD31+/CD34+ progenitor phenotype. These cells were then divided into three groups: unsorted, single sorted (CD34+), or double sorted (CD31+/CD34+). Magnetic-activated cell sorting (MACS) was used to positively enrich for the respective proteins in the sorted groups. Each group was then cultured and passaged in smooth muscle growth supplement (SMGS) medium to enhance further differentiation into pSMCs. qPCR was performed on the differentiated pSMCs at P3 and P5 to evaluate expression of pluripotency/tumorigenesis-associated genes such as Nanog, Oct-4, TRA-1-60, KLF4, c-Myc, and CD30. Flow cytometry analyses at P4 and P7 were performed to assess cell subpopulations using the following markers: TRA-1-60, SSEA-3, CD30, CD31, CD34, and α-smooth muscle actin (αSMA). With cell passaging in SMGS from P3 to P5, pluripotency gene expression declined to levels equivalent to those in bladder smooth muscle controls and did not differ significantly between the three groups. Flow cytometry data also showed no significant difference in the percentage of cells with pluripotency markers between unsorted and sorted cells, while all groups revealed appropriate differentiation with >90% αSMA expressing cells. All groups showed a similar, drastic decline in pluripotency markers/cells, close to undetectable level, with increasing passage. These data suggest that mechanical sorting during mesenchymal differentiation from pluripotent stem cells does not significantly improve final cell purity compared to cell passage and expansion with differentiation medium.

Funding source: This project was funded by the California Institute for Regenerative Medicine (CIRM) TRAN1-10958, PI B. Chen

Keywords: cell sorting, cell purity, mesenchymal differentiation

NEURAL

TSC277

ASYMMERTRIC INHERITANCE OF ENDOLYSOSOMAL VESICLES IN HUMAN NEURAL STEM CELLS DURING MITOSIS

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Stem cells are defined by both their ability to self-renew, and their ability to produce cells that differentiate to somatic cells. Symmetric and asymmetric cell divisions are conserved strategies by which stem cells can accomplish these two tasks, either giving rise to qualitatively identical, or different daughter cells, respectively. In this study we became interested in the identification of cellular determinants, which are differentially distributed between daughter cells during the cell division of human neural stem cells. As experimental model system we used human pluripotent stem cell-derived long-term self-renewing neuroepithelial stem cells (lt-NES cells) which we maintained in a self-renewing state by exposure to growth factors. We identified intracellular vesicles marked by the proteins LAMP1/2/3 to be asymmetrically distributed during metaphase in some of the dividing cells. These three proteins are known as surface markers of the endolysosomal system and were confirmed to be located at the same subcellular compartment in our model system. Withdrawal of the growth factors from the culture medium increased the occurrence of asymmetrically segregated LAMP-positive vesicles, potentially linking asymmetric mitotic events to the differentiation of lt-NES cells. When investigating potential signaling molecules present in these vesicles, we identified Notch1/2 receptors – well known cellular determinants controlling cell fate decisions of stem cells. Upon exposure of the cells to recombinant Notch ligand, the ligand is internalized by the cells and gets shuffled into LAMP-positive vesicles within 30 minutes, which might be associated with the activation of Notch signaling via γ-secretase cleavage in the acidic environment of the endolysosomal compartment. Our observation might reflect a so far unrecognized endolysosomal sorting mechanism distributing information asymmetrically between daughter cells, thereby directly influencing the fate of the cellular progeny.

Keywords: asymmetric cell division, lysosomes, Notch signaling

TSC278

LRP1 AS A MODULATOR OF CXCR4 IN HIPPOCAMPAL NEUROGENESIS AND NEURODEGENERATION

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Neurodegenerative disease causes the death of 1/3 of senior citizens, with a prevalence projected to triple by 2050. This expected public health crisis will be exacerbated by a lack of disease-modifying treatments. As the most commonly diagnosed neurodegenerative disorder, Alzheimer's disease (AD) is characterized by a pathological loss of memory function due to hippocampal degeneration. Preservation of hippocampal neurogenesis protects from memory loss in AD, suggesting that improved understanding of molecular processes underlying neurogenesis could lead to efficacious therapies. Neural stem cells (NSCs) migrate from the subgranular zone niche to integrate into hippocampal circuitry. The chemokine receptor CXCR4 plays a fundamental role in this migration—loss of CXCR4 activity causes hippocampal memory deficits in mouse models, and CXCR4 polymorphisms were identified as risk factors for several neurodegenerative diseases. Our lab has identified a novel regulator of CXCR4 – low density lipoprotein receptor related protein 1 (LRP1), which is also implicated in AD. LRP1 expression is decreased in AD patients. LRP1 is also a receptor for ApoE4 and amyloid beta, which each play a role in AD pathogenesis. We used a Nestin-Cre inducible mouse model to knockout LRP1 in NSCs of adult mice. We found that LRP1 knockout caused a 10-fold loss of CXCR4 expression and deficits in ischemia-stimulated migration from the subventricular zone. These mice also displayed memory deficits at 8 months of age, suggesting dysregulated hippocampal function. Given this, we hypothesize that LRP1 regulates CXCR4 in the subgranular zone NSCs to enhance hippocampal memory function. Ongoing research is testing our hypothesis via hippocampal functional tests and in vitro trafficking/expression assays. We expect our research to elucidate a previously unknown link between three independently identified effectors of neurodegenerative disease: LRP1, CXCR4, and neurogenesis.

Funding source: Owen's Foundation and Veteran's Administration CDA2

Keywords: Neurodegeneration, Neurogenesis, Memory

TSC283

EXPLORING DIRECT CONVERSION OF HUMAN SOMATIC CELLS INTO INDUCED NEURAL STEM CELLS AS AVENUE FOR NEUROREGENERATION

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With the advent of cell programming, several strategies for disease modeling and cell replacement have become available. While directly converted somatic cell-derived induced neurons largely preserve age-associated traits, induced pluripotent stem cells (iPSCs) and their derivatives represent an embryonic-like stage. We investigated whether age-related cellular properties are also conserved upon direct conversion of adult human peripheral blood cells (PBCs) into induced neural stem cells (iNSCs). Employing Sendai virus (SeV)-mediated expression of SOX2 and cMYC, we generated a bona fide NSC population from PBCs and despite the lack of a pluripotency transit, we found that even iNSCs converted from >85-year-old donors display a remarkable degree of epigenetic rejuvenation and lack of age-associated hallmarks. This might make iNSCs a promising alternative for regenerative applications and a blueprint for somatic cell rejuvenation. Indeed, iNSCs survive neurotransplantation in mice and generate electrophysiologically active neurons as well as glial cells upon engraftment. Furthermore, iNSC-derived neurons undergo synaptic integration into the host brain as shown by rabies virus-based monosynaptic tracing. We became interested in whether this direct conversion approach can be extended to microglia. To address this question, we used iPSC-derived microglia-like cells (iPSdMG). Generated via a developmentally informed and standardized protocol,

these cells express typical microglial markers, and respond to inflammatory stimulation by increasing phagocytotic activity and production of reactive oxygen species. Two weeks after infection of iPSdMG with temperature-sensitive SeV vectors, we observed emerging colonies consisting of neuroepithelial-like-shaped cells expressing NSC markers such as DACH1 and NESTIN. Temporary cultivation at 39°C yielded transgene-free iNSCs, which could be further proliferated for multiple passages and, upon spontaneous differentiation, gave rise to neurons and astrocytes. Considering their epigenetically rejuvenated state, their amenability to in vivo application and the fact that they can be generated from different somatic cell types, iNSCs provide interesting prospects for disease-related research and neuroregeneration.

Funding source: This work is supported by the German Federal Ministry of Education and Research (01EK1603A), the European Union (H2020-SC1-BHC-2018-2020-874758) and from the Innovative Medicines Initiative 2 Joint Undertaking (115976).

Keywords: Direct Conversion, Cellular rejuvenation, Neuroregeneration

TSC291

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF TWO BRAIN ORGANOGENESIS PATHWAYS WITHIN A COLONIAL CHORDATE

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Understanding how the central nervous system develops and the mechanisms by which it regenerates neurons, is a major area of interest in regenerative medicine. Here we characterized central nervous system development in a unique model organism, the colonial chordate Botryllus schlosseri. These marine organisms have two pathways to develop into their adult body, embryogenesis and blastogenesis. Through embryogenesis, a larval stage specific brain is formed for a swimming larva to complete its passage from 'hatching' to settlement on a subtidal surface, after which the larval brain is resorbed. The embryonic phase also develops a second brain, connected with the larval brain, but which persists after metamorphosis; this brain is regenerated during every weekly blastogenic cycle, perhaps analogous with mammalian neurogenesis from persisting central nervous system stem cells. Following metamorphosis, the colony founder individual undergoes asexual reproduction through budding to create genetically identical individuals, a process that continues weekly throughout the life of the colony. During these cycles new neural systems are developed in the colony's buds while the nervous system in the adult individuals are degenerated. By combining transcriptome sequencing with confocal, two-photons, electron microscopy and digital 3D reconstructions, we generated a complete atlas linking gene profile with key organogenesis stages, describing the molecular and morphological signatures associated with brain development and regeneration. Here we will highlight the similarities and differences between the two molecular pathways suggesting different regulation between development and regeneration.

Keywords: embryogenesis, blastogenesis, regeneration

TSC300

IMPROVED MIDBRAIN DOPAMINE NEURON DIFFERENTIATION BASED ON A SPOTTING METHOD

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The progressive death of midbrain dopamine neurons (mDANs) in the substantia nigra pars compacta is the principal cause of motor dysfunction of Parkinson's disease (PD). Cell replacement therapy using human ESC- or iPSC-derived cells relies on finely tuned differentiation protocols to yield healthy functional mDANs that can restore motor function. We sought to improve the efficiency of the mDAN differentiation protocol by introducing a novel "spotting" method that divides the conventional monolayer into smaller isolated portions, so as to limit cell attachment to designated areas in the culture dish, by pre-coating circular areas ("spots") of ~5 mm diameter using 10 µl of Matrigel on cross points of a 2 x 2 cm grid to maintain isolation between spots. This approach resulted in significantly decreased cell loss and in the production of healthier mDA cells, with markedly fewer dead or dying cells. In contrast, traditional monolayer culture conditions becomes significantly more acidic regardless of frequency of media change, likely contributing to a much higher proportion of dead or dying cells.

In vitro differentiation of hESCs and hiPSCs using this spotting method generated fully functional mDA cells, as determined by gene expression analyses, electrophysiological properties, dopamine production, and behavioral recovery in rodent models of PD following transplantation. We successfully scaled up this protocol at a GMP facility to produce clinically relevant quantities of high quality mDA cells.

Funding source: This work was supported by NIH grants (NS070577, NS084869, and OD024622) and NRF Grants 2017R1A2B4008456, as well as the Parkinson's Cell Therapy Research Fund at McLean Hospital and Massachusetts General Hospital.

Keywords: Neuroscience, Stem cell; Dopamine neuron, Parkinson's disease

TSC303

HUMAN IPS-DERIVED INTERNEURONS ENHANCE FUNCTIONAL RECOVERY AFTER CORTICAL STROKE

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Stroke is the leading cause of adult disability. Around 800,000 individuals experience this disease for the first time, and few completely recover. Therapy and effective treatment options for individuals' post-stroke is limited and difficult to find. A natural tissue repair process does occur post-stroke in the peri-infarct tissue, but it falls short of allowing a more complete remapping and regaining of sensory or motor functions. The use of transplanted stem cells to promote neural repair has long been hypothesized but is limited by poor progenitor survival and differentiation in vivo. Recently, the advent of a bioengineered hyaluronic acid (HA)-based self-polymerizing hydrogel that can be enriched with vascular endothelial growth factor (VEGF) has allowed for the survival and accurate differentiation of stem cells in the stroke brain. Previous cell-based treatments with human-derived neural progenitor cells (hNPCs), delivered within hydrogel, displayed a feasible approach to treat stroke. Interneuron progenitors may present an improvement on past work due to their diversity and integrative properties. The human induced- pluripotent stem cells derived into interneurons (hiPS-3i), in combination with cV-HA, will be transplanted into the stroke core at two different time points, the sub-acute (1-week) and the chronic (1-month) stages post-stroke. We report that post-transplantation of hiPS-3i cells encapsulated in hydrogel + VEGF presents a significant increase in motor function, as well as a regrowth of axonal projection and decrease in cortical loss post- transplantation. These results were both seen independently of whether the transplantation of cells was done at the 1-week or 1-month time points post-stroke. Overall results provide promising options for stem cell-based therapy for stroke patients.

Funding source: Supported by CIRM DISC1-08723

Keywords: Stem Cell Based Therapy, Interneurons, Human induced pluripotent stem cells

TSC313

IMMOBILIZED NRG1-FC ENHANCES DIFFERENTIATION OF HUMAN EPIDERMAL NEURAL CREST TO SCHWANN CELLS AND PROMOTES RADIAL SORTING

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Schwann Cell (SC) differentiation from Neural Crest (NC) Stem Cells plays a pivotal role in the myelination of the peripheral nervous system during embryonic development. Recently, we identified an easily accessible source of multipotent SOX10+/PAX3+/FOXD3+ NC cells isolated from human inter-follicular Keratinocytes (KC) isolated from glabrous neonatal foreskin. Transcriptomic analysis utilizing single cell RNA-seq, showed upregulation of pre-SC genes indicative of the propensity of these cells to differentiate into myelinating SC. In order to recapitulate the in vivo SC differentiation signal topology, radial sorting and myelination, we employed differentiation inducing ligands that were immobilized on a hydrophobic surface. Interestingly, immobilized NRG1 induced sustained activation of pathways downstream of the ERBb2/3, as evidenced by phosphorylation of ERK1/2, MAP38 and AKT, which, in turn, lead to the upregulation of mature SC markers. Indeed, after two weeks of culture on NRG1 surface, mature SC markers PLP1, PMP22, KROX20 and MPZ were significantly. q-RT-PCR verified the transcriptional upregulation of SC specific genes and downregulation of dedifferentiation genes (c-Jun, CDK1 and FGF2). In order to demonstrate SC function, we attempted to imitate the in vivo process of radial sorting. To this end, we used fibrous biomaterials with immobilized bioactive signals, which led to significantly increased mRNA levels of SC genes that are known to participate in cytoskeletal reorganization (α7β1, Proffilin, N-WASp, CDC42); as well as improved alignment on the fibers than undifferentiated NC. Finally, we employed an assay based on rat DRG neurons supplemented with NC-derived SC and showed decreased proliferation and increased alignment along extended neurons. Our current work focuses on the in vitro myelination of fibrous biomaterials or rat DRG neurons as well as transplantation of SC in the corpus callosum of the myelin deficient shiverer (shi/shi) mouse model. Given the accessibility of human skin, our SC differentiated from epidermal NC represent a potentially useful source of autologous multipotent stem cells for treatment of demyelinating diseases or spinal cord injuries, as well as potential in vitro demyelination assays that can be used for drug development.

Funding source: This work was supported by grants from the National Institutes of Health R01 EB023114 (S.T.A.) and the New York Stem Cell Science NYSTEM (Contract #C30290GG, S.T.A.).

Keywords: Schwann cells, Neural Crest, Radial sorting

NEW TECHNOLOGIES

TSC323

IMAGE PROCESSING PIPELINE FOR AUTOMATED HIPSC CULTURE AND HIGH THROUGHPUT 3D LIVE CELL MICROSCOPY PIPELINE

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The Allen Institute for Cell Science combines genomics, gene-editing, and 3D live cell imaging of the cell organization to identify and define the various normal and pathological states in which stem cells reside and to unlock the mechanisms by which the cells transition among these states. We do this using a collection of human induced pluripotent stem cell (hiPSC) lines expressing GFP tagged to proteins identifying major cellular organelles and structures (The Allen Cell Collection at Allencell.org). To generate imaging plates with uniform and reproducible cellular confluency and morphology, we developed an automated hiPSC culture procedure using the Hamilton Robotics Microlab Star platform. Using the brightfield overview images collected with Celigo imaging cytometer, we developed an image-based colony segmentation pipeline with CellProfiler to measure and track colony growth characteristics on 6- and 96- well plates. The colony area measurements from 6-well plates are used to calculate well confluency and resuspension volume of media for hiPSC passaging, eliminating the need for manual counting. The image processing pipeline also provides daily ranking of wells from 96-well plates for 3D live cell microscopy measurements. Cell confluency, number of colonies, and colony size and distribution are among the metrics we use to rank wells. These measurements are also used to track trends, identify potential issues with the platform or cell lines, and exclude plates not meeting quality control criteria. The top ranked plates are imaged on spinning disk confocal microscopes to visualize the 3D organization and dynamics of cellular structures under diverse but defined conditions, creating a holistic picture of the changes in cellular organization. These data are collected and processes using highly standardized, quality controlled and automated 3D live cell imaging. This imaging pipeline includes the following steps: an automated selection of areas within colonies, followed by 3D images captured from this position list, then 3D segmentation of individual cells, and uploads to a data storage management system. These large and standardized datasets are made publicly available for visualization and download using The Cell Feature Explorer at Allencell.org.

Keywords: Automated cell culture, microscopy pipeline, hiPSC

TSC327

EVALUATION OF A NOVEL COMPOSITE OF PLATINUM-COATED CARBON NANOTUBES ON ADULT MESENCHYMAL STEM CELLS AND CANCER STEM CELLS

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Functional polymers and multiwalled carbon nanotubes (MWCNT) are receiving a great deal of attention for cancer treatment, in particular cancer stem cells (CSCs), which may present the main cause for cancer recurrence or relapse. Thus, finding a therapeutic target for CSCs is of great importance. We hypothesize that novel-formulated platinum nanoparticles (Pt-NPs) supported on polybenzimidazole (PBI)-functionalized MWCNT will target CSCs more effectively and help prevent cancer recurrence. MWCNT/PBI/Pt-NPs composite was synthesized and characterized using scanning-transmission electron microscope, thermal gravimetric analysis, and X-ray photoelectron spectroscopy. MWCNT/PBI/Pt-NPs was incubated with bone marrow stem cells (BM-MSCs) and breast CSCs (CD44high CD24low). Evaluation of the biological properties of the composite was based on proliferation rate, viability assays, and gene expression. Our results showed that MWCNT/PBI/Pt-NPs was successfully synthesized and this was confirmed by different spectroscopic analyses. The obtained Pt-NPs were homogenously distributed on the surface of MWCNT/PBI composite. Incubation with MWCNT/PBI/Pt-NPs for 48 hours with CSCs resulted in a dramatic decrease in their proliferation ($p<0.001$). However, MWCNT/PBI/Pt-NPs had no significant effect on BMSCs proliferation ($p=0.077$). These data were contrary to the effect of cisplatin, which resulted in a significant decrease in the proliferation of BMSCs ($p=0.027$). The expression of apoptosis-related markers TP53 and BAX was significantly higher in cisplatin-treated CSCs compared to those treated with MWCNT/PBI/Pt ($P<0.0001$). Cells treated with MWCNT/PBI/Pt showed significantly lower expression of epithelial-mesenchymal transition markers (EMT) and downregulated SNAIL, SLUG and PARP compared to cisplatin-treated cells. Furthermore, quantitative expression of CDk4/6 mRNA revealed that MWCNT/PBI/Pt induced cell cycle arrest in CSCs. In summary, MWCNT/PBI/Pt exhibited specific cytotoxic effect on breast CSCs but not on adult stem cells. Further investigation is needed to test the possible synergistic effect when combining chemotherapy with MWCNT/PBI/Pt.

Funding source: Science and Technology Development Fund 5300

Keywords: Breast cancer, Platinum nanoparticles, Mesenchymal stem cells

TSC338

METABOLITES CAN REGULATE MESENCHYMAL STEM CELL BEHAVIOR IN A SIMILAR TREND TO THAT UNDER HYPOXIC CONDITIONS

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Stem cell therapy has long been considered a promising mode of treatment for many incurable diseases. Human mesenchymal stem cells (hMSCs) have provided the most promising results to date for regenerative medicine. Nevertheless, due to several obstacles such as difficulty in sourcing and characterizing hMSCs, they remain largely unavailable for clinical use. The signaling requirements for maintaining stem cell function have been studied widely, but little is known about how metabolism contributes to stem cell function. hMSCs have been shown to promote therapeutic efficacy in hypoxic conditions through metabolic conversion. According to published studies, certain metabolites are able to convert stem cell metabolism from oxidative phosphorylation to glycolysis. In this study, we selected several metabolites (fructose-1,6-bisphosphate (FBP), Phosphoenolpyruvic acid (PEP) and sodium oxalate (OXA)) to examine the relation between metabolites and stem cell functions. In addition, we investigated the ability of selected metabolites to induce rapid expansion of this cell population. Our results indicate that selected metabolites stimulate stem cell proliferation by induce glycolytic metabolism via AKT/STAT signaling.

Keywords: Mesenchymal stem cell, Hypoxic condition, Stem cell metabolism

TSC342

LOCALIZED IMMUNE TOLERANCE FROM FASL-FUNCTIONALIZED PLG SCAFFOLDS FACILITATES ENGRAFTMENT AND DIFFERENTIATION OF ISLET ORGANOIDS

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Type I diabetes (T1D) is a chronic autoimmune disease that affects an estimated 1.53M Americans. Exogenous insulin treatment, which is the standard of care for T1D patients, could negatively affect the quality of life due to chronic complications. Allogeneic islet transplantation has proven effective in preventing severe hypoglycemic events but with at least 3 critical barriers to the progress in the field of clinical islet transplantation; i) a limited supply of donor islets, ii) liver transplant site could compromise immediate post-graft islet engraftment and long-term survival, and iii) immune rejection, precipitated by both innate and adaptive immune responses. This study overcame these 3 obstacles by transplanting human pluripotent stem cell (hPSC)-derived islet organoids into the epididymal fat pad onto the engineered microporous polymer scaffolds (PLGA) that positionally attached streptavidin-Fas Ligand (SA-FasL). The localized immunomodulation with SA-FasL converted scaffolds into an immune-privileged site that supports long-term survival of islet hPSC-derived organoid without chronic immunosuppression. Stage-4 islets organoids were seeded on the PLGA scaffold attached with SA-FasL and transplanted to the epididymal fat pad of the streptozotocin-induced diabetic mice. The engineered PLGA scaffold with SA-FasL enhanced engraftment at early stages through its regulation of macrophage phagocytic function and DC cross-talk with adaptive immunity. SA-FasL further augmented the engraftment/survival of islets organoids by eliminating various innate immune cells with upregulated Fas expression in response to inflammation. Downregulation of proinflammatory and upregulation of anti-inflammatory cytokines/chemokines were observed and a more rapid restoration of euglycemia was achieved with a greater cell survival combined with an enhanced maturation due to a less inflammatory microenvironment that can skew differentiation. The application of immunomodulation to the hPSC-derived islet organoids has the potential to revolutionize the treatment of T1D by avoiding immunosuppression and overcoming the shortage of available donor islets. This concept will also improve clinical practice as scaffolds as an immune-privileged extrahepatic site to stabilize blood glucose levels.

Keywords: Islets organoid, Biomaterial, Immune tolerance

TSC352

HUMAN STEM CELLS IN REGENERATIVE MEDICINE: WHEN NATURAL MOLECULES JOIN NANOTECHNOLOGIES

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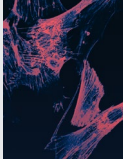
Stem cells are involved in maintaining tissue homeostasis providing self-renewal and new cellular elements. Skin repair in adulthood is tricky, because of the aging process and the continuous exposure to dangerous agents. In adult skin basal layer, the number of stem cells is very low, and their use in regenerative medicine is strictly related to the optimum isolation methods, and culturing conditions. Plants within the Mediterranean area contain molecules exerting biological activities, commonly used for various diseases in several formulations. It is well know that Myrtus Communis, an evergreen bush rich in phytochemicals, shows antioxidant, antiaging, and anti-inflammatory proprieties. In the present study, we aimed at investigating the effect of these polyphenols from myrtle



extracts, combined with polycaprolactone nanofiber (PCL) on skin stem cells features. In particular, stem cell pluripotency and aging under stressing conditions in the presence or absence of the combined extracts-nanofibers have been evaluated. The concerted analyses of the stemness-related transcripts OCT4, SOX2, and NANOG, the senescence-associated p16 and p19 genes and beta-galactosidase activity, the cell cycle regulating genes p53, p21, and the human telomerase gene TERT revealed that our products can protect skin stem cells from stressing conditions, and premature aging. Our results suggest the possibility to use the combination of myrtle extracts and nanofibers to obtain novel products with bioactive release suitable for skin regeneration and maintenance.

Keywords: Nanotechnologies, Tissue regeneration, Gene expression

TSC358

MONITORING GENOMIC STABILITY IN THE CHROMOSOME 20 BCL2L1 REGION BY DDPCR

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Copy number increases involving chromosome 20q with gain of the gene BCL2L1 are a prevalent form of genomic instability in hPSCs. In addition to large aneuploidies, findings in this region often include microamplifications that are too small to detect by G-banded karyotyping. Here we discuss risk factors inherent to gene editing and describe an optimized strategy for detecting BCL2L1 copy number increases in hPSC cultures using duplexed droplet digital PCR (ddPCR). This procedure generates same-day screening results for 1 to 96 samples, providing a convenient option for screening hPSC cultures before and after gene editing.

Keywords: genomic stability, hPSC quality control, gene editing quality control

TSC361

IMPROVED CELLULAR MAINTENANCE OF HUMAN PLURIPOTENT STEM CELLS IN A SERUM-FREE, XENO-FREE CULTURE SYSTEM FOR CLINICAL APPLICATIONS

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Pluripotent stem cell (PSC) culture has advanced significantly since the use of feeder layers, yet each innovation poses new challenges as pluripotency and stability may become compromised. While there are many media and substrate options for PSC culture, most contain variable, animal-derived components. As stem cell research evolves, the creation of better performing, more defined systems for the derivation, expansion, and application of clinical quality PSCs is required to avoid a bottleneck during clinical translation. More specifically, in order to navigate this regulatory environment, it is recommended that cells are cultured under xeno-free (XF) conditions. Utilizing two commercially available XF media and substrate combinations, episomally-derived human induced PSCs were cultured and compared to cells cultured in conventional PSC media on an animal-derived substrate. Cells from all conditions were then transitioned to the most supportive XF media while examining the cells' ability to adapt from traditional, poorly defined, animal-containing media to a cleaner, more clinically applicable condition. Throughout each step, all cultures were analyzed for cellular pluripotency and stability. Cells cultured in the serum-free (SF) NutriStem® hPSC XF Medium seeded on vitronectin exhibit, via flow cytometry, high levels of pluripotency markers TRA-1-60 and SSEA-4, as well as low levels of SSEA-1 while cells in the alternative XF culture system were found to exhibit lower levels of TRA-1-60 and SSEA-4 with higher expression of SSEA-1. Additionally, while ICC staining for Nanog and TRA-1-60 further confirmed the pluripotency of NutriStem hPSC cultures, cell colonies in the alternative XF system displayed atypical morphology and reduced marker expression intensity. Overall, SF, XF culture methods exist that can effortlessly maintain pluripotency and cellular health, but not all systems are made equally. Nevertheless, transitions from conventional culture conditions to clinically relevant ones can be achieved with minor adjustments facilitating long-term success, and these more streamlined culture systems can easily support the maintenance of key hPSC qualities essential for downstream differentiation and future clinical application.

Keywords: Pluripotent Stem Cells, Cell Therapy and Clinical Translation, Current Good Manufacturing Practices

PLACENTA AND UMBILICAL CORD DERIVED CELLS

TSC370

MANUFACTURE OF CLINICAL GRADE PLACENTA-DERIVED MESENCHYMAL STEM/ STROMAL CELLS FOR IN UTERO FETAL REPAIR OF MYELOMENINGOCELE

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Myelomeningocele (Spina Bifida, SB) is a congenital birth defect leading to life-time paralysis of the patient. The current standard of care is surgical intervention during the second trimester of pregnancy by closure of the defect using skin or extracellular matrix (ECM). To augment this fetal repair, we sought to apply early gestation placenta-derived mesenchymal stem/stromal cells (PMSCs) procured from the chorionic villus of donor placenta. Our pre-clinical studies demonstrated that PMSCs are neuroprotective, secrete neurotrophic growth factors (brain-derived growth factor (BDNF) and hepatocyte growth factor (HGF)), and rescue apoptotic neurons in vitro. In an ovine model of SB, PMSCs seeded on a small intestinal mucosa ECM (SIS-ECM) and applied onto a surgically created defect were found to rescue neurons and significantly improve ambulation of fetal lambs. For clinical applications of PMSCs we sought to manufacture a clinical grade cell bank in a current Good Manufacturing Practice Facility. Seventeen consented donors were initially screened for infectious diseases. PMSCs generated from eight donor placentas were expanded and cryopreserved as seed banks. Of these eight, only five passed the criteria for PMSC growth rate and viability and were subsequently expanded to product banks. After in-process testing for growth factor secretion and an in vitro neuroprotection assay, the cells were cryopreserved as product banks. All five product banks were then RNA sequenced and screened for MSC phenotype, pluripotency, karyotype, growth factor secretion and neuroprotective capability. They also underwent testing for viability, sterility, mycoplasma and adventitious viruses. Two of the five product banks were identical in all these assessments and outperformed the other three cell banks. Of these two PMSC banks, one donor was CMV IgG positive, suggesting an infection prior to tissue collection. Hence, we chose the other cell bank (generated from a CMV IgG negative donor) for clinical use. These cells are currently undergoing further testing for safety in a murine model, as well as safety and efficacy testing in the ovine SB model. Once these studies are completed, we will submit an IND application, paving the way for clinical application of the PMSC-ECM product to augment in utero repair of myelomeningocele.

Funding source: California Institute for Regenerative Medicine (CIRM), Grant Number-CLIN1-11404

Keywords: Spina Bifida, in-utero fetal repair, clinical grade mesenchymal stem/stromal cells

SATURDAY, JUNE 27, 2020

POSTER SESSION V
05:00 – 07:00

Theme: Cellular Identity

ADIPOSE AND CONNECTIVE TISSUE

CI328

ALTERNATIVE SPLICING IN MESENCHYMAL STEM CELL DIFFERENTIATION

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The differentiation and maturation of mesenchymal stem cells (MSCs) to mesodermal and other lineages are known to be controlled by various extrinsic and intrinsic signals. The dysregulation of the MSC differentiation balance has been linked to several pathophysiological conditions, including obesity and osteoporosis. Previous research of the molecular mechanisms governing MSC differentiation has mostly focused on transcriptional regulation. However, recent findings are revealing the underrated role of alternative splicing (AS) in MSC differentiation and functions. In this review, we discuss recent progress in elucidating the regulatory roles of AS in MSC differentiation. We catalogue and highlight the key AS events that modulate MSC differentiation to major osteocytes, chondrocytes, and adipocytes, and discuss the regulatory mechanisms by which AS is regulated.

Keywords: Mesenchymal stem cells, Alternative splicing, Mesenchymal stem cell differentiation

CARDIAC

CI107

IMPACT OF CELL CULTURE FACTORS IN CELL FATE DETERMINATION DURING HESC DIFFERENTIATION

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During embryogenesis, various lineages and cell types emerge to form tissues, organs and finally an organism. Cell fate determination is greatly influenced by the local environmental factors, including cell density, environmental pH, extracellular matrix and growth factors. We use human embryonic stem cells (hESCs) to understand the key local factors involved in embryogenesis. We report here that local factors play essential roles in mesodermal cell fate determination. These factors from stem cell niche not only influence mesodermal commitment, but also the subsequent differentiation toward cardiac and endothelial cell fates. These factors regulate both signal transduction and cellular metabolism. Our findings demonstrate the dynamics between stem cells and their niche, and reveal the relevant signaling circuits that are critical for cell fate determination.

Keywords: Stem cell niche, Cardiac differentiation, Metabolism

CI113

AN ADHESION GPCR, LATROPHILIN-2 INDUCES CARDIAC DIFFERENTIATION THROUGH CDK5, CONTRIBUTING TO REPAIR AFTER MYOCARDIAL INFARCTION

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Recently, there has been an increasing demand for cardiomyocyte (CMC) for research into cardiovascular disease and the toxicology of drug metabolites. We identified a G protein-coupled receptor, latrophilin-2 (Lphn2), as a functional marker for cardiomyogenic lineage cells during both in vitro differentiation from ESCs/iPSCs and in vivo heart development. Lphn2 was selectively expressed on CPCs and CMCs during the differentiation of mouse and human PSCs, and cell sorting with an anti-Lphn2 antibody promoted the isolation of populations highly enriched in CPCs and CMCs. Lphn2 knock-down iPSCs did not express cardiac genes. Heterozygous Lphn2-KO mice (Lphn2+/–) were alive and fertile. Homozygous Lphn2–/– mice were embryonically lethal and showed underdevelopment of the ventricular myocardium. Interestingly, the hearts of Lphn2–/– embryos revealed the disrupted conotruncal septation of ventricles at E13.5. Lphn2 induces the expression of the central transcription factors Gata4, Nkx2.5, and Tbx5 in mouse heart development. The amino acid sequence of Lphn2 is highly conserved across humans and mice (95.8% identical). For the purpose of cardiac regeneration, we transplanted PSC-derived Lphn2+ cells into the infarcted heart. Transplantation of Lphn2+ cells significantly reduced the fibrosis area and length compared with those in the PBS-injected control group and Lphn2– cells. Echocardiography revealed significantly small LV dimensions at the systole and diastole, and the LV systolic function was higher in the Lphn2+ cell group than in the control and Lphn2– cell groups. To investigate the molecular mechanism underlying the induction of cardiac differentiation by Lphn2, we used the Phospho Explorer Antibody Array, which encompasses nearly all known signaling pathways. Lphn2-dependent phosphorylation was strongest for CDK5 at Tyr15. We identified CDK5, Src, and P38MAPK as key downstream molecules of Lphn2. Our findings provide a valuable tool for identifying CPCs and CMCs differentiated from PSCs, as well as revealing novel insights into cardiac development. Furthermore, the results of this study could be of potential use in regenerative cell therapy for the restoration of cardiac muscle.

Funding source: This study was supported by “Strategic Center of Cell and Bio Therapy for Heart, Diabetes & Cancer” (HI17C2085) through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Republic of Korea.

Keywords: Cardiac Differentiation, G protein-coupled receptor, Repair

CI326

GENETIC MATERIAL AND SCAFFOLDS TOWARDS CARDIAC DIRECT REPROGRAMMING

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Direct reprogramming of cardiac fibroblast (CFs) to induced cardiomyocytes (iCMs) is a promising approach for cardiac regeneration. However, its potential is limited due to the low yield of reprogramming cells and the underlying mechanism. We previously, demonstrated that delivery of muscle-specific miRNA using nanocarriers significantly increased the percentage and the total number of cardiac marker positive iCMs. Furthermore, in another study, we showed that Porous PLLA scaffold is an excellent substrate for the cell adhesion and proliferation of the cardiac fibroblast. We observed that regardless of the surface modifications, the metabolic rate of the cells was significantly high in comparison with conventional cell culture and the proteome profiling revealed that the scaffold substrate not only served as an anchorage platform for cells but also showed a significant expression of the cellular proteome with many crucial proteins responsible for cardiac fibroblast growth, proliferation and differentiation. Herein, we fabricated electrospun bioactive genetic polymeric nanocomposite scaffolds with surface-immobilized polymeric nanocarriers encapsulated with dual

muscle-specific miRNA to mimic the naive intramyocardial environment. The electrospun scaffolds serve as an ideal in-situ delivery system which has potential in regulating the cell fate but also helps in the mimic the intramyocardial environment. The combinational approach of scaffold mediated delivery system of genetic factor can significantly enhance reprogramming efficiency. Henceforth, this study can demonstrate the importance of genetic regulators and extracellular matrix in dictating cell fate.

Funding source: Priyadharshni Muniyandi would like to gratefully acknowledge the support by Ministry of Education, culture, sports, science and technology (MEXT), Japan for the financial support during her research from 2017.

Keywords: Cardiac Direct reprogramming, Genetic reprogramming, miRNA, Electrospinning, Porous polymeric fibers

EARLY EMBRYO

CI124

RECONSTRUCTING GENE REGULATORY NETWORKS DURING IPS CELL REPROGRAMMING AND X CHROMOSOME REACTIVATION

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X chromosome reactivation (XCR) represents a paradigm to study epigenetic regulation and the reversal of chromatin silencing, although how it is linked to the pluripotency gene regulatory network, pluripotency transcription factors (TFs) and chromatin processes remains largely unexplained. Several pluripotency TFs have been linked to XCR through binding to the long non-coding RNA gene Xist, but whether other regulatory elements are involved is unclear. We show that reprogramming to iPS cells induces acquisition of chromatin accessibility at specific sites on the inactive X chromosome which is subsequently propagated to other regulatory elements including enhancers and promoters. For distinct categories of chromatin regions that become accessible at different times, different sets of TF motifs are enriched, including the pluripotency TF NANOG, supporting the link between pluripotency TFs in XCR. To further explore the relationship between pluripotency TFs and XCR, we construct the gene regulatory networks active during iPS cell reprogramming using single-cell RNA sequencing and computational analyses. We reveal gene targets of 311 TFs expressed across reprogramming to iPS cells. Using this single-cell regulatory atlas, we show that X-linked genes are preferentially targeted by several pluripotency TFs. Our results suggest a new model where XCR may be coordinated by direct targeting of regulatory elements on the X chromosome by pluripotency TFs, concomitant with step-wise acquisition of chromatin accessibility. Altogether, our results demonstrate how gradual acquisition of a new gene regulatory network during reprogramming of cellular identity is linked with dynamic induction of chromatin accessibility and overcomes stable chromatin silencing on the inactive X chromosome.

Keywords: Chromatin, Cellular reprogramming, Gene regulatory networks

CI128

NONSENSE DECAY LINKS TRANSLATION REGULATION TO CELL FATE CONTROL

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Cell fate changes during embryonic development require the irreversible reshaping of transcription networks to establish and stabilise lineage identity. The cell fate transition from naïve to formative pluripotency in mouse ES cells provides an experimentally tractable model to dissect key principles of cell fate change. The exit from naïve pluripotency is executed within 24 hours after withdrawal of self-renewal cues. Such a rapid cell fate change requires mechanisms to ensure extinction of transcription programmes. Regulation solely at the enhancer level cannot provide sufficiently rapid kinetics. Indeed, recently performed genome wide screens for exit factors indicates an involvement of several RNA- binding proteins in the exit from naïve pluripotency. Top ranking in several screens are components of the nonsense mediated mRNA decay (NMD) machinery. NMD tags target mRNAs for endonucleolytic (through Smg6) or exonucleolytic (through a Smg5 / Smg7 heterodimer) degradation. We find that disruption of Smg5, Smg6 or Smg7 leads to variable degrees of differentiation delays, where Smg5 depletion produces the strongest and Smg7 deficiency leads to a robust but the weakest phenotype. mRNA profiling indicates a near identical set of genes deregulated in all three KOs, indicating strong overlaps in target mRNAs. Interestingly, mRNA deregulation follows the same graded pattern as the phenotype (Smg5>Smg6>Smg7). This indicates that Smg5 and Smg7 can, in contrast to the proposed mechanistic mode of action of NMD, exhibit heterodimer independent functions. Partial redundancy between NMD factors is illustrated by synthetic genetic effects upon NMD factor double depletion. Intersection with transcriptome wide half-life measurements results in a list of potential direct targets for NMD. Among these we identified a translation initiation factor. Indeed, NMD KO ESCs show increased translation levels and increased protein levels of naive TFs, like Esrrb. Co-depletion of the translation factor with Smg proteins leads to rescue of the differentiation delay and overexpression leads to differentiation defects in wild-type ES

cells. Our results show a link between mRNA stability regulation and translational control that regulates cell identity decisions at the exit from naïve pluripotency.

Keywords: cell fate, nonsense mediated mRNA decay, exit from naive pluripotency

CI135

MIXL1 ACTIVATES ANTERIOR PRIMITIVE STREAK MARKERS AND PLAYS A ROLE IN GERM LAYER SELECTION IN A MOUSE EPIBLAST STEM CELL MODEL OF GASTRULATION

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During mouse gastrulation, the transcription factor MIXL1 is vital for mesoderm and definitive endoderm germ layer specification. Mouse Mixl1 knock-out models fail to progress past gastrulation while cells without a functional Mixl1 gene do not contribute to definitive endoderm lineages in mouse chimera studies. Previous attempts at determining the role of Mixl1 using mouse embryonic stem cells showed that activation of Mixl1 resulted in an increased efficacy of mesoderm and endoderm formation. More recently, within mouse epiblast stem cells (mEpiSC) that have undergone undirected differentiation, an endodermal cell fate appeared to be a correlated with the timing and intensity of Mixl1 expression. However, a genome-wide assay to identify Mixl1 targets has never been completed and it is unknown how Mixl1 plays a dual role in both mesoderm and endoderm specification. Using a novel model of Mixl1-timed activation within mEpiSC, we identified that indeed early induction of Mixl1 during undirected differentiation results in the immediate upregulation of anterior primitive streak markers as well as the upregulation of endoderm markers by the end of differentiation. To elucidate the functional role of Mixl1 during this narrow window, we performed bulk ChIP-seq, RNA-seq and ATAC-seq studies of differentiating cells with an early activation of Mixl1. Mixl1 was found to act primarily as a transcriptional activator on genes that are implicated in mesendoderm formation (e.g. Mesp1, Lhx1, Gsc and Cer1) and repression of ectoderm lineage genes via an evolutionarily conserved double ATTA motif. Furthermore, the induction of Mixl1 results in an increase in the accessibility of mesendodermal enhancers and regions harbouring double ATTA motifs, and a decrease in that of ectodermal enhancers and regions harbouring motifs for POU and SOX transcription factors. Taken together, these results suggest that the timing of Mixl1 expression influences germ layer specification during gastrulation by direct transcriptional activation and downstream chromatin remodelling that makes lineage specific enhancers less accessible.

Funding source: This research was supported (partially or fully) by the Australian Government through the Australian Research Council's Discovery Projects funding scheme (project DPDP160103651).

Keywords: Gastrulation, Epigenetics, Mesendoderm

CI156

IDENTIFYING NOVEL REGULATORS OF HUMAN PLURIPOTENCY AND EMBRYOGENESIS

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The transcriptional regulatory circuitry underlying human pluripotency is still unclear. Bona fide pluripotency exists in the preimplantation epiblast, which gives rise to embryonic tissues and hESCs. However, conventional hESCs represent a state of "primed" pluripotency. "Naïve" hESCs, which are more transcriptionally and epigenetically akin to the human epiblast, have been developed. Recent single-cell transcriptome analyses of human embryos have enabled us to identify novel transcription factors, including the zinc-finger protein KLF17, that are enriched in the human epiblast and activated in naïve hESCs, but switched off during standard hESC derivation. We are investigating a putative role for KLF17 in regulating human pluripotency. Using ectopic expression of KLF17 in conventional hESCs, we have shown KLF17 to be sufficient to upregulate other naïve-specific genes, including DNMT3L and REX1/ZFP42. That KLF17 alone activates a more naïve profile in primed culture conditions is suggestive of a functional role. However, using CRISPR/Cas9-mediated knockout of KLF17 prior to resetting to naïve pluripotency, we have observed that KLF17 is not required for naïve pluripotency in vitro, as KLF17-knockout cells survive for at least 10 passages. We are investigating the transcriptional profile of wild-type and KLF17-null naïve hESCs to identify any genes that are misexpressed upon KLF17 loss-of-function. Through these studies, we hope to elucidate any role of KLF17 in naïve pluripotency in vitro.

Keywords: Human embryonic stem cells, Naive pluripotency, Transcriptional regulation

CI158

HYPERDYNAMIC SILENCING: H3.3 TURNOVER SET ENDOGENOUS RETROVIRAL EXPRESSION LEVELS

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Mouse embryonic stem cells can silence retroviral elements by the recruitment of the Trim28 and the histone variant H3. The accumulation and eviction of H3.3 from the nucleosome is tightly related to transcription, but the dynamic state of H3.3 on ERVs is not yet characterized. Here we show that neither H3.3 nor Trim28 enrichment are regulating ERVs expression directly, but rather the rate of H3.3 turnover. Using our 'Time-ChIP' protocol, we measured the accumulation rate of H3.3 and examined the chromatin composition of the 20 most dynamic ERV elements. These hyperdynamic ERVs are significantly enriched with H3K9me3 and strictly silenced. Yet, the enrichment of Trim28 and ESET is high in all H3.3 enriched ERVs and is not affected by turnover-rate, which supports the notion that Trim28 is responsible for H3.3 deposition on these elements. Even though ESET is enriched on all H3.3 marked ERVs, ESET KO has a significant and specific effect on the fast turnover group, which becomes upregulated. Thus, on those specific ERVs, the silencing is a dynamic event that requires regional regulation of H3.3 turnover on silenced ERVs. However, whether the high turnover is the cause or the effect of the silencing is yet to be answered.

Funding source: Israel Science Foundation (ISF) 761/17

Keywords: ENDOGENOUS RETROVIRUSES;, H3.3 turnover, epigenetics

CI159

LIS1 RISC EPIGENETIC MODES TO RESOLVE LINEAGE COMMITMENT IN EARLY MAMMALIAN DEVELOPMENT

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LIS1 mutations and deletions have been associated with lissencephaly; a condition wherein the cerebral cortex of the patients assumes a smooth shape. The LIS1 protein is involved in several key functions including cell proliferation and neuronal migration, and is involved in regulation of the molecular motor cytoplasmic dynein and the cytoskeleton. Increase in the dosage of the LIS1 gene also causes mild brain malformations and developmental delay. During early development, following embryonic day 3.5, regulation of RNA at the transcriptional and post transcriptional levels plays a pivotal role in the regulation of pluripotency and differentiation. Lis1 knockout mice are early embryonic lethal and the role of this protein during early development together with its function in the nucleus still remains to be elusive. Immunostaining of mouse wild type blastocysts affirms that LIS1 co-localizes predominantly in inner cell mass cells. Here, in this study, LIS1 is detected in the nucleus of mouse embryonic stem cells in association with chromatin embedded proteins, and most notably, chromatin modifiers, the RISC complex and splicing factors. Genome wide studies and proteomics using Lis1 mutant mouse ES cells (mESCs) showed that LIS1 together with Argonaute factors physically associate with splicing and transcription factors. Further, LIS1 mutant human embryonic stem cell (hESCs) lines were generated using CRISPR/Cas9 genome editing. We developed a novel on-chip platform to grow 3D cortical organoids from mutant hESCs and modelled reduced folding. Extra cellular matrix (ECM) related genes were differentially expressed when wild-type and LIS1 +/- organoids were compared at different growth stages. Our study reveals novel molecular roles of LIS1 in ESCs and during early stages of brain development, and provide a model system to understand the mechanism associated with lissencephaly.

Keywords: Embryonic stem cells & pluripotency, RNA metabolism & epigenetics, Organoid modelling - Lissencephaly

CI309

FOXH1 REGULATION DURING CELL CYCLE PROGRESSION UPON DIFFERENTIATION IN HUMAN EMBRYONIC STEM CELLS

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Balance between differentiation and proliferation must be achieved to ensure the production of the correct amounts of each cell type during development. Despite the importance of these interplays, little is known about the molecular mechanisms orchestrating transcriptional networks and epigenetic modifiers during progression of the cell cycle upon differentiation. In this study, we hypothesized that key transcription factors could be directing differentiation in a cell cycle regulated manner. To dissect the molecular mechanisms involved, we first established a culture system allowing the differentiation of human embryonic stem cells (hESCs) synchronised for their cell cycle by combining nocodazole blockage in G2/M with our well-established endoderm differentiation protocol. We then focused on the forkhead transcription factor FOXH1 as it is known to play a key role in activating endoderm genes during differentiation. Accordingly, inducible knock down of FOXH1 during hESCs differentiation inhibits endoderm differentiation thereby confirming its essential role in endoderm specification in human. Interestingly, we observed that FOXH1 pattern of expression is cell cycle regulated during differentiation. Furthermore, chromatin immunoprecipitation followed by qPCR revealed that FOXH1 binds to its target genes as soon as cells re-enter the G1 phase of the first cell cycle upon differentiation. Surprisingly, this binding seems to be lost in the S phase of the same cycle. Thus, FOXH1 transcriptional activity could vary during cell cycle progression and FOXH1 could play a role in coordinating the initiation of differentiation with cell cycle progression. Taken together, these data confirm that studying cell cycle regulated transcription factors during differentiation could lead to unravelling mechanisms involved in the temporal acquisition of cell identity. This knowledge could benefit the regenerative medicine field as these mechanisms are likely to play a role in tissue repair and organ regeneration.

Keywords: Cell cycle, Cell identity, FOXH1

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CI180

TRANSLATIONAL REGULATION OF MITOCHONDRIAL REMODELING DURING HEPATOGENIC DIFFERENTIATION

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Mitochondrial remodeling supporting the metabolic shift from glycolytic toward OXPHOS-based metabolism has been shown as regulating stem cells differentiation. Recently, our team characterized this mitochondrial remodeling in a model of human Bone Marrow Mesenchymal Stem Cell (hBM-MSCs) hepatogenic differentiation. Interestingly, hBM-MSCs undergoing hepatogenic differentiation showed a decrease of 4E-BP1 (eukaryotic initiation factor 4E-binding protein 1) expression, a protein belonging to the mTOR pathway that is involved in the translational control of several nuclear-encoded mitochondrial mRNAs. In addition, recent data pointed out a correlation between mTOR pathway activation, mitochondrial remodeling and expression of hepatocyte-specific functional markers in a model of human iPSCs hepatogenic differentiation. Considering the key role of translational control during differentiation, we hypothesized that translational reprogramming, possibly through mTOR-pathway, could co-regulate mitochondrial remodeling and hepatocyte-specific features acquisition of differentiating iPSCs. iPSCs undergoing hepatogenic differentiation surprisingly showed a global decrease in protein synthesis, suggesting that the potential impact of mTOR activation on translation is restricted to specific transcripts. Polysome profiling will thus be performed in order to identify the differentially translated genes during hepatogenic differentiation. Altogether, this research project should shed light on the translational regulation that participates to the interplay between mitochondria, stem cells and differentiation.

Funding source: FRIA grant (fonds pour la formation à la recherche dans l'industrie et dans l'agriculture), FNRS

Keywords: Hepatogenic differentiation, Mitochondria, Translational regulation

CI181

LIVER CELLS FROM INDUCED PLURIPOTENT STEM CELLS: SELECTION CRITERIA DEFINED FOR PRECISION DRUG DISCOVERY

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Liver toxicity remains the leading cause of drug attrition. The state of the art animal models, primary liver cells and hepatoma cell lines are inadequate for predicting human drug responses accurately. Induced pluripotent stem cells (IPSCs) have unprecedented potential for preclinical drug discovery and disease modelling. The comparative analysis of the pluripotent state of IPS cells and human ES cells have revealed subtle differences which may impact downstream differentiation as well as functionality. This study aimed at an in-depth analysis of IPS cells differentiation to liver cell (Hepatocytes) and functionality assessment for drug metabolism with comparatives analysis of Human embryonic stem cell (hESC) lines for selecting key markers for early detection of stable lines based on functionality. A compliant biobank of IPSC lines representing both human genders, different age groups and ethnicities was established by reprogramming multiple cell types (skin cells, blood cells and liver cells) using a non-integrating vector strategy. The selection criteria for IPSC lines is based on transcriptomic profiling for pluripotency as well as differentiation into derivatives of three germ layers (spontaneous as well as directed differentiation). The IPSC lines differentiated to Hepatocyte like liver cells (HLCs) included characterisation of endodermal and hepatic stage-specific marker expression (definite endoderm, hepatic progenitor and functional hepatocytes). The liver-specific assessment included protein and gene expression profiling for Albumin, E-CAD and CK19, urea synthesis and fibrinogen secretion. Further, the assessment of Cytochrome P450 activity and mitochondrial toxicity conducted for a wide range of CYP substrates. The genotyping study (Single-nucleotide Polymorphism) included CYP1A1, CYP2D6 and CYP3A4 in human hepatocytes derived from IPSCs. The study proved the potential of induced pluripotent stem cell-derived liver cells and potential markers for early selection of stable IPSC lines for liver cell differentiation, simulating drug metabolism and mechanism of toxicity for precision drug discovery.

Funding source: This research is funded by Stemnovate limited and Co funded by Innovate UK.

Keywords: IPS, Hepatocytes Drug metabolism, IPS derived liver cells, IPS-Liver cells drug metabolism

CI300

A 3D ENVIRONMENT AND THE ALPHA-BETA CELL RATIO ARE MODULATING FACTORS TO DECREASE OXIDATIVE STRESS

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Oxidative stress is a determining factor in cell identity and fate. For example, high Hif1alpha expression due to low oxygen during late stages of pancreatic development inhibits differentiation towards the insulin-producing beta cells. We sought a better understanding of how oxidative stress is affected in a three-dimensional (3D) environment of a self-organized pseudoislet comprising pancreatic alpha and beta cells. Monolayer culture and pseudoislets were established by seeding the alpha cell line alphaTC1 and the beta cell line INS1E in the following ratios: 100:0, 80:20, 50:50, 20:80, and 0:100. Oxidative stress was induced by hydrogen peroxide and viability, oxidative stress and the level of endogenous antioxidant glutathione (GSH) were determined. We found that both a 3D environment and increasing the percentage of beta cells conferred greater resistance to cell death induced by oxidative stress. Increasing the prevalence of beta cells in monolayer culture diminished induced oxidative stress in alpha cells. In contrast, increasing the prevalence of alpha cells did not affect the oxidative stress in beta cells in a monolayer. Oxidative stress decreased GSH independently of the ratio between alpha and beta cells. Combining both cell types in a ratio of 50:50 prevented oxidative stress in beta cells and decreased oxidative stress in alpha cells in pseudoislets. In summary, the 3D environment protected against oxidative stress, and the beta cells decreased the oxidative stress in alpha cells in monolayer culture, while in 3D aggregates the 50:50 ratio was protective. This indicates that establishing a 3D niche at a specific cell ratio is essential for protecting against oxidative stress in pseudoislets.

Funding source: We would like to thank the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 694801) for the funding.

Keywords: Oxidative stress, Islets of Langerhans, 3D environment

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

CI199

IN SITU GELABLE CELL-LADEN TUBULAR MICROGELS FOR BLOOD VESSEL

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Drug screening using engineered blood vessels (EBVs) faces considerable barriers in approximating the conditions of an in vivo environment. To address this issue, we have introduced a microfluidic system for cell-laden tubular microgels. N-Carboxyethyl chitosan crosslinked with oxidized dextran was used for in situ gelable tubular scaffolds. The microfluidic system consisted of four glass capillaries that generated a coaxial flow of pre-polymer and phosphate buffered solutions. It rapidly produced cell-laden tubular microgels inside glass capillaries. The mechanical strength of the tubular microgels was suitable for their application as EBVs, with a maximum Young's modulus of 12.2 ± 1.9 kPa. In vitro cell studies using human umbilical vein endothelial cells verified the biocompatibility and non-cytotoxicity of the gelation and fabrication process. Thus, in situ gelable cell-laden tubular microgels can be a potential platform for screening drugs to treat blood vessel diseases.

Keywords: Blood vessle diseases, Drug screening, Tubular cell-laden microgel

EPITHELIAL

CI200

LGR4 MAINTAINS THE TUMOR STEMNESS OF HUMAN PROSTATE CANCER

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Prostate cancer (PCa) is among the most common cancers for men, and the mortality ranks the second place in the developed world. Therapeutic failure and tumor relapse may be caused by the incomplete elimination of tumor cells, especially the so-called cancer stem cells (CSCs), which are related to tumor initiation, cancer progression, metastasis and resistance to therapies. LGR4, a G protein coupled receptor, plays pivotal roles in prostate development and is involved in WNT signaling during carcinogenesis. Here, we found that LGR4 enhances the transcription of stem cell marker genes in PCa cell lines and LGR4 overexpression promotes the prostasphere forming ability. Knockdown LGR4 in PCa stagnated the tumor formation ability of prostate CSCs in nude mice. Furthermore, we found that the stemness-maintaining and tumor-enhancing ability of LGR4 is antagonized by another membrane protein. Domain mapping demonstrated the importance of extracellular domains for protein-protein interaction. In addition, LGR4 overexpression in pathological specimens correlated with poor prognosis of PCa. In summary, our results provide evidence to support the roles of LGR4 and its inhibitor in CSCs.

Funding source: Ministry of Science and Technology, 106, Sec. 2, Heping E. Rd., Taipei 10622, Taiwan, R.O.C.

Keywords: cancer stem cell, prostasphere prostate cancer, LGR4, G-Protein Coupled Receptor 48

CI206

FLOW CYTOMETRY ANALYSIS OF A CD44LOW/ CD133LOW CANINE COMPLEX MAMMARY ADENOMA CELL LINE

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In this study, we have obtained primary canine complex mammary adenoma cells and sorted in a flow cytometry using CD133 and CD44 antibodies. First of all, the tumor tissues were mechanical isolated and enzymatic dissociated by 0.25% trypsin-EDTA and 1mg/mL collagenase type IV. After digestion, trypsin-collagenase mixture was inactivated and then enzyme dissociated tumor cells were cultured in advanced DMEM (ADMEM) containing 0.5% FBS. After first subculture, ADMEM medium was replaced with serum-free DMEM/F12 medium containing 1X N2 supplement, 1X B27 supplement, 10ng/mL basic fibroblast growth factor (bFGF) and 10ng/mL epidermal growth factor (EGF). The complex mammary adenoma cell line (named as K9-102618(DG)) has grown in a cluster. We performed flow cytometry analysis on a complex mammary adenoma cell line at passage 4 and sorted with CD133 and CD44 antibodies. In the presence of EGF and bFGF, the CD44low/CD133low cell line has grown with or without ECM treatment showing representative epithelial markers such as E-cadherin and CD44. The mRNA expression levels of markers (breast cancer marker – HER2; cell proliferation marker – c-myc; and cell migration marker – Glis1) were more detected in the CD44low/CD133low cell cultured on ECM-coated plates. Since this cell line is a benign tumor, it is presumed that CSC markers expression is relatively low. Further studies are needed to characterize various types of CMT cell lines and to study the tumor microenvironment.

Funding source: Acknowledgement : This work was supported, in part, by a grant from the “Korea IPET (grant number: 318016-5 & 819029-2), funded by MAFRA” and “GRDC Program through the NRF funded by the MEST (2017K1A4A3014959)”, Republic of Korea.

Keywords: Canine, Mammary gland tumor, Tumor microenvironment

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

CI209

HUMAN GENOME MODIFICATION: TOWARD A REGULATORY FRAMEWORK IN AUSTRALIA?

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Around the globe, scientists are carrying out research on the innovative CRISPR-Cas/9 (Clustered Regularly Interspaced Short Palindromic Repeats) (CRISPR), removing gene variations that produce transmissible illnesses and conditions. However, there are various ethical issues associated with this novel technology. This presentation provides a critical analysis of the current Australian regulation on human genome editing. Claims that Chinese babies have been born with modified genomes could mean that the Australian law on this subject may need to be revised. Before deciding whether amendments in the law is necessary, first, it is critical to conduct a legislative review in Australia. In this presentation, I strongly recommend that it must to be organised to explore whether there is a need for legal updates to govern human genome editing in Australia. Since 2011, there has been no review on the current regulation. Thus, a law review which entails in-depth debates among many stakeholders and open submissions is outstanding.

Keywords: CRISPR, regulation, Australia

EYE AND RETINA

CI210

THE THERAPEUTIC EFFECT OF MESENCHYMAL STEM CELLS IN SJOGREN’S SYNDROME

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Sjögren’s syndrome (SS) is a systemic autoimmune disorder that is characterized by the inflammatory destruction of salivary and lacrimal glands (LG). Mesenchymal stem cells (MSC) are multipotent stem cells that has shown promising potential in treating various autoimmune diseases. Thus, this current study will aim to elucidate the effect of MSCs on the amelioration of SS dry eyes. MSCs were injected once into either the conjunctiva or the lacrimal glands of mice and sacrificed after 7 days. In order to observe the effects of MSCs on dry eye parameters, tear secretion by phenol red, corneal staining score, conjunctival goblet cells by PAS staining, and inflammatory gene expression of the cornea-conjunctiva and the LG were observed. Seven days after injection of MSC (5x105 cells) into the conjunctiva or the lacrimal glands, it would be observed that tear secretion as well as PAS staining scores were alleviated compared to the untreated mice. Corneal staining scores did not show statistically significant decreases compared to the untreated mice. Inflammatory cytokine levels in the conjunctiva and the LG of MSC treated mice showed decreases compared to untreated mice. This study demonstrated that dry eye severity was attenuated with the administration of MSCs in either the conjunctiva or the lacrimal glands as shown through decreases in tear secretions and PAS staining, as well as decreased inflammatory cytokine expressions compared to the untreated mice in SS animal model. Thus, MSC treatment offers promising potential to be utilized as treatment for SS dry eyes.

Funding source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2018R1C1B6008748).

Keywords: Sjögren’s syndrome, Mesenchymal stem cell, Dry eye

GERMLINE

CI212

SINGLE-CELL CHIP-SEQ COUPLED WITH FLUORESCENCE IMAGING REVEALS H3K4ME3 SIGNATURE FOR ACQUISITION OF MOUSE OOCYTE DEVELOPMENTAL COMPETENCE

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Chromatin immunoprecipitation and sequencing (ChIP-seq) offers genome-wide profiling of histone modifications and can provide insight into gene regulatory networks. Mapping by ChIP-seq is routinely carried out using large pools of cells, and the resulting data does not take heterogeneity within the cell population into account. Some recent studies have taken steps towards achieving single-cell resolution, however the applicability of these technologies is limited by their need for relatively large amounts of starting material (50,000 – millions of cells), modest yield in terms of unique reads per cell (1,000 – 10,000 reads), and/or requirements for specialised equipment, such as multiple microfluidic devices. None of these methods demonstrate the capacity to start out with a single cell, nor produce high yields. Current techniques involve barcoding and parallelisation with an emphasis on high throughput, but as a result they come with bias due to cell duplets/multiples in the 5-20% range, which could affect biological interpretations. Methods to map histone modifications based on Tn5 transposase or MNase claim to surpass ChIP-seq as they do not require immunoprecipitation, however these strategies come with their own biases and limitations. Here we present ChIP-seq from pico-scale input (picoChIP-seq), and show that we can obtain higher quality data from 50 cells as compared to state-of-the-art small-scale ChIP-seq from 1,000 cells. Furthermore, we carry out ChIP-seq starting from a single cell and demonstrate preservation of the signal pattern and the signal-to-noise ratio. Compared to existing technologies, we improve the read numbers by orders of magnitude. PicoChIP-seq has the advantage that it can be established in any lab using standard laboratory equipment. We further show that we can combine imaging of global chromatin organisation with scChIP-seq from the very same cell. Here we apply this method to compare H3K4me3 establishment in actively transcribing and silenced GV oocytes. We identify an epigenetic programming that is linked to the establishment of meiotic and developmental competence. Our work opens a new avenue for the study of fertility and infertility in humans, livestock and mammalian model organisms, as well as for characterisation of tissues with single-cell resolution.

Keywords: scChIP-seq, picoChIP-seq, Mouse oocyte epigenome Germline epigenome, Epigenetic reprogramming of gametes

CI324

CHARACTERIIZNG PUTATIVE PIRNAS IN HUMAN FETAL TESTES DURING THE WINDOW OF EPIGENOMIC LANDSCAPE ESTABLISHMENT AND CROSS SPECIES COMPARISON

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Along with the genome-wide epigenomic reprogramming during germ cell development, transposable elements (TEs) are largely de-repressed, which may damage the genome integrity. In animals, an evolutionarily conserved PIWI-interacting RNA (piRNA) pathway emerges to regulate TE expression either by guiding PIWI proteins to cleave targeted transcripts or via deposition of repressive epigenetic marks. For human fetal testes, however, it is difficult to dissect these processes, due to the obvious scarcity of available samples. In the present study, to clarify the potential role of PIWI/piRNAs machinery in human germ cells during the epigenomic reprogramming process, fetal testes were collected for investigation from abortuses in gestation weeks (GW) 16~22. We found that by immunostaining, PIWI pathway-related proteins, including PIWIL2, PIWIL4, VASA, and MAEL, were detected within the tissue sample. Furthermore, we have created a bioinformatic pipeline to determine piRNA representation from the RNA sequencing reads between 24~34 nucleotides that were mappable to the human genome without

allowing any sequence mismatch, and excluded from known and predicted rRNA/tRNA, miRNA species. By referring to human piRNA database, some of the piRNA candidates could be designated with putative piRNA identity. These putative piRNAs were further categorized based on being originated from or targeting TEs, genes, TE/gene fusion transcripts and intergenic sequences. Of note, in a GW19 testis, up to 25% putative piRNAs were annotated to repeat elements that mostly belong to LINE family of TE. Altogether, our data of piRNA analysis support the idea that the human PIWI-piRNA pathway may involve in the regulation of TE activity in fetal testis during development. We will also compare and contrast the expression of piRNA machinery and other epigenetic modulators during the window of male germ cell epigenetic landscape establishment in mouse, chicken, and human, to discuss the evolutionary implications of our finding.

Keywords: piRNA pathway mediated epigenetic regulation, germline, genomic imprinting

MUSCULOSKELETAL

CI227

A NOVEL HUMAN SKELETAL MUSCLE IN VITRO MODEL USING OPTI-OX MEDIATED CELLULAR REPROGRAMMING OF INDUCED PLURIPOTENT STEM CELLS

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Skeletal myocytes play roles in a number of biological processes ranging from limb movement to the regulation of nutritional homeostasis, and are implicated in the pathophysiology of a variety of diseases involving muscle dysfunction and wasting. There is a pressing need for reliable models of mature human skeletal muscle to permit investigations into physiological and disease mechanisms, and to facilitate the generation of new therapeutics. While human induced pluripotent stem cells (hiPSCs) offer a promising starting material for skeletal muscle cells, their broad use has been hampered by difficult to reproduce, complex differentiation protocols. We have developed an optimised inducible system (opti-ox) that enables tightly controlled expression of transcription factors (TFs) improving cellular reprogramming approaches for the differentiation of hiPSCs. Through the targeting of genomic safe harbour loci, we used opti-ox to achieve homogenous, inducible expression of the myogenic regulator MYOD1. Induction of MYOD1 through opti-ox leads to the rapid shutdown of the core pluripotency network, and activation of core myogenic factors. We demonstrate robust expression of myosin heavy chain isoforms, coupled with the transition of immature myosin heavy-chain isoform (MYH3 and MYH8) to mature isoforms (MYH1) expression, in a time dependent manner. Skeletal myocytes derived through opti-ox express Desmin, Dystrophin and Titin, and form striated multinucleated myotubes that contract in response to acetylcholine. Critically for metabolic studies, robust expression of the insulin-regulated glucose transporter GLUT4 is also detected. Opti-ox reprogramming of hiPSCs provides a unique scalable model of human skeletal myocytes, opening new avenues for high throughput screening and academic research applications, for consistent study of normal and pathological muscle biology.

Keywords: Human Pluripotent Stem Cells, Reprogramming, Myocytes

NEURAL

CI241

THE OXYGEN-SENSOR AND TRANSCRIPTIONAL COREPRESSOR CTBP2 IS REQUIRED FOR PROPER DEVELOPMENT OF THE MOUSE CEREBRAL CORTEX

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C-Terminal Binding Proteins (CtBPs) 1 and 2 are transcriptional co-repressors which have been shown to be critically involved in embryonic development and various cancer disease, partly depending on the regulation of epithelial-to-mesenchymal transition (EMT). The CtBPs are oxygen sensing molecules, and we have previously demonstrated an important role for CtBP1 in integrating oxygen levels and BMP-signaling to influence neural progenitor fate choice (Cell Reports, 2014). In turn, CtBP2 has been associated with neurodevelopment and tumors such as high grade glioma, and we have shown that CtBP2 acetylation and dimerization, that are required for proper transcriptional activity, are regulated by microenvironmental oxygen levels (ECR, 2015). Yet, the putative function of CtBP2 in mammalian cortical development and neurogenesis in vivo is still largely unknown. Here we show that CtBP2 was widely expressed by neural stem and progenitor cells (NSPCs) as well as neurons during cortical development in mice. By using in utero electroporation of siRNA to reduce the levels of CtBP2 mRNA and protein in the developing

mouse brain, we found that the NSPC proliferation and migration were largely perturbed, while glial differentiation under these conditions remained unchanged. Our study provides evidence that CtBP2 is required for the maintenance and migration of the NSPCs during mouse cortical development. Taken together with previous reports, our results point to a mechanism for how oxygen levels influence proliferation and migration of cortical NSPCs via CtBP2.

Funding source: The Swedish Research Council (VR-MH), the Swedish Cancer Society (CF), and the Swedish Childhood Cancer Foundation (BCF) to O.H..

Keywords: Oxygen levels, Epigenetics, Neural stem cells

CI246

AN INDUCIBLE GENETIC SWITCH FOR THE SCALABLE REPROGRAMMING OF HUMAN IPSCS TO DISTINCT NEURONAL SUBTYPES

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Human brains differ remarkable in size, shape and cellular composition from the rodent models used in pre-clinical research. Differences at the molecular and cellular level sets our brains even further apart from these models. In particular, human cortical neurons have in comparison to rodents larger dendritic trees, distinct electrophysical properties, different types of protein isoforms and 30% additional proteins present in their synapses. Therefore, it is not surprising that for neuronal indications less than 10% of the findings derived from animal models can be translated to the clinic. Human iPSC-derived neurons, besides applications for biological and disease modelling studies, offer an attractive in vitro model for high content drug screens and could reduce costs, improve screen specificity and potentially save lives. However, iPSC differentiation protocols that use extrinsic factors are often complex, lengthy, expensive, inconsistent and generate mixed cell population. To overcome these problems, we have developed a proprietary discovery platform and genetic switch (opti-ox) to express optimal sets of transcription factors (TFs) to rapidly and robustly reprogram human iPSCs towards pure somatic cell types. With our unique reprogramming method, we are already able to consistently manufacture highly pure cultures of glutamatergic neurons (>80% VGLUT1/2), as well as GABAergic neurons (VGAT1, GABA), which show homogenous molecular phenotype at single cell transcriptomics resolution. We show that the cellular reprogramming process is very consistent and synchronised for both neuronal subtypes, giving fully differentiated functional neurons in only 14 days. We will discuss single cell transcriptome data of these differentiating neurons and a trajectory analysis of their in vitro lineages. Furthermore, we will present the use of our discovery platform to identify optimal TF combinations for the generation of oligodendrocytes and astrocytes by opti-ox cellular reprogramming. Our technology, by allowing the consistent, scalable production of human neurons and glial cells from iPSCs, offers novel avenues for the development of robust in vitro models necessary to support basic science and health care innovations.

Keywords: iPSC Reprogramming, GABAergic and glutamatergic neurons, single cell sequencing

CI249

GENERATION OF INTEGRATION-FREE INDUCED NEURONS USING GRAPHENE OXIDE-POLYETHYLENE

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The direct conversion of somatic cells into induced neurons (iNs), without inducing pluripotency, has great therapeutic potential for central nervous system diseases. Reprogramming of somatic cells to iNs requires the introduction of several factors to drive cell fate conversion. Common direct reprogramming strategies to deliver these factors into somatic cells use viruses. However, novel gene delivery systems that do not integrate transgenes into the genome should be developed to generate iNs for safe human clinical applications. In this study, we investigated whether graphene oxide-polyethylenimine (GO-PEI) complexes are an efficient and safe system for mRNA delivery for direct reprogramming into iNs. The GO-PEI complexes created in this study exhibited a low cytotoxicity, high delivery-efficiency, and non-integration features for direct conversion into iNs. Moreover, in vivo transduction of reprogramming factors with GO-PEI complexes into the brain was sufficient to facilitate conversion into iNs which alleviated symptoms in mouse models of Parkinson's disease. Thus, these studies demonstrate that the GO-PEI delivery system could be used to obtain safe iNs, and provide a promising for direct reprogramming-based therapies of neurodegenerative diseases.

Keywords: Graphene oxide-polyethylenimine (GO-PEI), Direct conversion, Fibroblasts, Neuron, Gene delivery, Non-integration, mRNA

CI299

A SHH/GLI-DRIVEN THREE-NODE TIMER MOTIF CONTROLS TEMPORAL IDENTITY AND FATE OF MOUSE NEURAL STEM CELLS.

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Time is a central axis of information during embryogenesis but few mechanisms explaining the timing of developmental events have been resolved. In the forming central nervous system (CNS), defined pools of multipotent neural stem cells (NSCs) produce distinct cell types in a specific sequential order and over defined timeframes. In this process, ageing NSCs become progressively restricted in their developmental potential by losing competence to generate early-born cell types and genome-wide analyses have revealed that NSCs undergo dynamic transcriptional changes over time. However, the composition and functional properties of time-encoding circuitries determining timeframes and point of transitions have not been resolved in any model system. To approach the question, we focused on a relatively well-defined lineage in the ventral brainstem that is induced by Sonic hedgehog (Shh) and sequentially produces motor neurons (MNs), serotonergic neurons (5HTNs) and oligodendrocyte precursors (OPCs). By combining experiments and computational modelling, we defined a Shh/Gli-driven three-node timer underlying the sequential generation of MNs and 5HTNs in the brainstem. The timer is founded on temporal decline of Gli-activator and Gli-repressor (GliR) activities established through downregulation of Gli transcription. The circuitry conforms an incoherent feedforward loop, whereby Gli proteins promote expression of Phox2b and thereby MN fate, but also account for a delayed activation of a suppressive Tgfβ-node that triggers a MN-to-5HTN fate switch by repressing Phox2b. Data suggest a model in which activation of the Tgfβ-node is temporally gated by a Gli inhibitor-titration mechanism whereby induction of Tgfβ2 by Gli1 is prohibited until GliR has been titrated out. Biological timers based on accumulation or titration of activators or repressors have previously been reported and Gli decay is formally sufficient to mediate timer function without Tgfβ, raising the question why timing of the MN-to-5HTN fate switch involves a more complex network architecture. Computational modelling suggest that spatial averaging enabled by the diffusible and self-activating properties of Tgfβ, in combination with hysteresis, produces prompt suppression of Phox2b and a coordinated switch at the population level.

Keywords: Ventral brainstem, Temporal neural patterning, Shh/Gli signaling

NEW TECHNOLOGIES

CI260

A NEW HUMAN REPROGRAMMING STRATEGY TO ERASE SOMATIC CELL EPIGENETIC MEMORY AND REDUCE DIFFERENTIATION BIAS

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A process of massive epigenome reconfiguration is required during the reprogramming of adult cells into induced pluripotent cells (iPSCs) for cells to lose their somatic cell identity and transition to a state that resembles an embryonic stem cell (ESC). However, during human reprogramming the somatic cell epigenome is not fully reset, leaving iPSCs with an epigenetic memory of their progenitor cell type. More specifically, the epigenetic memory has been observed as regions where DNA methylation and histone modification states in iPSCs resemble the state observed in fibroblast cells, making iPSCs distinct from ESCs. Importantly, this epigenetic memory can be retained through differentiation. However, there has been considerable debate regarding the existence, extent, and consequences of epigenetic memory retention during reprogramming. To obtain a comprehensive understanding of how the epigenome is reconfigured during reprogramming, we performed an integrative multi-omics time-course analysis of the reprogramming of human fibroblasts. First, we confirmed previous reports of the existence of epigenetic memory. Second, through studying the epigenome dynamics in this system and comparing our observations with the epigenome reconfiguration during human embryonic development, we devised a new reprogramming strategy to minimize the epigenetic memory retained in human iPSCs so that they more closely resemble ESCs. We observe that genomic regions harboring epigenetic memory are concentrated in large domains of repressive chromatin that distinguish fibroblasts from ESCs, and that our new strategy can change both the repressive histone and DNA methylation states within these domains to obtain cells that, epigenetically, more closely resemble ESCs. Importantly, iPSCs obtained through this approach show reduced differentiation bias towards the mesoderm lineage compared to conventionally reprogrammed iPSCs, and reduced emergence of fibroblast-like cells when differentiating into neural stem cells.

In summary, we have developed a new reprogramming strategy that minimizes the somatic cell epigenetic memory to produce iPSCs that more closely resemble ESCs.

Keywords: Reprogramming, Epigenetics, Human

CI267

MOGRIFY: A COMPUTATIONAL FRAMEWORK TO CONVERT BETWEEN CELL TYPES

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Mogrify has developed a predictive system that combines gene expression data with regulatory network information to predict the reprogramming factors necessary to induce cell conversion by systematically controlling the cellular transcriptomic network underlying cellular identity. This platform can identify the key regulatory factors necessary to convert any cell type into any other cell type without going through stem cell state, a process called transdifferentiation. We have applied Mogrify to 173 human cell types and 134 tissues, defining an atlas of cellular reprogramming including both known transcription factors used in transdifferentiations and new ones, never implicates in these cellular conversions. Mogrify in silico predictions have been validated in vitro in over 20 cell conversions, including generation of endothelial cells, astrocytes and cardiomyocytes. This technology also allows the development of enhanced differentiations and reduce the costs of current cell therapies.

Keywords: Cell therapy, Computational Research, Cell Identity

CI269

A ZINC-FINGER C2H2 TYPE PROTEIN ZNF REGULATES PLURIPOTENCY OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) and human induced pluripotent stem cells (hiPSCs) are characterized by its abilities to proliferate infinitely (self-renewal) and differentiate into almost all cell types in our body. However, the ultimate functions of controlling pluripotency by zinc-finger protein family remain unknown. Here, an uncharacterized protein ZNF, a zinc-finger C2H2 type family member protein, was found enriched in hPSCs. ZNF knockdown impaired the pluripotency of hPSCs which were shown by downregulated key pluripotent markers such as OCT4, SOX2, NANOG, KLF4, PODXL. Meanwhile, ZNF overexpression can partially delay cell differentiation. ZNF knockdown also significantly inhibited iPSC reprogramming. Inducible ZNF knockout approach also blocked the pluripotency of hPSCs and the teratoma formation in the mice model. Using protein sequence alignment, the ZNF protein shared 92% similarity with CTCF. Moreover, ZNF possessed DNA-binding ability through ChIP-seq analysis. Combined with transcriptomic target gene analysis after loss of ZNF, ZNF ChIP-seq indicated that ZNF was potentially involved in chromatin remodeling in hPSCs.

Keywords: Zinc-finger C2H2 type protein, pluripotency, chromatin

CI270

CONSERVED EPIGENETIC REGULATORY LOGIC INFERS GENES GOVERNING CELL IDENTITY

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Understanding genetic control of cell diversification is essential for establishing mechanisms controlling biological complexity. We analyzed 111 NIH epigenome roadmap data sets to identify distinguishing features of genome regulation associated with cell-type specification. We show that the a priori deposition of H3K27me3, which we call a gene's repressive tendency (RT), provides genome-wide enrichment for genes governing fundamental mechanisms underlying biological complexity in cell differentiation, organ morphogenesis and disease. We tested the ability to infer regulatory genes controlling theoretically any somatic cell by interfacing genome-wide RT values with cell-specific genome-wide sequencing data. Using more than 1 million genome-wide data sets from diverse omics platforms including bulk and single cell RNA-seq, CAGE-seq, ChIP-seq and quantitative proteomics, we identify cell-type specific regulatory mechanisms underlying diverse cell-states, organ systems and disease pathologies. Since regulatory control of cell identity is highly evolutionarily conserved across species, we demonstrate that this computational logic enriches for cell- type specific regulatory genes from species across the animal kingdom including chordates and arthropods. Furthermore, we demonstrate its application to identify key genetic drivers of disease pathology and highlight cell type-specific regulatory processes underlying disease states, such as melanoma and heart failure. Lastly, we use this computational inference approach for novel gene discovery. We identify and genetically validate SIX3 and RNF220 as novel regulatory drivers of development using scRNA-seq cardiac data from in vitro differentiation of human iPSCs and in vivo heart field specification of the tunicate Ciona robusta. This study demonstrates that the conservation of epigenetic regulatory logic provides an effective strategy for utilizing large, diverse genome-wide data to establish quantitative basic principles of cell-states to infer cell-type specific mechanisms that underpin the complexity of biological systems.

Keywords: Cellular Identity, Epigenetics, Computational approaches

CI273

GENERATION OF CANINE INDUCED PLURIPOTENT STEM CELLS USING NON-INTEGRATING METHOD

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Canine is an ideal model organism for comparative human disease, already share many diseases in our life. Many countries are collaborating to collect and study from large cohorts of dogs suffering from a range of carefully defined diseases of relevance to human health. Part of this, canine induced pluripotent stem cells (ciPSCs) are an attractive source for regenerative medicine serve as a disease model for human and veterinary field. Since discovery of Yamanaka's factor, reprogramming tools are developing for the increasing efficiency and safety. In this study, we were challenge to reprogramming of ciPSCs using canine fibroblast that was produced by the combination of reprogramming mRNAs [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28] on feeder free condition. After transduction, reprogrammed ciPSC morphologies were appeared seems like canine embryonic stem cells that was maintained under serum-free condition. For characterization, ciPSC-like colonies were identified with stemness marker such as Alkaline Phosphatase, OCT4, and SSEA-1 by immunocytochemistry. This is the first ciPS generation report by mRNA reprogramming methods that non-integrating method shows an available for reprogramming and ciPSCs may useful source for stem cell study, comparative oncology, and disease model. In the further study, ciPSCs will be required to investigate their availability of the long-term culture and comparison with other mammalian iPSCs, including human iPSCs.

Funding source: This research was supported by the National Research Foundation of Korea(NRF) funded by the Ministry of Education [2015H1D3A1066175(KRF), 2016R1D1A1B03933191, 2017K1A4A3014959(GRDC) and 2019R1I1A1A01059554].

Keywords: Canine induced pluripotent stem cells, iPSCs, Reprogramming

CI277

LONG-READ INDIVIDUAL-MOLECULE SEQUENCING REVEALS CRISPR-INDUCED GENETIC HETEROGENEITY IN HUMAN ESCS

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Accurately quantifying the genetic heterogeneity of a cell population is essential to understanding of biological systems. We develop a universal method to label individual DNA molecules for analyzing diverse types of rare genetic variants, with frequency as low as 4x10e-5, using short- or long-read sequencing. Our method includes two parts, IDMseq (Individual

DNA Molecule sequencing) and VAULT (Variant Analysis with UMI for Long-read Technology), that can be adapted to all current sequencing platforms, such as Illumina, Oxford Nanopore, and PacBio. It enables base-resolution haplotype-resolved quantitative characterization of rare variants. It provides the first quantitative evidence of persistent nonrandom large deletions and insertions following DNA repair of double-strand breaks induced by CRISPR-Cas9 in human pluripotent stem cells.

Funding source: The research of the Li laboratory was supported by KAUST Office of Sponsored Research (OSR), under award # BAS/1/1080-01 and URF/1/3412-01-01.

Keywords: CRISPR-Cas, Oxford Nanopore, DNA repair

PLACENTA AND UMBILICAL CORD DERIVED CELLS

CI293

GLUTATHIONE DYNAMICS DETERMINE THE THERAPEUTIC EFFICACY OF MESENCHYMAL STEM CELLS FOR GRAFT-VERSUS-HOST DISEASE VIA CREB1-NRF2 PATHWAY

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Glutathione (GSH), the most abundant non-protein thiol functioning as an antioxidant, plays critical roles in maintaining the core functions of mesenchymal stem cells (MSCs), which are used as a cellular immunotherapy for graft-versus-host disease (GVHD). However, the significance of GSH dynamics in MSCs remains elusive. Genome-wide gene-expression profiling and high-throughput live-cell imaging assays revealed that CREB1 enforced the GSH-recovering capacity (GRC) of MSCs through NRF2 by directly up-regulating NRF2 target genes responsible for GSH synthesis and redox cycling. MSCs with enhanced GSH levels and GRC mediated by CREB1-NRF2 possessed improved self-renewal, migratory, anti-inflammatory, and T-cell suppression capacities. Administration of MSCs overexpressing CREB1-NRF2 target genes alleviated GVHD in a humanized mouse model, resulting in improved survival, decreased weight loss, and reduced histopathologic damages in GVHD target organs. Collectively, these findings demonstrate the molecular and functional significance of the CREB1-NRF2 pathway in maintaining MSC GSH dynamics, determining therapeutic outcomes for GVHD treatment.

Funding source: National Research Foundation of Korea(NRF) funded by the Ministry of Education (NRF-2017R1D1A1B03031379), (NRF-2018R1D1A1B07047450) National Research Foundation of Korea(NRF-2018R1A2B2001392).

Keywords: Glutathione, CREB1, Graft versus Host Disease

Theme: Clinical Applications

CARDIAC

CA109

CONTROLLED STIRRED TANK BIOREACTORS FOR LARGE-SCALE MANUFACTURE OF HUMAN IPSC MODELS FOR CELL THERAPY

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Cell and gene therapies show promise in providing new therapeutic strategies for a wide range of indications. Advancements in induced pluripotent stem cell (hiPSC) technologies have substantially expanded access to many human cell types to accommodate the future demand for such therapies. However, the differentiation and manufacture of iPSC-derived cells can face significant challenges in obtaining the necessary production scales, quality standards and high reproducibility between batches for cost-effective cell therapy research and clinical application. Currently, the development and production of iPSC-derived cell types is often performed in a small-scale culture unsuitable for robust generation of a large number of cells. Using the example of cell-based therapy for heart failure, it is estimated that 109 cells are required per patient. Stirred-tank bioreactors have emerged as promising culture systems for large-scale cell manufacturing from hiPSC sources. These systems allow full automation and conduction in closed systems, resulting in cultures with comparable characteristics from batch to batch. Closed-system, parallel processing with increased automation is also critical to minimize error and contamination from human interaction with cell products. Ncardia has established a controlled stirred-tank bioreactor platform that is shown to routinely yield high numbers of hiPSC-derived cardiomyocytes and additional cell models that are currently used in cell therapy, safety and efficacy applications. Using a Quality by Design approach, we demonstrate a robust and controlled process for large-scale manufacturing (>3x1010) of iPSC-derived cardiomyocytes to a purity of >95% in a serum-free protocol. The bioreactor-derived cells are shown to be a relatively mature model recapitulating a human cardiomyocyte's contractile and electrophysiological profile. We demonstrate the implementation of a flexible process development workflow comprised of state-of-the-art bioreactor systems that allows for optimization of processes at 15 mL scale, validation of promising conditions at mid-scale (100 - 250 mL) and manufacture from a diverse set of hiPSC lines to yield the required scale in the tens of billions.

Keywords: cell therapy, IPSC manufacturing, drug discovery

CA111

STIR TANK REACTOR EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AND DIFFERENTIATION TO CARDIOMYOCYTES: PROCESS DEVELOPMENT THROUGH TO PRECLINICAL ANIMAL MODELS FOR MYOCARDIAL INFARCT

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Heart failure costs Australia approximately 1 billion p.a. One in 2 Class IV cases die within a year of diagnosis due to a lack of effective treatments. Pharmacotherapy is reserved for Class III and IV while heart transplants have a limited availability necessitating a treatment that is more readily available. Cardiomyocytes (CM) derived from human pluripotent stem cells (hPSC) have emerged as a leading candidate to restore heart function after myocardial infarct but there have been difficulties in reaching desired cell numbers in a reproducible fashion. In order to reach patient relevant doses of CMs (approximately 1E9), defined, scalable and robust process for hPSC expansion and differentiation is required that can be translated to a GMP grade procedure. In this work we outline the development of a scalable expansion process for hPSC and their differentiation to CMs using stirred tank reactors and a thermo-responsive polymer to aid expansion, the Nanobridge system. The Nanobridge system utilizes a poly N-isopropyl acrylamide (PNIPAM) polymer decorated with recombinant fibronectin to bind to and bridge adjacent cells and form aggregates at 37°C. Here we have utilised the Nanobridge system to develop a clinically relevant production process of hPSC-CMs and demonstrate effective engraftment of these cells into rats and pigs using a modified version of the hPSC line H9. In the first stage we established master and working cell banks that were characterised for quality control, free of gross genetic abnormalities, >90% positive for pluripotency markers and negative for adventitious agents. For expansion in STRs, we demonstrate a consistent aggregate formation across multiple runs with a >1.5 fold expansion over 3 days in the presence of the Nanobridge system. For differentiation optimisation, we investigated multiple parameters including CHIR99021 concentrations, timings for WNT inhibition and addition of the mTOR inhibitor rapamycin and identified optimal conditions for H9. Prior to cryopreservation of hPSC derived CMs, cells were heat-shocked at 42 °C and treated with a pro-survival cocktail to enhance engraftment. The quality control at all stages of H9 expansion and differentiation has resulted in a reproducible method for the production of CMs that engraft and show electrical coupling in both rat and pig models.

Keywords: Cardiac, Preclinical, Bioreactor

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CA121

AMNIOTIC FLUID MESENCHYMAL STEM CELLS ATTENUATE CHRONIC ALCOHOL-INDUCED LIVER INJURY IN ALDO-KETO REDUCTASE 1A1 KNOCKOUT MOUSE MODE

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Alcoholic liver disease (ALD) covers a wide range of hepatic lesions, which depend on the amount and duration of alcohol consumption, from the early and reversible condition, a hepatic steatosis, to the severe lesions, including steatohepatitis, alcoholic fibrosis to irreversible cirrhosis. With limited therapeutic options, stem cell-based cell therapy has already showed a promising efficacy in improving liver disease. In this study, we aimed to evaluate the regenerative and repair ability of amniotic mesenchymal fluid stem cells (AFMSCs) in a novel hepatic disease mouse model after chronic alcohol exposure. An aldo-keto reductase family member, AKR1A1, which may participate in the aldehyde detoxification to their corresponding alcohols and protects cells against oxidative stress in an NADPH-dependent manner. Male AKR1A1-/- and ICR control mice were divided into five groups that fed either the Lieber-Decarli diet containing 5 % ethanol or an isocaloric control diet for 8 weeks. In comparison with ethanol fed-ICR control mice, the ethanol fed-AKR1A1-/- mice showed a significant increase in the serum alanine aminotransferase and aspartate transaminase levels, and the levels of pro-inflammatory cytokines (TNF-α and IL-1β), hepatic triglycerides and cytochrome P4502E1(CYP2E1), whilst decreased expression of antioxidant enzymes, including hepatic glutathione and superoxide dismutase. Histopathologic and molecular examination observed that transplantation of AFMSCs to ethanol fed-AKR1A1-/- mice integrated into the hepatic structure and restore the liver function through relieve oxidative stress and inflammatory response, and reducing hepatic triglyceride and lipid accumulation, and also decreasing CYP2E1 expression level. We conclude that transplantation of AFMSCs showed a potential liver regenerative ability and reverse a novel ethanol induced liver disease in AKR1A1-/- mouse model.

Funding source: MOST 108-2313-B-005-039-MY3 from the Ministry of the Science and Technology, Taiwan

Keywords: alcoholic liver disease, amniotic fluid mesenchymal stem cells, AKR1A1

CA122

EFFECTS OF AMNIOTIC FLUID MESENCHYMAL STEM CELLS ON PANCREATIC ADENOCARCINOMA DEVELOPMENT

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Pancreatic ductal adenocarcinoma (PDAC) is notorious as one of the malignant cancer results from late-diagnose that emphasizes the significance of treatment. However, current chemotherapy is not as effective as expected, especially to variant mutation of PDAC, the alternative tumor-targeted therapy is required. Amniotic fluid mesenchymal stem cells (AFMSCs) have been reported to suppress tumor growth in various cancer but PDAC and therefore become a strong candidate in treating PDAC. In this study, we aimed to evaluate the cell therapy efficacy of AFMSCs on PDAC development in subcutaneous and orthotopic pancreatic cancer mouse models. We established an in vitro cell co-culture system to evaluate the tumorigenicity through invasion, migration, and angiogenesis analysis and subcutaneous or orthotopic implantation of PANC-1 cancer cells to nude mice also established to evaluate the effects of AFMSCs on the tumor development in vivo. Results showed that the migration, invasion, and tube formation abilities significantly decreased in AFMSC-co-cultured-PANC-1 compared to PANC-1 control group. The expression of chemokine receptors, CXCR4 and CCR7, were upregulated in co-cultured AFMSCs, which may enhance more AFMSCs migrate to the tumor sites, whist the expression level of migration, invasion and angiogenic-related genes, N-cadherin, Vimentin and VEGF, also downregulated in AFMSCs-co-cultured-PANC-1 group through RT-qPCR analysis. We conclude that AFMSCs suppress the migration, invasion and tube formation ability of PANC-1, also providing the insights of interaction between PANC-1 and AFMSCs.

Funding source: MOST 107-2813-C-005-070-B from the Ministry of Science and Technology, Taiwan

Keywords: Amniotic fluid mesenchymal stem cells, Pancreatic cancer, Tumorigenicity

CA126

IPS-DERIVED INTESTINAL ORGANOIDS FROM CYSTIC FIBROSIS PATIENTS ACQUIRE CFTR ACTIVITY UPON TALEN-MEDIATED GENE CORRECTION OF THE P.F508DEL MUTATION

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Cystic fibrosis (CF) is the main genetic cause of death among Caucasian children. The disease is characterized by abnormal fluid and electrolyte mobility across the epithelia of numerous tissues. The first manifestations occur in early childhood, generally affecting the respiratory tract, and later extending to other organs. It is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. CFTR encodes a cyclic adenosine monophosphate (c-AMP)-dependent, phosphorylation-regulated chloride channel required for transport of chloride and other ions through cell membranes. There are more than 2000 mutations affecting CFTR, but one of them, p.F508del, is responsible for approximately 70% of the cases of CF worldwide. Here we present the results of applying genome editing techniques to the restoration of CFTR activity in p.F508del patient-derived iPS cells. Gene-edited iPSCs were subsequently used to produce intestinal organoids on which the physiological activity of the restored gene was tested in forskolin-induced swelling tests. The seamless restoration of the p.F508del mutation resulted in normal expression of the mature CFTR glycoprotein, full recovery of CFTR activity and normal response of the repaired organoids to treatment with two approved CF therapies: VX-770 and VX-809

Funding source: This work was supported by grants from the Spanish Ministry for Science and Innovation (PLE2009-0091, RTC-2014-2207-1 and IPT-2011-1402-900000) and the European Commission, (H2020, PHC-667079)

Keywords: Cystic Fibrosis, Gene correction, Organoids

CA246

HYPOIMMUNOGENIC INDUCED PLURIPOTENT STEM CELL LINES AS A PROMISING SOURCE OF CELL REPLACEMENT THERAPY FOR AUTOIMMUNE TYPE 1 DIABETES

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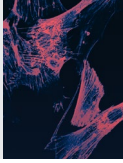
Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by loss of insulin-producing pancreatic beta cells and leads to cardiovascular complications and other comorbidities. Despite substantial research efforts, there is no reliable cure for T1DM. Pancreas and islet allotransplantation is limiting due to notable safety and availability issues, and exogenous insulin therapy still remains the most common treatment option for patients with T1DM. Stem cell-based replacement therapy is an alternative approach that may provide an extensive renewable source of insulin-producing beta



cells. However, either embryonic (ESC) or induced pluripotent stem cells (iPSC)-derived graft requires immunoprotection to prevent the recipient's rejection due to HLA mismatching. Although patient-specific iPSC-based product may overcome this hurdle, it takes time and costs a lot. The recent progress in genome editing technologies allows to create universal human iPSC lines by removing HLA antigens. We aimed to generate hypoimmunogenic iPSC-derived insulin-producing cells which could be a potential tool for T1DM therapy in patients of any HLA type. In order to establish safe iPSC line, we used non-integrating RNA reprogramming. Healthy donor-derived dermal fibroblasts were transfected with RNA vector expressing OCT4, KLF-4, SOX2, GLIS1, c-MYC and puromycin resistance gene. After antibiotic selection ESC-like colonies were picked and expanded. The resulting hiPS-VD1 cell line had a typical ESC-morphology and expressed endogenous pluripotency markers. To generate immune-compatible iPSC lines, we knocked out the beta-2-microglobulin (B2M) gene in the hiPS-VD1 cells by CRISPR/Cas9 system. As B2M is required for HLA class I presentation, its disruption would suppress cytotoxic T cell-mediated immune response. We used double-nicking strategy with paired guide RNAs. Two gRNAs targeting exon 1 in the B2M locus were designed and cloned into Cas9 nickase vectors containing GFP or puromycin selection cassette, respectively. A total of about 50 cell clones obtained after transfection and selection were analysed by sequencing. Finally, two successfully edited B2M-/- and B2M+/- hiPS-VD1 cell lines were chosen for further characterization and differentiation into functional beta cells.

Funding source: The work was supported by Russian Foundation for Basic Research, grant 19-29-04121-mk.

Keywords: iPSC, immunogenicity, genome editing

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

CA261

COVID-19: A COMPREHENSIVE STUDY OF THE EMERGING FIELD INCLUDING THE ROLE OF STEM CELL RESEARCH

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The outbreak of Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), has thus far killed over 300,000 people and infected near 5,000,000 worldwide, resulting in great catastrophe for humans. Similar to its homologous virus, SARS-CoV, which caused SARS in thousands of people in 2003, SARS-CoV-2 might also be transmitted from the bats and causes similar symptoms through a similar mechanism. However, COVID-19 has lower severity and mortality than SARS but is much more transmissive and affects more elderly individuals than youth. In response to the rapidly increasing number of publications on the emerging disease (near 13,000 based on the keyword of COVID-19), this article attempts to provide a timely and comprehensive review of the swiftly developing research subject. We will cover the basics about the epidemiology, etiology, virology, diagnosis, treatment, prognosis, and prevention of the disease. Interestingly, stem cells play an increasingly important role in the disease modeling, drug screening, and treatment of the disease. Although many questions still require answers, we hope that this review helps in the understanding and eradication of the threatening disease.

Keywords: SARS-CoV-2, COVID-19, stem cells

HEMATOPOIETIC SYSTEM

CA152

UNRAVELING CELL ADHESION MECHANISMS AT THE INTERFACE OF HEMATOPOIETIC STEM CELLS CD34-NEGATIVE AND ENDOTHELIUM

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True hematopoietic stem/progenitor cells (HSPCs) represent a rare cell population that are difficult to isolate and expand. Expression of CD34 is often used to isolate the therapeutic stem cells but the lack of CD34 is associated with the HSPC's long term repopulation capacity. However, numerous studies have shown that these cells do not function well when injected in vivo and this deficiency may be correlated a lack in the expression of some key adhesion molecules necessary for migration and engraftment. The aim was therefore to determine if such adhesion systems (i.e. proteins and their carbohydrate modifications) were lacking on this population of HSPCs and, if so, to enhance their ability to migrate and engraft through modifications of key mechanistic steps in the adhesion cascade. Following the purification of these rare cells from umbilical cord blood, using 13 lineage markers (CD2, CD3, CD4, CD11b, CD14, CD15, CD24, CD10, CD41, CD20, CD66c, CD127, CD33) and dividing the cells into a CD34pos (13LinnegCD34pos) fraction and a CD34neg fraction (13LinnegCD34neg), we found that the CD34neg HSPCs expressed considerably lower levels of the key homing molecule, sLex, compared to CD34pos fraction. sLex modifications on proteins and lipids bind to the endothelial cell receptors, the selectins, and here we sought to determine whether we could create these selectin ligands through ex vivo glycosyltransferase treatment of the HSPC cell surface. To this end, we successfully expressed and purified highly active recombinant human glycosyltransferases using eukaryotic expression systems (Yeast and Silkworm) and treated the HSPC populations with these enzymes. Notably, the 13LinnegCD34neg HSPCs gained sLex expression and was able to bind E-selectin following treatment. Further studies to identify this population's ability to migrate to the bone marrow of mice when injected intraveneously are currently underway. This study represents the

first direct analysis of E-selectin ligand expression on this long term hemaopiotic stem cell population and will potentially shed light on methods to optimally utilize these very valuable long-term HSPCs in clinical bone marrow and cord blood transplants worldwide.

Keywords: hematopoietic stem cells, long-term repopulating stem cells, cells homing, E selectin ligand., Fucosyltransferase, ex vivo treatment, yeast system, silkworm system., Bone marrow transplantation, blood disease.

CA155

GENERATION OF HIGH DENSITY RED BLOOD CELLS FROM PLURIPOTENT STEM CELLS IN A BIOREACTOR AND APPLICATION IN AN ACUTE TRANSFUSION INJURY MOUSE MODEL

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Globally an estimated 112.5 million units of blood are collected for transfusion applications from blood donors. Continual advancements in the fields of lineage differentiation, bioprocessing and scale-up culture have brought closer the reality of using hiPSCs-differentiated cells for therapeutic applications and regenerative medicine. One such potential is the use of hiPSCs to generate O –ve universal RBCs for transfusion applications. However, unlike most cell therapies, generating RBCs for clinical application poses unique bioprocessing and manufacturing challenges. The need to generate 2 trillion RBCs for each transfusion unit of blood (equivalent to 300 ml of donated blood) requires the development of ultra-high density cultures of cells. We demonstrate significant progress in solving the manufacturing challenge by: 1. Implementing efficient reprogramming of hiPSC in suspension microcarrier cultures at the start of the process. Screening hundreds of clones and selecting dozens with both features of high expansion capability (greater than 10-fold) and differentiation to early hematopoietic lineage, positive for CD34 and CD43 markers (above 70%). 2. Initiating of the mesoderm differentiation in suspension culture and selection of clones with at least 20-fold expansion and production of T-bra and KDR +ve cells (> 30% expression) 3. Simplifying differentiation with implementation of designs of experiments to use small molecules and reduced cytokine cocktails towards the erythroblast lineage. Screening a second stage for high expandability to erythroblasts, > 20,000 fold or more; while decreasing cost of goods by 10 fold. 4. Applying high intensity culture methods using ultrasound to concentrate erythroblast expansion to achieve greater than 25 million cells/ml in controlled bioreactor cultures. 5. Applying shortened, simplified enucleation protocol with inactivated OP9 co-cultures and screened plasma sources to drive enucleations rates up by 10 fold or more from 6% to 65%. 6. Testing the final RBCs, injecting 1 billion cells per animal in an acute transfusion injury mouse model to demonstrate their ability to restore oxygenation and longer survival. We present solutions en route to developing a scalable-process for generating high density culture of functional RBCs from hiPSCs.

Funding source: A*STAR

Keywords: Bioprocessing;, haematopoietic stem cells;, acute transfusion model

NEURAL

CA182

APPLICATION OF MESENCHYMAL STEM CELLS IN TREATING CISPLATIN-INDUCED HEARING LOSS IN MICE

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Mesenchymal stem cells (MSCs) have been demonstrated to be isolated from different origins of tissues, such as bone marrow, placenta, umbilical cord, adipose tissues, skin tissues and etc. They share similar characteristics and can secret many anti-inflammatory molecules and growth factors for tissue repair and remodeling in many inflammatory disorders. However, whether the skin-derived MSCs (SMSCs) could exert beneficial effects on repairing the cochlear damage and improving the hearing loss remain unclear. Cisplatin is an extensively used chemotherapeutic agent, and one of its most adverse effects is ototoxicity through inflammation and oxidative stress. In this study, we investigated the therapeutic effects of SMSCs on cisplatin-induced hearing loss in mice. Two independent experiments were designed for modelling the cisplatin-induced hearing loss in mice: (i) chronic toxicity: 4 mg/kg dose for the intraperitoneal injection once a day for 5 consecutive days; (ii) acute toxicity: 25 mg/kg, one intraperitoneal injection on Day one. These mice were then treated with 1x106 or 3x106 SMSCs through tail vein injection into mice three days after cisplatin injection. Our data suggested that 3x106 SMSCs could significantly improve the hearing loss and rescue the loss of cochlear hair cells in the cisplatin-treated mice. Furthermore, whole transcriptome analyses of SMSCs and the mouse cochlear tissues by RNAseq revealed the underlying mechanisms of the therapeutic effects of SMSCs on cisplatin-induced hearing loss in mice. In conclusion, SMSCs showed the potentials for clinical application in treating cisplatin-induced hearing loss.

Keywords: hearing loss, mesenchymal stem cells, cochlear hair cells

CA191

NOVEL NEUROPROTECTIVE EFFECTS OF MELANIN CONCENTRATING HORMONE IN PARKINSON'S DISEASE

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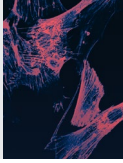
Acupuncture has shown the therapeutic effect on various neurodegenerative disorders including Parkinson's disease (PD). While investigating the neuroprotective mechanism of acupuncture, we firstly found the novel function of melanin-concentrating hormone (MCH) as a potent neuroprotective candidate. Here, we explored whether hypothalamic MCH mediates the neuroprotective action of acupuncture. In addition, we aimed at evaluating the neuroprotective effects of MCH and elucidating underlying mechanism in vitro and in vivo PD models. First, we tested whether hypothalamic MCH mediates the neuroprotective effects of acupuncture by challenging MCH-R1 antagonist (i.p.) in mice PD model. We also investigated whether



MCH has a beneficial role in dopaminergic neuronal protection in vitro primary midbrain and human neuronal cultures and in vivo MPTP-induced, Pitx3− / − , and A53T mutant mice PD models. Transcriptomics followed by quantitative PCR and western blot analyses were performed to reveal the neuroprotective mechanism of MCH. We first found that hypothalamic MCH biosynthesis was directly activated by acupuncture treatment and that administration of an MCH-R1 antagonist reverses the neuroprotective effects of acupuncture. A novel finding is that MCH showed a beneficial role in dopaminergic neuron protection via downstream pathways related to neuronal survival. This is the first study to suggest the novel neuroprotective action of MCH as well as the involvement of hypothalamic MCH in the acupuncture effects in PD, which holds great promise for the application of MCH in the therapy of neurodegenerative diseases.

Keywords: Parkinson's disease, MPTP, melanin-concentrating hormone

CA255

DIRECT REPROGRAMMING OF HUMAN FIBROBLASTS TO EXPANDABLE INDUCED DOPAMINERGIC NEURONAL PROGENITORS FOR PARKINSONS DISEASE THERAPY

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Personalized cell therapy is being explored as a promising treatment strategy for incurable diseases including Parkinson's disease (PD). Here, we directly reprogrammed human fibroblasts into induced dopaminergic neuronal progenitors (hiDPs) as an improved material for PD therapy. Using the method, we rapidly and safely obtained autologously transplantable cells. The hiDPs showed high expressions of genes reported which found positive correlation with therapeutic outcomes after transplantation. Transplantation of the hiDPs to PD mice model recovered the movement defect without ectopic cell growth. We also found the advantages of hiDPs such as high expansion capacity together with genomic stability during long-term culture, efficient differentiation to dopaminergic neurons with about 90% over total neurons without serotonergic neurons, and reproducible hiDP reprogramming from various adult cells. Thus, our hiDPs are expected to be a practically advanced option for the development of personalized and regenerative PD therapies.

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Keywords: Reprogramming, Parkinson's disease, Cell therapy

CA256

HUMAN ASTROCYTES FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS): PRECLINICAL AND CLINICAL DATA

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Amyotrophic lateral sclerosis (ALS) is a multifactorial disease, characterized by a progressive loss of motor neurons (MNs) that eventually leads to paralysis and death. Despite the selective MN death in ALS, there is solid evidence that malfunctional

astrocytes play a crucial role in the progression of the disease as well as other neurodegenerative diseases. Thus, transplantation of healthy astrocytes can potentially compensate for the function of the diseased astrocytes. We developed a GMP protocol for generation of astrocytes from human embryonic stem cells (hESC). The first stage of our protocol is derivation of astrocyte progenitor cells (APC) from hESCs. These APCs can be expanded in large quantities and stored frozen as cell banks. Further differentiation of the APC yields an enriched population of astrocytes (AstroRx®) that highly express GFAP, CD44 and GLAST. In vitro, AstroRx® possess the activities of functional astrocytes, including glutamate uptake, promotion of axon outgrowth and protection of MNs from oxidative stress. A secretome analysis showed that AstroRx also secrete several inhibitors of metalloproteases as well as variety of neuroprotective factors (e.g. TIMP-1&2, OPN, MIF and Midkine). Intrathecal injections of the AstroRx® to transgenic hSOD1G93A mice and rats significantly delayed disease onset and improved motor performance as compared to sham-injected animals. Safety study in immunodeficient mice showed that intrathecal transplantation of AstroRx® is safe. Transplanted AstroRx® cells attached to the meninges along the neuroaxis and survived for the entire duration of the study without formation of tumors or teratomas. First-in-Human phase I/IIa clinical trial to evaluate the safety and efficacy of AstroRx® in ALS patients is currently ongoing (clinical.gov ID: NCT03482050). Results from six-month post-treatment follow up of the first cohort showed no treatment-related serious adverse events (SAEs) or dose-limiting toxicities. In addition, assessment of ALSFRS-R slopes before and after treatment demonstrated encouraging preliminary efficacy of AstroRx® to slowdown disease progression.

Keywords: Astrocytes, Amytrophic Lateral Sclerosis, cell therapy

NEW TECHNOLOGIES

CA197

PROCESS DEVELOPMENT FOR SCALABLE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS IN SPINNER FLASKS AND BIOREACTOR

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Human pluripotent stem cells (hPSCs), either embryonic (hESCs) or induced (hiPSC), as well as their derivatives are considered as powerful cell-based tool for diverse clinical and industrial applications but only if mass production can be reached. Traditionally, adherent culture (2D) has been used as the standard technique for in-vitro expansion of hPSCs. However, clinical and industrial applications require billions of cells that cannot be produced by 2D culture. In addition, the standard culture media which enables self-renewal and pluripotency of the undifferentiated cells is based on expensive growth factors (GFs) and cytokines. Therefore, mass production of cells requires many resources, both laborious work and high costs. Accellta has developed a carrier-free suspension platform for culturing hPSCs as cell aggregates or single cells at high densities. This platform allows also the directed differentiation of hPSCs towards representative cells and progenitors of the three embryonic germ layers in suspension. As a complementary technology to the suspension culture platform, Accellta has developed a novel culture media, suitable for industrial scale, supplemented with small molecules (SM) as a cheap substitute for the expensive GFs. Thus, Accellta's unique technologies provide the solution for mass production of cells needed for cell-based therapy in a cost-effective manner. Here we present, a process development for culturing hPSCs in non-adherent, carrier-free suspension culture in standard culture media as well as in SM-based media in spinner flasks and in Ambr250 stirred bioreactor, followed by mesenchymal differentiation. Following 8 days of culture in spinner flask and Ambr250, > 61-fold increase in cell number, total of ~2 billion cells, was observed while culturing hPSCs in standard media. During the suspension culture the cells maintained their pluripotent characteristics. Moreover, following 7 days of cultivation in growth media, the hPSCs were subjected to mesenchymal differentiation. The resultant characterized cells can be defined as bona-fide MSCs. The establishment of cost effective and scalable processes is a major step forward for the development of hPSC-derived products for clinical applications as well as for industrial uses.

Keywords: MSC, STEM CELLS, hPSCs, hESCs, bioreactor, suspension culture

CA200

EXPLOITING GENOME EDITING TO GENERATE UNIVERSALLY TRANSPLANTABLE HUMAN CELLS

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Immunologic incompatibilities caused by the highly polymorphic Human Leukocyte Antigen class I (HLA-I) molecules between donor and recipient are limiting organ transplantation. Indeed, HLA mismatches will activate the recipient immune system, resulting in graft rejection. By exploiting the biology of these interactions, we developed a single-step gene editing approach to generate human pluripotent stem cells (PSCs) that, once differentiated into the cell type of interest, can be transplanted across HLA barriers. This strategy foresees insertion of one or more tolerogenic molecule within the β2 microglobulin (B2M) gene, a constituent of HLA-I. Consequently, engineered donor cells lack expression of their polymorphic HLA while stably expressing the tolerogenic molecules to inhibit Natural Killer (NK) and T-lymphocytes activation, major players in graft rejection. Beyond the already known HLA-E and HLA-G, we designed new constructs combining these invariant HLA-I molecules with an immunomodulatory small protein of viral origin. As proof of principle, we targeted the tolerogenic molecules within the B2M gene of different human cell lines using CRISPR/Cas9 technology, obtaining the transgene stable cell surface expression. The immune evasive capacity of sorted engineered cells was tested by co-culturing them with primary human NK cells. These assays showed that the expression of HLA-G, HLA-E or HLA-E in combination with the viral protein partially protects from lysis, decreasing cell death of 1.3, 1.6, and 2-fold in comparison to control, respectively. Giving these results, we have subsequently generated engineered PSCs lines and we are now differentiating them into clinically relevant cell types, as insulin-producing cells, to assess their in vitro and in vivo immune escape capability towards NK and T-lymphocytes. If successful, these studies will allow to obtain universally compatible, off-the-shelf transplantable human cells that inhibit graft rejection by interfering on different immune pathways.

Keywords: Universal donor cells, Genome editing, Immunomodulation

CA206

A NOVEL 3D FEEDER-FREE CULTURE SYSTEM OF HUMAN INDUCED PLURIPOTENT STEM CELLS BASED ON CHITOSAN MEMBRANES

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Human induced pluripotent stem cell (hiPSC) technology has promising potential in regenerative medicine so that culturing hiPSC pluripotency is a crucial issue for advanced applications. Although recombinant matrix-based feeder-free hiPSC culture systems have been developed, the expensive and complicated manufacturing process still hampers the progress of hiPSC technology. Chitosan, a versatile biocompatible polysaccharide, has been reported to serve as a three-dimensional (3D) cell culture system that promotes the physiological activities of mesenchymal stem cells and cancer cells. In the current study, we demonstrated that chitosan membranes sustained the proliferation and pluripotency of hiPSCs in long-term culture. On that membranes, hiPSCs were self-assembled into 3D spheroids with the average diameter ~100 μm. These hiPSCs could be directly differentiated into three-germ layers with 3D structures such as neural stem cell-, cardiomyocyte-, or hepatocyte-like spheroids. Collectively, chitosan membranes not only sustained the long-term culture of hiPSCs but also efficiently manipulated the lineage-specific differentiation. This convenient biomaterial platform allows effective maintenance and expansion of hiPSCs for tissue engineering, disease modeling, and drug screening.

Funding source: MOST 104-2320-B-002-047 and 108-2321-B-002-047

Keywords: human induced pluripotent stem cell, 3D feeder-free culture system, chitosan membranes

CA217

EFFICIENT IN VIVO GENOME EDITING MEDIATED BY HUMAN STEM CELLS-DERIVED EXTRACELLULAR VESICLES CARRYING DESIGNER NUCLEASES

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Precise genome modification mediated by designer nucleases (DNs) has become an indispensable procedure in basic research, biotechnology and, more recently, in therapeutic applications. Such technology allows for insertions, deletions or single nucleotide exchange at specific and predefined genomic locations. In most instances, the activity of DNs is needed only transiently, since prolonged expression may lead to increased off-target activity and genotoxicity. However, the currently used transient DNs delivery methods are either inefficient or associated with considerable risk of immune response in the host cells. Particularly, efficient genome editing in vivo remains a challenge. Thus, in this work, we employed extracellular vesicles (EVs), which naturally mediate horizontal gene transfer, as carriers of DNs. First, we used umbilical cord-derived mesenchymal stem cells (UC-MSCs), which are the least immunogenic cells known so far, as source cells for isolating EVs carrying zinc finger nucleases, transcription activator like effector nucleases and the clustered regularly interspaced short palindromic repeat/Cas9 nuclease (CRISPR/Cas9) system. In our proof-of concept study, we achieved up to 6% of enhanced green fluorescent protein (EGFP) gene knockout in vitro and importantly, up to 50% of EGFP gene knock out in vivo, particularly in the liver of the experimental animals. Second, we validated human induced pluripotent stem cells-derived EVs as delivery vehicles of the CRISPR/Cas9 system for therapeutic targeting of proprotein convertase subtilisin/kexin-9 (Pcsk9) gene, which overexpression is implicated in hypercholesterolemia. We achieved over 40% of Pcsk9 gene knockout in mouse liver, as verified by next generation sequencing. Together, our results provide strong evidence that stem cells-derived EVs constitute a very useful tool in delivering DNs in vivo, which may be harnessed for future clinical applications to treat human diseases.

Funding source: This work was supported by the projects: Homing Plus/2013-7/3 from the Foundation for Polish Science and SONATA12: UMO-2016/23/D/NZ3/01310 from the National Science Centre of Poland to SBW.

Keywords: genome editing, stem cells, extracellular vesicles

CA221

HUMAN PLURIPOTENT STEM CELL-DERIVED EXOSOMES AND NANOVESICLES RESCUE DERMAL FIBROBLASTS FROM PHOTO-INDUCED AND NATURAL AGING

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Stem cells secreted numerous paracrine factors, such as growth factors, cytokines, and some extracellular compartments. Among them, extracellular vesicles (EVs) derived from various types of stem cells have emerged as a therapeutic resource for regenerative medicine, because they can stably deliver valuable cellular contents to other recipient cells. However, to date, therapeutic effect of human pluripotent stem cell-derived EVs have not been clearly elucidated. In this study, we purified exosomes, a representative EV with a diameter of 30-120 nm from the medium conditioned by human induced pluripotent stem cells (iPSCs) and examined the effects on the photo-aged and naturally senescent human dermal fibroblasts (HDFs). In addition, we prepared cell-negineered nanovesicles (CENVs) by serial extrusion of human iPSCs through membranes with different pore sizes and investigated their therapeutic effect on the aging of HDFs as well as the physicochemical characteristics. The photoaging and natural senescence of HDFs were induced by UVB (315 nm) irradiation and repetitive sub-cultures over passage number 28, respectively. MTT and transwell assay demonstrated that both iPSC-exosomes and CENVs promoted proliferation and migration of aged HDFs. Quantitative real-time PCR analysis also revealed that the two types of human iPSC-derived vesicles restored aging-related alterations of gene expression, including constituent of extracellular matrix, matrix-degrading proteinases, and tumor suppressors. These results indicate that human iPSC-derived exosomes and CENVs have potentials to ameliorate the dermal fibroblasts aging. Meanwhile, it is interesting that the CENVs have a therapeutic effect similar to the exosomes, while the yield of the CENVs was fifty to hundreds of times than that of the exosomes. Taken together, these finding could provide a technical advance toward the application of human pluripotent stem cell-derived nanovesicles for treatment of skin aging.

Funding source: This study was supported by the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (NRF- 2018R1C1B6007644).

Keywords: human induced pluripotent stem cell (iPSC), cell-derived nanovesicles, skin aging

CA222

PEPTIDES-MEDIATED IMPROVEMENT OF THERAPEUTIC POTENTIAL OF HUMAN PLURIPOTENT AND MESENCHYMAL STEM CELLS

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Developing a highly defined culture condition is crucial for the clinical application of pluripotent or mesenchymal stem cells (PSCs or MSCs). For efficient culture of PSCs and MSCs, we tried to coat the surface of culture dishes with the peptides derived from adhesion motifs of the extracellular matrix (ECM) proteins, such as integrin, fibronectin, and laminin, and extracellular motifs of growth factor receptors and growth factors. We evidenced the capacity of the fibronectin-derived peptide, PHSRN-GRGDSP, in enhancing the adhesion, alkaline phosphatase activity, and pluripotency-related gene expression in stem cells, compared to the extracellular matrix (ECM) mimetics and Matrigel. Moreover, we tested the capacities of growth factor receptor-derived peptides, namely MCR-1, MCR-2, MCR-3, and MCR-4, on the proliferation and the differentiation of PSCs and Wharton's jelly-

derived MSCs. Interestingly, our study revealed the enhanced proliferation and the osteogenic differentiation of MSCs upon culture with the MCR-2 peptides. Moreover, the injection of MCR-2-cultured cells into the joints of an experimental arthritis animal model showed a marked improvement of the arthritis symptoms. Taken together, our results demonstrated the peptides for setting up a defined culture condition for the efficient culture of PSCs and MSCs, and for the enhanced osteogenic differentiation of MSCs with robust in vivo anti-arthritis activity.

Funding source: This research was supported by a grant from National Research Foundation (NRF) funded by the Korean government (Ministry of Education, Science, and Technology) (grant no.: 2019M3A9H1030682).

Keywords: anti-arthritis, ECM motif-derived peptides, Growth factor receptor-derived peptides

CA270

DIFFERENTIATION OF IPS CELLS CAN BE EVALUATED BY QUANTIFYING EXOSOME CONCENTRATION AND CD63 EXPRESSION IN CELL CULTURE SUPERNATANT

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Quantitative evaluation of cell conditions during cell culture is important for improvement of cell production efficacy. In cell manufacturing, the major verification method for cell culture is optical microscopic observation so far. Another aproach is metabolites measurement in cell culture supernatant. However, microscopic images or metabolites do not necessarily accurately reflect cell conditions. In recent years, exosomes have attracted attention as cancer biomarkers. Because exosomes possess wide variety of information of source cells, we expect them as effective index of cell conditions. In this study, we demonstrated evaluation of exosomes in cell culture supernatant to verify over time alteration of exosome concentration and exosome specific marker expressions of CD63 during differentiation of iPS cells. First, iPS cells (201B7-Ff) were differentiated to dopaminergic progenitor cells and cell culture supernatant was obtained on day 0 (undifferentiated), 8 (differentiation stage) and 12 (differentiated) after cell seeding. Then cell culture supernatant was ultracentrifuged and exosome concentration was analyzed by resistive pulse sensing. As the results, exosome concentration was reduced over time during differentiation. Next, we analyzed expression of CD63 in supernatant by ELISA. We also analyzed correlation between CD63 expression on day8 and CORIN (floor plate marker) expression in cells on day12. As the results, expression level of CD63 was transiently induced on day8 and CD63 expression on day8 was highly correlated with CORIN expression on day12. We showed that exosome concentration in supernatant was reduced during differentiation, which indicated that it could function as index of differentiation for iPS cells. Exosome marker expression of CD63 in supernatant during differentiation was highly correlated with differentiation marker in cells after differentiation. This fact suggested that differentiation level of final product is able to estimate by quantifying CD63 expression in supernatant. We conclude that exosome can be a novel evaluation index not only for cancer diagnostics, but also for cell differentiation during cell culture.

Funding source: This research was partly supported by the KBIC Research Grants-in-Aid for Young Researchers.

Keywords: exosome, monitoring cell culture, iPS cell

PLACENTA AND UMBILICAL CORD DERIVED CELLS

CA243

HUMAN PLACENTAL MSC-SECRETED IL-1B ENHANCES NEUTROPHIL BACTERICIDAL FUNCTIONS DURING HYPERVIRULENT KLEBSIELLA INFECTION

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Hypervirulent Klebsiella pneumoniae (hvKP) causes severe infections even in healthy individual by escaping surveillance and killing from polymorphonuclear neutrophils (PMNs), the first-line leukocytes in bacterial infections; moreover, emergence of multidrug-resistance strains further limit treatment options. We therefore assessed whether multilineage mesenchymal stem cells (MSCs), best known for immunomodulation towards T cells, could be therapeutic towards highly virulent bacterial infections via modulation of PMNs. We found that both bone marrow MSCs and placental MSCs (PMSCs) preserve in vitro PMN survival, but only PMSCs significantly enhance multiple PMN bactericidal functions including phagocytosis through secretion of interleukin-(IL-)1β. PMSC treatment of hvKP-infected mice suppressed T and NK responses as expected, but could preferentially recruit PMNs and enhance anti-bacterial functions to allow for disease survival; IL-1β knockdown in PMSCs significantly decreased hvKP clearance, worsening survival and resulting in 100% lethality. Our data strongly implicate possible use of PMSCs for infections of PMN-resistant hvKP strains.

Keywords: Placental mesenchymal stem cells (PMSCs), Polymorphonuclear neutrophil (PMN), Klebsiella pneumoniae (KP)

Theme: Modeling Development and Disease

ADIPOSE AND CONNECTIVE TISSUE

MDD105

UTILIZING TGFBR1 MUTATED AND CORRECTED HIPSC LINES FOR DISEASE MODELLING OF LOEYS-DIETZ SYNDROME

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Loeys Dietz Syndrome (LDS) is a connective tissue disorder that predisposes patients to premature death due to aggressive aneurysms, dissections, and rupture of vasculature. There is no known cure and a lack of full understanding of etiology. The emergence of hiPSCs for disease modelling provides a virtually limitless source of autologous cells, which allows insight into the pathophysiology of human diseases. This research project is set out to model LDS. It aims to recapitulate the disease phenotype in vitro through the differentiation of LDS patient-derived iPSCs into endothelial cells (ECs), smooth muscle cells (SMCs), and fibroblasts, which are key players of the vascular network. CD34+ cells from three LDS Type I patients were reprogrammed into iPSCs and characterized concerning pluripotency marker expression, in vitro differentiation potential, and genomic stability. LDS-Patient iPSCs have been successfully differentiated into CD31+/CD144+ ECs through a mesodermal intermediate stage, with high differentiation efficiencies. In addition, LDS-Patient ECs express typical EC markers, form networks on a matrix, and exhibit sheet migration. With a few modifications, this protocol was able to generate CD140b+ SMC-like cells that express known SMC markers TAGLN/ACTA and TAGLN/CNN1. These cells can support ECs in the formation of networks on a matrix and contract slightly upon stimulation with carbachol. LDS-Patient iPSCs were differentiated into fibroblast-like cells by activation of epithelial-to-mesenchymal transition during mesoderm induction. The protocol generates CD44+/CD90+/CD73+/CD105+/Vimentin+ fibroblast-like cells that are capable of being cultured on plastic. The fibroblast-like cells also show positive staining for extracellular matrix proteins Collagen I, Collagen III, and Fibronectin. These LDS-hiPSC derived vascular cell types should now serve as a basis for disease modelling purposes. Recapitulating the vasculature in vitro can offer a robust platform for elucidating disease pathology and developing LDS-patient specific stem cell based therapies.

Keywords: hiPSC-derived vascular cells, disease modelling, TGFBR1 mutation

CARDIAC

MDD115

THE NOVEL FUNCTIONS OF MOLECULAR CHAPERONE UNC45B IN CARDIAC CELL DEVELOPMENT

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The sarcomere is a building block of contraction muscle, and it is precisely assembled by hundreds of proteins. It has been shown that mutations in the genes coding sarcomere proteins result in cardiomyopathy, the leading cause of morbidity and mortality worldwide. Although the function and structure of sarcomere have been well studied, the precise process of sarcomere formation remains to be elucidated. Molecular chaperones have been shown to play an important role in modulating protein folding, maintaining protein stability and preventing protein damage. Recently, we have successfully generated a human cardiac MYH6:mCherry reporter line and identified a molecular chaperone, UNC45B, that is highly expressed in MYH6:mCherry positive population. UNC45B is known to modulate myosin assembly and myosin head domain folding, knockdown of Unc45b in zebrafish resulted in impairment of heart looping and dysfunction of ventricle followed by severe cardiac edema. Furthermore, knockout of Unc45b in mice caused early cardiac defects in heart looping. These findings suggest that Unc45b not only has a function in myosin assembly, but also may have an additional function in early cardiac development. However, the role of UNC45B in human cardiac development remains unclear and whether mutations in UNC45B will cause cardiomyopathy still needs to be investigated. However, a human sample is always a bottleneck for studying human genes. Human embryonic stem cells (hESCs)-derived cardiomyocytes (CMs) provide great potential for studying cardiac development and serve as a disease model for pathological mechanisms studying. Here, we ablated UNC45B in hESCs using the CRISPR/Cas9 technique. Interestingly, UNC45B-/- hESCs can successfully differentiate into CMs, but possess disorganized sarcomere structure in

UNC45B-/--derived CMs. Furthermore, we demonstrated that the ablation of UNC45B impedes sarcomere formation. Notably, we performed a hypothesis-driven chemical screening and excitingly identified a combination of chemical compounds that can rescue disorganized sarcomere phenotype. These findings may provide a potential treatment for future cardiomyopathy.

Keywords: Sarcomere formation, UNC45B, hESC-derived cardiomyocyte

MDD122

TARGETED GENOME EDITING IN HUMAN INDUCED PLURIPOTENT STEM CELLS FROM ARRHYTHMOGENIC CARDIOMYOPATHY USING CRISPR-CAS9.

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Application of genome editing for cardiomyopathies has been recently suggested as a potential therapeutic intervention. We examined the ability of CRISPR/Cas9 to serve as a therapeutic intervention for arrhythmogenic cardiomyopathy (AC). Patient-specific-hiPSCs were generated from AC patient carrying a pathogenic mutation in PKP2. Patient's hiPSC mutation was genetically corrected using CRISPR/Cas9-based mutation correction and homologous recombination. The corrected cells served as isogenic-controls for the AC-hiPSCs. Molecular, structural and functional assessment was conducted to assess the phenotype of the diseased and corrected cells. Optical mapping was used to characterize signal propagation using engineered hiPSC-derived cardiac tissues. Finally, we established a model for stratifying the risk for arrhythmogenesis using an electrophysiological study in a dish (EPS-in-a-dish) approach. CRISPR/Cas9 corrected-hiPSC expressed normal mRNA and protein levels of PKP2 based on real-time PCR and western-blots /immunofluorescence, respectively. Lipid droplet accumulation was significantly reduced in the corrected-hiPSCs when compared to AC-hiPSCs and was at similar levels to that of healthy-control-hiPSCs. Optical mapping revealed a significant increase in conduction velocity in the corrected-hiPSCs 61±2cm/s compared with AC-hiPSCs 33±0.6cm/s ($p<0.001$) and was similar to that of control cells. The improved conduction velocity was associated with improved expression of Cx-43 and improved kinetics of INa in patch-clamp studies. Finally, the corrected-hiPSCs demonstrated decreased vulnerability for developing arrhythmia based on the EPS-in-a-dish study when compared to AC-hiPSCs. CRISPR/Cas9 based therapeutic genome editing results in the restoration of functional and structural abnormalities associated with AC and may serve as a potential therapeutic intervention for genetic cardiomyopathies.

Funding source: Israel Science Foundation

Keywords: Stem Cell, Arrhythmia, Cardiac

MDD133

CHARACTERIZATION OF VASCULARIZED HUMAN CARDIAC ORGANOIDS FOR CARDIAC HYPERTROPHY MODELLING

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Over the last few years, the self-assembly of cells into organoids has emerged as an interesting technique to create a 3D cell environment that can be used in cellular assays to study disease and test compounds. The more in vivo-like cell orientation, comprising a combination of relevant cell types, can potentially provide information from the organoids about the state of the cells that is not possible in traditional 2D cell cultures. Here, we report on the establishment of a co-culture 3D cardiac hypertrophy model in an organoid format. Cardiac organoids consisting of human induced pluripotent stem cell derived cardiomyocytes (hiPS-CMs), human primary cardiac fibroblasts (CFs), and human umbilical vein endothelial cells (HUVECs) were formed in AggreWells for 48h. The cardiac organoids were subsequently transferred to 6-well suspension plates in a shaking incubator and stimulated electrically for up to 4 weeks. For hypertrophy experiments, the cardiac organoids were exposed to endothelin-1 (ET-1) at 1 – 100 nM for up to 72 h. The cardiac organoids were analyzed based on beating properties, cell maturity and hypertrophy biomarkers. The cells within the cardiac organoids showed expression of cardiac troponin T, vimentin, and CD31, and networks of connected endothelial cells were observed. A hypertrophy disease state could be induced in the cardiac organoids as revealed by increased BNP expression. By electrically stimulating the organoids at different frequencies, their beat rate could be altered which was further used to study cell metabolism. In conclsuison, these cardiac organoid show potential to be used for hypertrophy modelling.

Keywords: Cardiac organoids, Disease modelling, Cardiac hypertrophy

MDD444

MINIATURIZED ENGINEERED HEART TISSUES FROM HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CO-CULTURE

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Engineered Heart Tissues (EHTs) on-chip are of growing interest as tools to study the ef-fects of drugs and disease on the human heart in vitro. EHTs form in 3D through the self-assembly of cell bundles around flexible pillars. EHTs have been reported to recapitulate human heart response more closely than standard 2D cultures. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from patients can be used to develop dis-ease models or personalize treatments. However, to enable industry-level high-throughput screening, miniaturized formats are of large interest. Moreover, the inclusion of somatic cells of the heart like fibroblasts and endothelial cells from hiPSC isogenic with the CMs may mimic physiological conditions even more closely. Here, we pre-sent three miniaturized EHT-chips of 3, 2 and 1 µL volume composed respectively of ~47000, 31000 and 16000 hiPSC-CMs with hiPSC-derived cardiac fibroblasts in a colla-gen/Matrigel based extracellular matrix. The chips were fabricated by a combination of sili-con micromachining and PDMS-based soft lithography. Contractile activity was recorded and evaluated using MUSCLEMOTION software. The miniaturized EHTs started spontane-ous contraction after 72 hours. As expected, upon increasing pacing frequency a decrease in contraction duration, relaxation time and peak-to-peak time was seen in all chips. Moreover, stimulation up to 1.6 Hz did not decrease contraction ampli-tude as typically seen in (more immature) 2D cultures. Isoprenaline decreased contraction duration, relaxation time and peak-to-peak time as expected in all chips. Immunostain-ing showed troponin-T and alpha-actinin-positive cardiac sarcomere network. This research shows that the miniaturized EHT-chips containing solely hiPSC-derived cells, exhibited physiologically relevant contractile responses to pacing and Isoprenaline. The miniaturized EHT-chips we propose thus facilitate first steps towards high-throughput (personalized) drug screening and disease modeling.

Funding source: The work described in this paper was supported by the Netherlands Organ-on-Chip Initia-tive, an NWO gravitation project funded by the Ministry of Education, Culture and Science of the government of the Netherlands (024.003.001).

Keywords: Engineered Heart Tissue, Cardiac, Organ-on-chip

MDD455

ISOGENIC SETS OF HIPSC-CMS HARBORING KCNH2 MUTATIONS CAPTURE LOCATION-RELATED PHENOTYPIC DIFFERENCES

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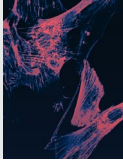
Congenital long QT syndrome type 2 (LQT2) is one of the most common genetic channelopathies of the heart leading to life-threatening cardiac arrhythmias and sudden cardiac death (SCD). Patients exhibit a broad range of phenotypes as a result of mutations in the potassium ion channel gene KCNH2. The phenotypic variation is strongly associated with the location of the mutation within the ion channel, e.g, mutations in pore region tend to manifest a more severe clinical course than non-pore mutations. Thus, understanding the underlying genetic basis of the disease not only has significant diagnostic implications, but also prognostic and therapeutic value. Patient-derived hiPSCs can be used to investigate the pathogenicity of these mutations, but interline variability complicates the use of these models to reflect genotype-phenotype relationships. To overcome this issue, we created a set of isogenic hiPSCs by introducing KCNH2 mutations into a well-established and validated wild-type hiPSC line. Aiming to compare the effect of variants in different regions of the protein, we introduced mutations either in the pore or in the C-terminal region of the KCNH2-encoded ion channel. Molecular and electrophysiological analysis indicated differing trafficking defect phenotypes depending on the mutation. We confirmed prolongation of action potentials and field potentials of the hiPSC-CMs, with differences detected between the mutations when measured as paced 2D monolayers. This was also reflected in the cytosolic Ca2+ transients and contraction kinetics of the different lines. Exposing the hiPSC-CMs to E-4031 (channel blocker) revealed different sensitivities between the lines, with the hiPSC-CMs containing a mutation in the pore region more susceptible to the proarrhythmic effects of the channel blocker. Our findings indicate that phenotypic differences related to the location of the KCNH2 mutation in LQT2 patients are reflected in hiPSC-CMs under genetically controlled conditions. The results



also demonstrate the capability of hiPSC-CMs to correlate the functional effects of KCNH2 mutations with subtle differences in disease phenotype and could ultimately facilitate patient risk stratification.

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Keywords: Long QT Syndrome 2, Disease modelling, Isogenic

EARLY EMBRYO

MDD144

DECIPHERING THE INTERACTOME OF LHX1 IN STEM CELL EMBRYOID BODIES AND MOUSE CHIMERAS

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Embryonic development is driven by a series of molecular instructions encoded by the transcription factors (TFs) that drive the formation of the body plan and the specialisation of tissue precursor cells. Analysis of mouse genetic models has demonstrated that key TFs such as LIM homeobox 1 (Lhx1) and Otx2 are indispensable for head and face development, with severe head truncation phenotype seen in the knock-out mice. The aim of this project is to identify and functionally characterise the interacting proteins and genetic targets of LHX1 to refine the gene regulatory network for embryonic head formation. We have conducted RNA-sequencing, ATAC-sequencing and used an improved mutant DamID method on gastrulating mouse embryos to identify genomic regions that are either directly regulated by LHX1 or Lhx1 downstream targets. We identified the TFs FoxD4, Kctd1, Zkscan2 and Zfhx4 as newly discovered targets of LHX1 that may have a key role during embryonic head development. LHX1 has been shown to act together with LDB1 and SSDP1 to regulate transcription. To discover interacting partners that may be present in this TF complex, we engineered embryonic stem (ES) cell lines to express a BioID2 moiety fused to Lhx1 by doxycycline activation. The BioID2-Lhx1 fusion protein was able to biotinylate proximal proteins in embryoid bodies, which were then identified by affinity-pulldown coupled to mass-spectrometry. Furthermore, we have we generated chimera mouse embryos using BioID2 expressing ES cell lines as a novel technique to identify protein interactors of TFs in vivo. This project has helped elucidate the composition of the LHX1 TF complex and is an integral step of elucidating the gene regulatory network for embryonic head formation. By determining novel gene regulators in the LHX1 network, we gained an insight into the cellular and molecular mechanisms building the body plan in the early mammalian embryo.

Funding source: Australian Research Council

Keywords: gene regulatory network, embryonic development, proteomics

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

MDD151

GENERATION OF IPSCS FROM PATIENTS WITH MODY2 AND NEONATAL DIABETES DUE TO GLUCOKINASE MUTATIONS

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Glucokinase (GCK, hexokinase IV) is a member of the hexokinase family that phosphorylates glucose to glucose-6-phosphate during glycolysis process in pancreatic β-cells and hepatocytes. This is the rate limiting step in glucose metabolism and enables pancreatic β-cells and hepatocytes to respond appropriately to blood glucose level. Mutations in GCK gene could alter glucose homeostasis causing either hyperglycemia or hypoglycemia. Heterozygous loss of function mutation in GCK gene causes maturity-onset diabetes of the young 2 (MODY2), a monogenic form of diabetes that is characterized by mild fasting hyperglycemia, while homozygous inactivating mutation leads to permanent neonatal diabetes mellitus (PNDM), which is characterized by severe hyperglycemia. Understanding the development of diabetes due to GCK mutations is not fully understood due to lack of human models. In the current work, we were able to generate induced pluripotent stem cells (iPSCs) from blood cells of two patients diagnosed with heterozygous and homozygous mutations in GCK. In patient's samples, the heterozygosity and homozygosity were confirmed using whole exome sequencing (WES) followed by Sanger sequencing. Three iPSC lines were established from each patient, in which the mutation was confirmed using Sanger sequencing. All iPSC lines were extensively characterized using different approaches, including immunostaining, RT-PCR, karyotyping, STR, and embryoid body (EB) formation. The generated cell lines expressed pluripotency markers at both mRNA and protein levels and were able to differentiate into all three germ layers upon spontaneous differentiation. To generate isogenic controls, we used CRISPR/Cas9 knock-in approach to correct the mutation in iPSCs with homozygous mutation. One corrected patient-specific iPSC line was established. Mutated and corrected iPSCs will be used to understand the role of GCK mutations in the development of MODY2 and neonatal diabetes. This human iPSC model can be used for understanding the disease pathogenesis, drug screening, and cell therapy.

Keywords: Glucokinase, MODY2, iPSCs

MDD162

ESTABLISHMENT OF HUMAN LIVER ORGANOID AS AN IN VITRO TOOL FOR PHARMACOKINETIC EVALUATION

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Assessing pharmacokinetic (PK) is essential for investigational new drug (IND) application. PK describes absorption, distribution, metabolism, and excretion, which determines the onset and duration of drugs. Liver plays important role in PK. Since PK differs between the species, it is impossible to use experimental animals to assess human PK. Human primary hepatocytes (PHs), liver microsomes, and recombinant drug metabolizing enzymes have been used to predict PK in human. Using human PHs is ideal, but they are expensive, disposable, and hard to obtain. In spite of these limitations, human PHs are requisitely needed to evaluate enzyme induction and metabolic profiling of drug candidate. Organoid is artificially grown mass that resembles in vivo organogenesis. A report describing method to cultivate adult stem cell derived human liver organoid (HLO) was published in 2015. HLO is originated from bile ductal cells and can be differentiated to hepatocytes. Hepatocyte derived from HLO, promising candidate to replace PHs, is relatively inexpensive to cultivate, can be obtained from relatively diverse sources including liver biopsies and small pieces of liver from alive or brain-dead donors. They can be frozen, thawed, and grow infinitely, which enables to perform replicate experiments. This study aims to establish PK model using HLO that can substitute human PHs. The major CYP expression patterns were analyzed in PHs and differentiated HLO. Unlike PHs, differentiated HLOs showed similar pattern of major CYP expression to human liver tissue, which suggested that using HLO might be better to evaluate metabolic profile of investigational drugs than PHs. HLOs were grown under various conditions to enhance the expression of major seven cytochrome P450 (CYP) enzymes that both FDA and EMA guidelines recommend to evaluate. Measuring the transcripts and activity of CYPs, phase II metabolic enzymes, and transporters in HLOs was conducted to find optimal clones that can substitute for PHs. The optimized HLO clones are to be used for evaluating PK and for drug development ultimately.

Keywords: Human liver organoid, Pharmacokinetics, Drug metabolizing enzymes

MDD164

PROGRESSIVE ENDOPLASMIC RETICULUM STRESS OVER TIME DUE TO HUMAN INSULIN GENE MUTATION CONTRIBUTES TO PANCREATIC BETA CELL DYSFUNCTION

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Insulin gene mutations have been reported to be a cause of permanent neonatal diabetes mellitus (PNDM), and are thought to act in a dominant negative way. Despite heterozygosity indicating the presence of one WT allele, most individuals with INS mutations still require lifelong insulin replacement. In this study, we focus on two heterozygous INS gene mutations: C109Y and G32V. The first mutation was reported in a Singaporean family, and the latter has not yet been reported, but was found in an Australian family. We hypothesize that both C109Y and G32V misfolded proinsulin restrict insulin secretion by directly binding to the WT proinsulin, or by increasing endoplasmic reticulum (ER) stress leading to β-cell death or dysfunction. We modelled the structures of both C109Y and G32V proinsulins, and observed conformational changes that will likely affect proinsulin processing into mature insulin. Then, we overexpressed C109Y and G32V human preproinsulin in a mouse insulinoma cell line, MIN6, and analysed the insulin granules using electron microscopy. We found that cells overexpressing mutant preproinsulin were different in terms of size and number of insulin granules compared to cells overexpressing WT preproinsulin. We also obtained skin fibroblasts from patients with the C109Y mutation as well as their WT family members, and reprogrammed their cells into induced pluripotent stem cells (iPSCs). These iPSCs were characterized before undergoing differentiation into β-like cells. These β-like cells were sent for single-cell RNA-sequencing (scRNA-seq), and gene ontology analyses revealed that cells with the heterozygous C109Y INS mutation upregulated genes involved in secretion and processing, as well as inflammatory response and apoptosis. Our findings revealed that heterozygous insulin mutations may result in initial compensation through upregulating genes involved in secretion. However, the β-cells have irregular insulin granules, as well as upregulated inflammation and apoptosis, which could lead to β-cell dysfunction in these patients with PNDM.

Keywords: Insulin mutation, ER stress, Pancreatic beta cell

MDD173

IMPROVING THE FUNCTIONALITY OF HUMAN PLURIPOTENT STEM CELL DERIVED HEPATOCYTES BY EMPLOYING KNOWLEDGE FROM HEPATOGENESIS

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Human pluripotent stem cells (HPSC) have successfully been differentiated into different cell types by mimicking the developmental stages as occurs in the embryo. The accuracy of this process accounts for the generation of functional specialized cells. HPSC-derived hepatocytes (HPSC-HEP) share many features with in vivo hepatocytes including cytochrome P450 enzyme (CYP) activity, glycogen storage, and expression of several drug transporters. However, they exhibit lower CYP activity, and produce lower levels of albumin. In addition, they still express fetal hepatic markers including AFP and other cell type markers such as the intestine marker CDX2, indicating to some extent incomplete programming. The differentiation protocol reported by Asplund et al. was shown to mimic the lever development and generates hPSC-HEP with enhanced functionality. The protocol starts with the differentiation of hPSC into definitive endoderm (DE). The DE cells are replated at day 7 and the differentiation continuous through hepatoblast into hPSC-HEP that exhibited mature hepatocyte features. In order to make further improvements of the differentiation protocol, the DE stage was modified to mimic, more accurately, hepatogenesis by the addition of the growth factor FGF7 and an inhibitor of the TGF-β/Activin/NODAL pathway. Herewith, generating ventral foregut cells already at day 7. The next stage was also modified to promote the hepatic fate from the ventral foregut by the addition of the growth factor FGF and BMP cytokines. Moreover, the hepatocyte growth factor was also provided to promote the proliferation of the hepatoblasts as emerges in liver development. Interestingly our results show, improved activity of several CYP enzymes further with increased hepatic functionality. Moreover, the albumin and urea secretion were significantly improved. In addition, the expression of the intestine marker CDX2 was also reduced. This study demonstrates the importance of precise and accurate reflection of liver development in the hPSC-HEP differentiation protocol to further improve the functionality of these cells. Additional improvements might be achieved by modifying later stages of the differentiation protocol by the addition of factors that promote the hepatocyte maturation by more precisely following in vivo hepatogenesis.

Keywords: hepatocytes, hepatogenesis, drug metabolism

MDD175

DECREASED GLUT2 AND GLUCOSE UPTAKE CONTRIBUTE TO INSULIN SECRETION DEFECTS IN MODY3/HNF1A HUMAN IPSC-DERIVED MUTANT BETA CELLS

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Heterozygous HNF1A gene mutations can give rise to maturity onset diabetes of the young 3 (MODY3), characterized by insulin secretion defects. However, the specific mechanisms of MODY3 in humans remain unclear due to lack of access to diseased human pancreatic cells. Here, we leveraged upon MODY3 patient-derived human induced pluripotent stem cells (hiPSCs) to study the effect(s) of a causal HNF1A+/H126D mutation on pancreatic cell function. Molecular dynamics simulations predicted that the H126D mutation alters the interaction of the residue with its neighbors, which could compromise its ability to bind to DNA and activate gene expression. Genome-wide RNA-Seq and ChIP-Seq analyses on MODY3 hiPSC-derived endocrine progenitors revealed numerous HNF1A gene targets affected by the HNF1A+/H126D mutation. We found a decrease in glucose transporter GLUT2 expression, which is associated with decreased glucose uptake function and decreased ATP production in the MODY3 hiPSC-derived β-like cells. We confirmed that human HNF1A directly upregulates GLUT2 expression and this regulation was disrupted by the H126D mutation. Overall, our MODY3-hiPSC-based findings reveal the importance of HNF1A in regulating GLUT2 and several genes involved in insulin secretion function that can account for the insulin secretory defect clinically observed in MODY3 patients. Modulation of these targets could serve as a viable means to restore the insulin secretory capacity of dysfunctional MODY3 β cells.

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Keywords: Diabetes, Beta cell, Insulin secretion

MDD176

THE TYPE 2 DIABETES RISK GENE STARD10 INFLUENCES PANCREATIC BETA CELL DIFFERENTIATION FROM HESC-DERIVED CRISPR/CAS9-MEDIATED KNOCKOUT CELLS

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There are to date hundreds of genetic loci that have been associated with the risk of type 2 diabetes (T2D), a chronic disease characterized by an absolute or relative lack of insulin due to pancreatic beta cell dysfunction coupled with insulin resistance. One such locus includes the STARD10 gene, which encodes a lipid-transfer protein, whose precise function in pancreatic beta cells remains unclear. It is a promising candidate gene as previous rodent studies revealed that it is involved in proinsulin processing and pancreatic beta cell insulin secretion. We hypothesize that STARD10 regulates human pancreatic beta cell maturation and function, and therefore dysregulation of STARD10 can contribute to the pathogenesis of T2D. We first aimed to evaluate the impact of STARD10 perturbation on human pancreatic beta cell differentiation using human embryonic stem cell (hESC)-derived cells with CRISPR/Cas9-mediated STARD10 knockout. STARD10 expression normally increases over the course of beta cell differentiation, similar to other well-known beta cell markers. STARD10-/- beta-like cells exhibited distinct transcriptomic changes, including a significant decrease in INS expression, as well as upregulation of PIRT and downregulation of PLA2G7 expression. PIRT and PLA2G7 respectively encode a phosphoinositide-interacting protein known to regulate ion channel activity, and a member of the phospholipase A2 group of proteins that may be involved in phospholipid metabolism. RNA-Seq analyses further revealed significant changes in cell cycle pathways. We also identified perturbations in a number of lipid species, such as triglycerides, in STARD10-/- beta-like cells, using lipidomics studies. Through studying the STARD10 gene, we seek to highlight actionable targets or lipid-related interventions that could pave the way for improved and more targeted strategies to tackle human beta cell dysfunction in T2D.

Funding source: IMCB A*STAR; NMRC OF-YIRG18May0040, MRC Programme Grant

Keywords: STARD10, pancreatic beta cells, CRISPR/Cas9

MDD439

RECAPITULATING HUMAN LUNG ALVEOLAR DEVELOPMENT USING ORGANOID TECHNOLOGY

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In the developing human lung the multipotent early tip epithelium sequentially generates the airway and then the alveolar structures. The tip cells spontaneously acquire alveolar features, such as lamellar bodies containing surfactant proteins, phospholipids and a more cuboidal shape, during the transition from airway branching to alveolar development. However, the underlying mechanisms which promote alveolar features in the tip epithelium in vivo remain unknown. We have developed a new self-renewing tip organoid model system recapitulating the distal lung tip epithelium at the alveolar stage using primary human lung cells. The new organoid system exhibits distinct alveolar signatures, which our previous branching stage-specific tip organoids do not have in self-renewing culture conditions. The alveolar stage tip organoids also exhibit heterogeneity, being comprised of at least two differentiated subpopulations and are largely committed to alveolar lineages. Through comparative analysis of the transcriptomes of the tip alveolar and branching stage organoids, we identified a combination of surface antigen molecules targeting the stage-specific, in vivo distal tip epithelial population. Using these surface markers, we discovered that the molecular acquisition of alveolar signatures precedes morphological changes during the transition from the branching to the alveolar developmental stages. Finally, we have used our tip organoid system to facilitate identification of developmental signaling cues and upstream transcription factors driving the onset of alveolar program during the transition period. Collectively, this study presents an ideal, lung organoid model for deciphering how the lung tip epithelium undergoes differentiation toward the alveolar lineage. It will provide further mechanistic insight to understanding further alveolar differentiation and maturation during the next stages of human lung development.

Funding source: MR/P009581/1

Keywords: organoids, alveolar differentiation, human lung development

MDD446

A HUMAN KIDNEY ORGANOID MODEL FOR CISPLATIN-INDUCED ACUTE KIDNEY INJURY

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Acute kidney injury (AKI) is a major global healthcare problem, and there is a need to develop human-based models to study AKI in vitro. Towards this goal, we have characterized human induced pluripotent stem cell (iPSC)-derived kidney organoids and their response to cisplatin, a chemotherapeutic drug that is associated with severe side effects on the kidney, particularly to the proximal segment of the kidney tubule. Different doses of cisplatin were added to the culture medium, and cisplatin-treated organoids (along with controls) were analysed by quantitative PCR and immunohistochemistry. We found that a single treatment with 50 µM cisplatin induces expression of kidney injury marker KIM1/HAVCR1 and inflammatory chemokine CXCL8, DNA damage and cell death in the organoids, but greatly impairs organoid viability. Co-localization of DNA damage marker γH2AX with the different cell types in the organoids revealed that

cisplatin predominantly targets proliferating cells. This suggests a general cytotoxic effect reminiscent of the drug's impact on tumour cells, contrary to the proximal tubule-specific damage observed in vivo. This lack of specificity correlated with low expression of cisplatin transporters in the organoids, providing one explanation for this observation. To improve viability, we developed a repeated low-dose regimen of 4x 5 µM cisplatin and found this caused less toxicity, while still inducing a robust injury response. Gene expression profiling of this regimen revealed a large set of classic AKI biomarkers among the highest induced transcripts, including TNFα, CXCL2, CCL2, HAVCR1 and LCN2. Gene Ontology and pathway analysis showed 'inflammatory response', 'apoptosis' and 'TNFα signaling' among the top enriched categories in cisplatin-treated organoids, in line with the major pathways that mediate AKI. Lastly, we found that differential gene expression in cisplatin-treated organoids overlaps significantly with previously published gene sets of AKI, thus underscoring the relevance of the model. This work validates the use of human kidney organoids to model AKI, with the potential to identify new biomarkers and develop better therapies.

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United States Army Medical Research and Development Command (W81XWH-17-1-0610)

Keywords: Human iPSC, Kidney organoids, Acute kidney injury

MDD457

MODELLING HUMAN NEPHROGENESIS USING HUMAN PSC-DERIVED PATTERNABLE AND FUNCTIONAL KIDNEY ORGANOIDS

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The incidence of end-stage renal disease (ESRD) is increasing at an alarming rate, but treatment options remain unchanged over the past 70 years. Directed differentiation of human pluripotent stem cells (hPSCs) into kidney organoids offers an unprecedented opportunity to study human kidney development and pathogenesis, for performing drug screening, and ultimately to generate functional kidney tissue for replacement therapy. Despite recent advances in generating kidney organoids form hPSCs, the ability to modulate various cellular components within kidney organoids has not yet been illustrated. Here, we report the establishment of a highly efficient protocol for differentiating hPSCs into kidney organoids comprised of segmented nephron epithelium, vascular endothelium and interstitium. Through stringent temporal modulation of canonical WNT-signalling pathway, we could preferentially modulate the choice of proximal versus distal lineage, as well as the vascular compartment in the kidney organoids. Within hPSC-derived kidney organoids, we identified that KDR+ vascular progenitors originated from a subpopulation of SIX2+SALL1+ cells that further differentiate and mature into CD31+ endothelial cells in response to VEGF-A secreted by podocytes. Following renal capsule implantation into an immunodeficient host mouse, kidney organoids acquired significant structural maturation, as represented by the formation of Bowman's capsule space surrounding glomerular capillary turfs of a human origin. The implanted kidney organoids exhibited size-selective dextran filtration and reabsorption; and the accumulation of putative filtrate within tubules, demonstrating functional maturation. Our work represents an advanced version of kidney organoids that promise to offer extensive utility in both basic science and clinical practice.

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Keywords: Organoid, Kidney, Patterning

MDD462

MODELLING AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE WITH PATIENT IPSC-DERIVED KIDNEY ORGANOIDS

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Autosomal recessive polycystic kidney disease (ARPKD) is a severe genetic disease occurring in neonates with poor prognosis and little therapeutic approaches. Mutations in either PKHD1 or DZIP1L were identified as drivers of this disease. Here, we have established a protocol to generate 3-dimensional (3D) kidney organoids using iPSCs derived from ARPKD patient fibroblasts. Compared to corrected-iPSC-derived 3D kidney organoids, ARPKD kidney organoids presented exacerbated cystic formation upon an increase of intracellular cAMP levels. Global mass-spectrometry analysis suggested energy reprogramming during cyst formation. Based on this, the drug targeting on the glycolysis was tested and showed significant

effects on cyst inhibition. Our work demonstrated that kidney organoids generated from ARPKD iPSCs could represent a powerful tool to model polycystic kidney disease for mechanistic studies and drug screening.

Keywords: Disease modelling, Kidney organoid, Drug screening

MDD483

STUDY OF NOVEL MOLECULAR DEFECTS IN HUMAN PANCREAS DYSFUNCTION

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Diabetes is a worldwide health problem caused by the loss or dysfunction of the insulin-secreting β-cells in the pancreas. Despite extensive research, fundamental processes during pancreas development remain to be determined, hampering our understanding of the disease mechanisms. Unelucidated forms of monogenic diabetes, arising from rare mutations in one single gene, represent invaluable models for identifying new targets of β-cell development and function. In this study, we identified novel putative disease-causing variants by next-generation sequencing of a cohort of patients with puberty-onset diabetes. Next, we focused on gene candidates, which play a transcriptional role during embryonic development, are abundantly expressed in mouse pancreas progenitor cells, and prioritized them for functional analyses. Specifically, we studied pathogenic missense variants identified in the gene glioma-associated oncogene homolog 2 (GLI2) encoding for the major Hedgehog pathway transcriptional effector. To investigate the role of this gene variant in pancreatic cell differentiation and function, we used the CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9-mediated genome editing in combination with human induced pluripotent stem cell (iPSC) directed β-cell differentiation. Employing these approaches, we engineered patient-like iPSC lines carrying the identified heterozygous missense variants. Functional assays showed that a variant in the C-terminal activation domain of GLI2 impairs human pancreatic endocrine development. Interestingly, we found that the variant alters the transcriptional activity of GLI2 and whole transcriptome analysis highlighted defects in downstream pathways during endocrine development. Thus, the identified variant in GLI2 might be responsible for a genetic predisposition to develop diabetes. Current experiments are ongoing to shed some light into the underlying disease mechanism.

Keywords: Diabetes, Pancreatic differentiation, Genome editing

MDD502

COMPARATIVE ANALYSIS OF HUMAN STEM CELL-DERIVED ISLET-LIKE CELL DIFFERENTIATION WITH SINGLE-CELL TRANSCRIPTOMICS

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Islets of Langerhans are crucial organs for sensing circulating glucose and nutrients and finely tuning blood glucose level. Destruction of islet tissues is one of the important causes of diabetes. Transplantable and functional islet-like cells differentiated from human stem cells are promising for diabetes therapy, but further studies are needed to fully understand the differentiation process. Here, we established two pancreatic differentiation systems, from human embryonic stem cells (hESCs) or endodermal stem cells (hEnSCs). hESC-derived cell clusters show similar composition of endocrine cell subtypes to human adult islets, robustly perform static and dynamic GSIS, and alleviate hyperglycemia in streptozotocin-induced type 1 diabetes mouse model. We reconstructed the hESC-pancreatic differentiation trajectory with single cell RNA sequencing (scRNA-seq). In hEnSC-pancreatic differentiation system, we also generate glucose-responsive β cells, but with poor hyperglycemia reversal capacity in vivo. In order to optimize the hEnSC differentiation system, we systematically characterized cells at posterior foregut (PF), pancreatic progenitor (PP), endocrine progenitor (EP), immature β cell (IB) and mature β cell (MB) stages during hEnSC differentiation and compared these stages with their counterparts during hESC-differentiation. In both systems, cells pass through posterior foregut, pancreatic progenitors, early and late endocrine progenitors, and hormone-expressing cells. However, these two systems also display different properties. At PF stage, hESC-derived cells, with high PDX1 expression, differentiate into pancreatic progenitors with high efficiency, while hEnSC-derived cells, with lower PDX1 expression, tend to be trapped in guttube-like status. At EP stage, hESC-derived cells, after multistep differentiation, become FEV-high EP cells, which majorly differentiate into β cells and minorly into enterochromaffin cells (ECs), while hEnSC-derived cells rapidly express FEV and generate more glucose-unresponsive ECs and less β cells. We identified pathways and conditions regulating β cell and EC fate determination by analyzing scRNA-seq data. Our study provides informative clues to generate more mature and functional human islet-like cells for diabetes treatment.

Keywords: Human islet cells, Human endodermal stem cells, Single-cell sequencing

EPITHELIAL

MDD192

IN VITRO MATURATION ENHANCES MUCUS SECRETION AND BACTERIAL COLONIZATION IN HUMAN INTESTINAL ORGANOID

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The human gastrointestinal (GI) tract has a large number of variety enterobacteria which commensals and pathogens is important for modulation of health and disease in vivo. Enterobacteria grow and colonize within the mucus layer overlaid intestinal epithelium that roles growth substrate but also ligands for enterobacterial adhesins. The in vitro model for host-enterobacteria direct interactions in the small intestine has limited by a lack of physiological and biological reconstituted intestinal epithelium model. Here, we established biological advanced in vitro human model for direct interaction between complex host epithelium and enterobacteria. We generated human pluripotent stem cells (hPSCs)-derived intestinal organoids (hIOs) applied in vitro maturation process and carried enterobacteria into intraluminal side of hIOs via microinjection. In vitro matured hIOs were enhanced cellular functionality such as proliferation, tight junctions and transporter activity for absorption of nutrients. Especially, in vitro matured hIOs were increased expression of glycoprotein-related genes and mucus layers thickness. We performed microinjection of enterobacteria into hIOs that increase of microbiome propagation in hIOs via scanning electron microscopy (SEM) imaging, colony forming (CFU) assay, fluorescence live imaging and observed binding of microvilli-microbiome in in vitro matured hIOs. Collectively, our study show that the obtained functional mucus layers in vitro matured organoid can be applied useful model for host epithelial-enterobacteria interaction 'symbiosis' study.

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Keywords: Human intestinal organoid, mucus layer, epithelial-bacteria interaction

MDD194

IN VITRO MATURE HUMAN INTESTINAL ORGANOIDS VIA STAT3 SIGNALING MAINTAINED ENGRAFTMENT AND MATURITY AFTER IN VIVO TRANSPLANTATION

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Human intestinal organoids (hIOs), derived from human pluripotent stem cells (hPSCs), have emerged in vitro as a new modality for the study of the intestinal molecule and cell biology. We recently developed an in vitro-maturation technique for generating functional hIOs from human pluripotent stem cells (hPSCs). To assess the portability of in vitro-mature hIO, hIO was transplanted under renal capsules of immunodeficient NOD-scid IL2Rgammanull (NSG) mice. Intestinal cell-specific markers were detected in all hIOs in vivo, and markers and function of intestinal maturation became evident only in the transplantation of in vitro-mature hIOs after short-term transplantation. We observed that mature hIO in vivo can successfully induce neovascularization in host vessels only one week after transplantation, even though CD31-positive cells were not detected before hIO transplantation. In addition, we constructed a STAT3 knockout (KO) human embryonic stem cell (hESC) line using CRISPR / Cas9-mediated gene editing to investigate the function of STAT3 in in vitro-maturation of hIOs. We found that STAT3 KO affects the maturation of hIO. STAT3 KO hIO shows an immature morphology with reduced size and budding in hIO even after in vitro-maturation and failed to maintain during in vivo transplantation. This study demonstrates that the key signaling pathway consisting of STAT3 controls the in vitro-maturation of hIO derived from hPSCs and plays an important role in engraftment after transplantation. Given that in vitro-maturation hIOs maintain a common karyotype, we suggest that they are an effective alternative source of cells that can be applied in vivo.

Funding source: This research was supported by a grant from the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT and Future Planning (2018M3A9H3023077)

Keywords: In vitro mature Human intestinal organoid (hIO), STAT3, in vivo transplantation

MDD195

REFINED TONSILLAR ORGANOIDS REVEAL LONGEVITY AND CROSSTALK BETWEEN TONSILLAR T CELLS AND EPITHELIUM

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Tonsil is an immune organ composed of T cell and B cell clusters surrounded by non-keratinized epithelial cells, playing first immune defense against environmental pathogens in oral cavity and gastrointestinal tract. Although the studies about lingual organoids were reported, tonsillar organoids were not fully recapitulated because its non-keratinized feature was not observed in vitro 3D culture. In this study, we established the recapitulation of tonsillar organoid using a novel tonsillar epithelium organoid culture system with optimized concentration of WNT3A and RSPO1. Our culture system showed that tonsillar organoids can be maintained for 6 months without defects in proliferation. In addition, epithelial markers such as CK19, CK13, CK14, CK8, and MUC1 were expressed in tonsillar organoids. Furthermore, we found the immune cells (B and T cells) can be maintained with organoids even after several passages. Thus, tonsillar organoid system enables us to investigate crosstalk between immune cells and epithelium and can be applied to immune cell education in vitro.

Funding source: This work was supported by the Basic Science Research Program through the NRF funded by the Ministry of Science and ICT (NRF-2018R1A2B3004269), Republic of Korea.

Keywords: Tonsillar organoid, Immune cells, Crosstalk

EYE AND RETINA

MDD233

HUMAN PLURIPOTENT STEM CELL DERIVED RETINAL ORGANOIDS: CREATING NEURONAL STRUCTURES AND CIRCUITS

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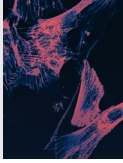
Retinal organoids derived from human pluripotent stem cells (PSC) represent a promising technology. PSC-derived photoreceptor transplantation is a major goal in the field and in vitro they are already used in disease modelling and gene therapy development. Amongst the abundant photoreceptors in these organoids, other retinal cell types have been noted, albeit in a disorganised fashion; despite their name, retinal organoids do not recapitulate the whole human retina particularly well and possibly as a result, most studies have focused on the



production, assessment and utilisation of photoreceptors or their close progenitors. This lack of organisation is an impediment to a more detailed study of retinal neuronal circuits which, to date relies on the sparse availability of post-mortem tissue. We therefore focused on improving the circuit architecture of retinal organoids, in order to provide a reliable model for functional studies. We sought to establish an optimized neuronal differentiation protocol that would enable the differentiation, organisation and sustained culture of retinal cell types in addition to photoreceptors. In particular, we are able to differentiate layered retinal structures, which contain the main neuronal cell types required for retinal computation, including well-developed photoreceptors, bipolar, amacrine and ganglion cells. Using imaging and electrophysiology techniques, we have found that this optimized culture methodology allows for the formation of identifiable plexiform layers, where the first microcircuitry of the visual system resides. The high level of organization is evidenced in spontaneous and coordinated activity that resembles a developing retina. Our optimized approach provides a viable solution to the generation of large numbers of retinal structures that will allow the study of function in health and disease and for drug efficacy and toxicity.

Funding source: Medical Research Council: MJB, MR, KP, EL, MK, EW European Reseach Council: MJB, MR, KP, AGC NIHR BRC for Ophthalmology at Moorfields and UCL IoO: MR, KP, EL, RM, MT, EW Retina UK: MB, EL, OS Moorfields Eye Charity: EL, MK, MT UCL: AGC

Keywords: Pluripotent, Retina, Neuronal

MDD237

SEMI-AUTOMATED PRODUCTION AND DEEP LEARNING-BASED ANALYSIS OF THE DIFFERENTIATION IN 3D MOUSE RETINAL ORGANOIDS

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We are developing deep learning-based computer algorithms to distinguish features of stem-cell derived organoids based on the brightfield imaging. The three-dimensional, “organoid” approach for the differentiation of pluripotent stem cells into retinal and other neural tissues has become a major in vitro strategy to recapitulate development. In part, that success is owed to the availability of fluorescent reporter cell lines that allow to quickly and non-invasively assess differentiation. However, design and validation of reporter cell lines with genetic background of interest is time consuming and not compatible with clinical application. We hypothesized that basic contrast brightfield images contain sufficient information on the tissue specification and it is possible to extract this data using convolutional neural networks (CNN). Retina-specific reporter Rx-GFP mouse embryonic reporter stem cells have been used for all of the differentiation experiments. To increase the throughput, we implemented an automated media exchange which allowed us to differentiate >10000 organoids required for the CNN training. The brightfield (BF) images of organoids have been taken on day 6 and fluorescent on day 9. To train the CNN we utilized a transfer learning approach: ImageNet pre-trained VGG19 CNN had been trained on 3 000 labeled BF images, divided into two categories (retina and non-retina), based on the fluorescent reporter gene expression. A comparison of CNN with the human-based classifier showed that the CNN algorithm performs better than the expert in predicting organoid fate: 77% vs 74% of correct predictions respectively, confirming our original hypothesis. Overall, we have demonstrated that computer algorithm can successfully predict retinal differentiation in organoids before the onset of reporter gene expression, which forms the basis for universal, non-invasive, scalable and rapid approach to assess the state of the cell and forecast its fate.

Funding source: The BrightFocus Foundation, The Department of Ophthalmology HMS, The Massachusetts Lions Fund, ARVO AMD Genentech Fellowship, Research to Prevent Blindness, NIH/NEI Core Grant P30EY003790, Russian academic Excellence project

Keywords: retina, deep learning, automation

MDD461

EXPLORING HUMAN LACRIMAL GLAND BIOLOGY USING ORGANOIDS AND SINGLE-CELL SEQUENCING

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The lacrimal gland is an essential organ for maintaining ocular surface homeostasis. When tear production by the lacrimal gland is impaired, pathologies such as dry eye disease arise. However, regenerative capacities of the lacrimal gland under homeostasis and disease conditions are largely unknown. Here, we establish organoids derived from mouse and human lacrimal gland that produce mature tear products. Using organoids, we show that the master regulator of eye development Pax6 is required for maturation of the lacrimal gland. Lacrimal gland organoids could be used as a screening platform for inducers

of crying and they engrafted upon orthotopic transplantation. We finally provide a single-cell atlas of the human lacrimal gland tissue and organoids, which unveils its cellular heterogeneity. Together, this study provides an experimental platform to study the (patho-)physiology of the lacrimal gland.

Funding source: M.B.-H. was supported by a grant from NWO-ZonW, Y.P. by a Leducq foundation grant, H.G., Y.E.B.E and H.C. by an ERC Advanced Grant, H.G. by a VENI grant from NOW-ZonMW and J.v.d.V. by CancerGenomiCs.nl

Keywords: Lacrimal gland, organoids, single-cell RNA sequencing

HEMATOPOIETIC SYSTEM

MDD241

DEVELOPMENT OF A HEALTHY LONGEVITY HAMATOPOIESIS /BLOOD SYSTEM FOR ANTI-CANCER/ANTI-AGING

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Erythroid Krüppel-like factor (EKLF/ KLF1) was initially identified as an erythroid-specific transcription factor and later found to be also expressed in other hematopoietic cells. However, little is known how EKLF acts in the hematopoiesis process. In an initial study, we focus on investigating the regulatory effects of EKLF on hematopoiesis. By comparative analysis of E14.5 fetal livers from wild type (WT) and Eklf gene knockout (KO) mouse embryos, we show that EKLF is able to express in the long-term hematopoietic stem cells (LT-HSC) and regulates its homeostasis. Incidentally, we have found that a healthy longevity of Eklf (K74R) mouse model, generated by amino acid substitution at the K74 sumoylation site of Eklf, displays extended lifespan and resistance to cancer incidence. This tumorigenesis resistance could be transferred by bone marrow transplanting to WT mice. Following these works, we are developing stem cell-based platform for transfer of tumorigenesis resistance and other healthy longevity characteristics in mice and later in human. Thus far, we have used CRIRSPR/Cas9-mediated genome editing to generate mouse embryonic stem cells (mESCs) carrying K74R mutation and human ESCs carrying K54R mutation, which is orthologous to the mouse EKLF (K74R). These ESCs will be induced to differentiate into hematopoietic stem/progenitor cells (HSPCs) in vitro for transplantation in vivo or ex vivo, for cancer therapy and for application in anti-aging and rejuvenance. This work will support EKLF plays a regulatory role on hematopoiesis as well as anti-cancer and/or healthy longevity by a genetically modified hematopoietic /blood system.

Funding source: Academia Sinica- IMB- OE0106426604

Keywords: EKLF, hematopoietic stem/progenitor cells, anti-cancer anti-aging

MUSCULOSKELETAL

MDD274

SCREENING OF HUMAN EMBRYONIC STEM CELL-DERIVED SKELETAL MUSCLE CULTURES FOR COMPOUNDS THAT ENHANCE MUSCLE FIBER SIZE

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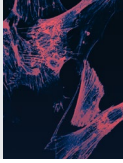
Sarcopenia is an age-associated progressive loss of muscle mass and function that leads to disability, reduced quality of life, and death. Currently there are no muscle building agents approved in the US to treat sarcopenia and drug development has mostly focused on androgen receptor modulators and myostatin inhibitors. To expand our knowledge of factors that regulate human muscle development, we set up an in vitro drug screening system using human embryonic stem cell-derived skeletal muscle cultures that form multinucleated syncytial monolayers. Mass spec analysis revealed these muscle cultures are primarily at the fetal stage of human muscle development. These cultures are excitable and show calcium flux and contraction in response to acetylcholine. In addition, the anabolic compounds testosterone or follistatin enhanced myofibril thickness, suggesting this readout may be useful to identify other compounds with similar activities. To this end, we screened 1056 compounds including 850+ FDA approved compounds and 200+ epigenetic/kinase modulators for the ability to augment myofibril density, or otherwise impact muscle morphology or nucleation, and an overview of these efforts will be presented. Together, our data highlight the utility of screening ES-derived skeletal muscle cultures to identify new compounds that can enhance muscle fibre size, some of which may have clinical value in treating sarcopenia or other diseases of muscle weakening.



Keywords: High Content Screening, Sarcopenia, Skeletal Muscle

MDD447

RECAPITULATING DELAYED MUSCLE REGENERATION IN A MOUSE MODEL OF PRESSURE ULCERS

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Human pressure ulcers exhibit delayed healing and are classified as chronic wounds, but animal models of pressure ulcers do not exhibit delayed healing unless additional bacteria or hyperglycemia are introduced. This lack of animal models impedes research. A mouse with inducible confetti-colored fluorescence satellite cells (muscle stem cells) was developed by crossing the Brainbow2.1 mouse with a conditional Pax7CreER mouse. The resulting mouse, upon tamoxifen induction, provides fluorescent colors for lineage tracing of muscle regeneration in vivo. Muscle pressure injuries were created in these mice by applying a pair of 12mm magnets to the dorsal skinfold and panniculus carnosus muscle, in two intervals of 12 hours. For normal healing baseline, other mice were injected with cardiotoxin to induce acute muscle death in the panniculus carnosus. Tissue sections were studied by histology. At 3 days after acute muscle death, large muscle volumes were removed, presumably by phagocytosis. At 10 days, substantial muscle regeneration had occurred; and at 16 days, muscle was completely regenerated. In contrast, at 3 days after pressure injury, muscle tissue was dead but not well infiltrated and not phagocytosed by immune cells. At 10 days after pressure injury, immune infiltration was significant and dead muscle was phagocytosed. At 40 days after pressure injury, muscle tissue was partially regenerated, accompanied by myoblast-like cells and immature myofibers, suggesting that muscle regeneration was still underway after 40 days. This indicates that prolonged tissue compression impairs subsequent processes of infiltration and clearance. Multiple split and deformed myofibers were also observed in the newly regenerated muscle. Thus, gradual induction of pressure injury causes extensive delay in muscle regeneration and abnormal geometry of the resulting fibers, compared with normal healing baseline. Creating delayed and disrupted regeneration in mice will advance research by mimicking a central pathology of human pressure ulcers.

Keywords: muscle regeneration, pressure ulcer, delayed wound healing

NEURAL

MDD278

INCREASING MITOCHONDRIAL NAD+ OFFERS NEUROPROTECTION FOR ALS MOTOR NEURONS

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by progressive loss of upper and lower motor neurons (LMNs) located at the spinal and bulbar level. There are two forms of ALS, familial and sporadic. The most common form is sporadic ALS (SALS) which accounts for 90-95% of all ALS cases where there is no known etiology. The remaining 5-10% of ALS cases are familial ALS (FALS) where there is an associated hereditary dominant inheritance factor. To date, FALS is clinically and pathologically indistinguishable from SALS, suggesting similar disease manifestation mechanism. Over the years, NAD+ metabolism has been found to have a role in ALS disease progression and serves as potential therapeutics for ALS. Despite efforts in understanding the roles of NAD+ metabolism in ALS, clear mechanistic insights between altered NAD+ metabolism and ALS disease manifestation is currently unknown. In this study, we investigated if reduced NAD+ availability could be the common pathway affected in both SALS and FALS. NAD+ availability analyses reveal a reduction of whole-cell NAD+/NADH ratio and mitochondrial NAD+ availability in both SALS and FALS motor neurons, suggesting cellular metabolism in ALS motor neurons may be affected. Indeed, metabolic flux analyses reveal a deficiency in mitochondrial respiration in both SALS and FALS motor neurons. By replenishing NAD+ availability with NAD+ precursors, we were able to reverse the defective metabolic profiles in both SALS and FALS motor neurons. Supplementation of NAD+ precursors also promote neurite regeneration and enhance ALS motor neuron survival. Therefore, our study demonstrated that dysregulated NAD+ metabolism could be a potential converging pathogenic mechanism between both SALS and FALS. Moreover, we demonstrate that NAD+ precursors supplementation can be exploited as potential therapeutics for ALS.

Keywords: Amyotrophic Lateral Sclerosis, NAD+ metabolism, Motor Neuron Regeneration

MDD302

POTENTIAL OF HESC-DERIVED PERIPHERAL SENSORY NEURONS IN GENERATING INNERVATED HUMAN SKIN EQUIVALENTS

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High numbers of skin tissue equivalents are developed for in vitro drug and cosmetic testing purposes. Majority of these contain only two cell types – (1) keratinocytes in the epidermis and (2) fibroblasts in the dermis. However, human skin tissue is more complex with numerous other cell types and vital structures like immune cells, blood vessels and nerves that collectively contribute to its proper functioning. Thus, existing commercial skin models do not provide comprehensive data as it lacks this complexity of native skin. Further, in compliance with the 3Rs principle (replace, reduce and refine) and questionable relevance to human skin, researchers are moving away from animal models. Hence, there is a current need to generate more complex and organotypic in vitro skin tissue equivalents that closely mimic native human skin tissues. Considering the above need, the present study was aimed to generate innervated human skin tissue equivalents. Peripheral sensory neurons (PSNs) were developed from hESCs as primary human sources are inaccessible for research. The pluripotent hESCs were sequentially differentiated to neuroectoderm, NCSCs and PSNs by dual SMAD inhibition/ WNT-β catenin activation. Differentiated PSNs had characteristic morphology and expressed sensory neuronal markers by transcriptome profile and immunocytochemistry. Calcium flux was also evident showing functionally active synaptic junctions with fluorescence calcium imaging. Further, an in vitro 3D culture platform was developed wherein hESC-derived PSNs (hESC-PSNs) were integrated together with fibroblasts in a dermal matrix composed of ECM-based hydrogel. In addition, keratinocytes were seeded on top to generate the epidermis and thus full-thickness innervated skin constructs. The resulting in vitro 3D innervated skin construct developed by using hESC-PSNs as a neural component, is versatile and closely organotypic than existing commercially available skin models. This platform provides opportunities for studying neurite functional analyses as well while testing for drugs and cosmetics. It also reveals new strategies that could be a critical milestone in the development of more complex in vitro skin tissue in future.

Keywords: human embryonic stem cells, peripheral sensory neurons, innervated skin tissue equivalents

MDD313

USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CEREBRAL ORGANOIDS TO INVESTIGATE THE ROLE OF APOLIPOPROTEINS E

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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. It is known that polymorphisms in the APOE gene are the major genetic risk factor. Carriers of two APOE4 alleles (APOE4/4) have 15 times higher risk to develop AD than homozygous carriers of the most common allele APOE3 (APOE3/3). Here, we generated isogenic induced pluripotent stem cell (iPSC) lines with different APOE genotypes (APOE4/4 and APOE3/3) using CRISPR/Cas9 editing. Following characterisation (pluripotency, genotyping and karyotyping), cells were differentiated into cerebral organoids for assessment of amyloid secretion and phosphorylation of Tau. After 6 months in culture, the pathological forms of amyloid β 1-42 and 1-40 were detected in organoid lysates and in secreted media with no statistical difference between organoids of different APOE genotypes. However, levels of amyloid β 1-42 were slightly higher in APOE4/4 than in lysates (2.71±0.68 vs 1.70±0.78 pmol/L, n=5-7) and secreted media (0.86±0.19 vs 0.39±0.11 pmol/L, n=7). Total Tau protein was also detected in organoid lysates and secreted media with no statistical difference between group. Levels of phosphorylated Tau at different amino acid sites were detected in organoid lysates but not in secreted media. Our data thus provides a quantitative snapshot of levels of amyloid and Tau in isogenic APOE cerebral organoids.

Funding source: This research was founded by the Yulgilbar Foundation and the Brain Foundation

Keywords: Cerebral organoids, Isogenic iPSCs, APOE, Alzheimers

MDD314

ESTABLISHMENT OF IN VIVO AND IN VITRO BRAIN TUMOR -INDUCIBLE PIG MODEL

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Pig models in brain imaging and neurosurgery are increasingly used in neuroscience because of human and brain similarities. In this study, we used a 2A peptide-dependent polycistronic expression vector capable of expressing DsRed, SV40LT and HrasV12 genes via the CreERT2 system. Whether the introduction of the transgene was confirmed by PCR and fluorescence activated cell sorter. Somatic cell nuclear transfer(SCNT) was performed using the established transgenic(TG) cell line and then whole seeding was performed using SCNT blastocysts. As a result, two TG porcine embryonic stem(pES) cell lines were

established. Both TG pES lines showed a primed form and showed bFGF-dependent properties. For in vitro organoid model, these pES lines were used to induce neural differentiation to produce the in vitro brain tumor model. pES were cultured using the SFEBq(serum-free floating culture of embryoid-body(EB)-like aggregates with quick reaggregation) method. The SFEBq culture method recreates the process of in situ generation by using the phenomenon(self-organization) that the cell group makes a spontaneous orderly structure. pES was cultured in in vitro until the final 61 days after neural induction(NI), neural patterning(NP), and neural expansion(NE). RSPO2, known to promote midbrain dopaminergic neurogenesis and differentiation, was added to the NP stage. The organoids were sampled at each step and the expression of Dopaminergic neuronal marker(TH) and Mature neuronal marker (MAP2) was confirmed by PCR. Expression of neural stem cell marker(PAX6), neural precursor marker(S100, SOX2) and early neural marker(MAP2, Nestin) was confirmed by immunostaining. For the in vivo model, SCNT embryos were transplanted once into surrogate mothers and five piglets were born. Umbilical cord analysis confirmed the insertion of tumor-inducing genes in all piglets. Two of them survived, and expression of EGFP was confirmed in both of them. After puberty, spermatozoa were confirmed and the expression of EGFP was observed. This suggests that the pig model produced would allow germline transmission of tumor-inducing genes. Therefore, this study successfully produced neuronal organoids derived from pES cells in in vitro and also produced tumor-inducing model capable of germline transmission.

Funding source: This work was supported by "Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries(IPET)" (grant number: 318016-5, 819029-2)

Keywords: Pig, tumor-inducible model, Embryonic stem cells

MDD317

EVIDENCE FOR COMPLEX NETWORK FUNCTION IN BIOENGINEERED NEURONAL ORGANOIDS

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Bioengineered Neuronal Organoids (BENOs) are highly interconnected neuronal networks in a macroscale format. They are generated by directed differentiation and self-organization of human induced pluripotent stem cells in a collagen hydrogel and consist of excitatory and inhibitory neurons, myelinating and non-myelinating glia. BENOs recapitulate cortical development and present hallmarks of brain network function: (1) glutamatergic-, gabaergic- and TTX-sensitive giant depolarizing potentials (d20); (2) the switch of GABA from excitatory to inhibitory neurotransmission (d40); (3) electrical point stimulation in fused BENOs results in spreading of electrical network activity to the remote BENOs; (4) paired pulse depression induced by electrical point stimulation can be alleviated by GABAR inhibition demonstrating inhibitory and excitatory neuron connections as described for the human brain; (5) complex bursting activity patterns (Mean firing rate 1.5 ± 0.2 Hz, Network burst frequency 0.08 ± 0.01 Hz, Burst Interspike interval 2.2 ± 0.2 ms) are observed between 1- 2 months of culture; (6) evidence for neuronal plasticity induced by electric stimulation was collected supporting a "learning and memory" function in BENOs. These complex features suggest that BENOs are attractive biological systems to model diseases; this will be exemplified in a model of epilepsy.

Keywords: human iPS-derived cortical neuronal organoid, Long-term potentiation & neuronal plasticity, Network function in neuronal organoids

MDD320

MODELLING MEDULLOBLASTOMA AND IDENTIFICATION OF THERAPEUTIC TARGETS WITH PATIENT IPS CELL-DERIVED NEURAL STEM CELLS

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Reprogramming, iPS cells and directed differentiation has opened up the field of human disease modelling. Disease relevant cell types can be derived and studied from individuals with or without a diagnose, carrying the genome causing or not causing disease. However, reprogramming and iPS cells has so far only occasionally been used for modelling the onset and progression of cancer. Here, we describe and utilize a cellular model of medulloblastoma created by reprogramming healthy skin cells from patients with a familial mutation in PATCH causing constitutively active sonic hedgehog signaling. Patient iPS cells were neurally induced and neuroepithelial stem (NES) cells with a hindbrain identity were established, a cell type very similar to the anticipated cell of origin for medulloblastoma. First, we performed in vitro investigations of proliferation, apoptosis and growth factor dependency in 2D/3D, with and without stress. We found that patient NES cells formed larger neurospheres and tolerated hypoxia better than healthy NES cells, however, the phenotypes of these parental NES cells were subtle. So, to model cancer onset and progression we transplanted NES cells to cerebellum of adult mice and studied the tumour formation using luciferas to find that 87% of mice injected with patient NES cells form tumour resembling medulloblastoma compared to no tumour formation with NES cells from healthy control individuals. We isolated human NES cells from the tumours (primary tNES), which we subsequently injected into the cerebellum to find that tumours were formed at a higher rate. Again, NES cells (secondary tNES) where isolated and investigated in vitro. We found that after two rounds of tumour formation in the mice these cells where no longer depended on growth factors for proliferation. Transcriptome investigation show upregulation of metastatic and inflammatory pathways, among the upregulated genes we found LGALS that we could confirm being a direct target of sonic hedgehog signaling. Further, inhibition of LGALS in secondary tNES significantly decreased cell viability. We are

now using our cellular model of the initiation and progression of medulloblastoma to screen for potential drugs depressing the viability of tNES cells.

Keywords: iPS cells, neural stem cell, Cancer modelling

MDD321

THE CHEMORESISTANCE TO THE COMBINATION OF OTS964 AND TEMOZOLOMIDE DURING THE SELF-RENEWAL OF HETEROGENEOUS POWER-LAW CODED GLIOMA STEM CELL POPULATIONS

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Glioblastoma is a fatal space-occupying lesion that resists chemotherapy. To improve the long-term prognosis, we need to establish a novel chemotherapeutic paradigm via addressing the issues of chemoresistance and tumor size. Glioma stem cell (GSC) populations, heterogeneous power-law coded populations in glioblastoma, are suggested to be responsible for the recurrence and tumor progressiveness. We employed a detailed quantitative approach using clonal glioma sphere (GS) cultures, measuring the sphere survivability and changes to growth during the self-renewal. We then reported that OTS964, an inhibitor for T-Lak cell originated protein kinase, suppressed the growth of the U87- and U251-GS population size, the total number of GS cells in a population, via dose-dependent clone elimination during the self-renewal; the OTS964-suvived GS clones adversely grow, and have become resistant to clone-eliminating efficacy of OTS964: OTS964 alone would not be enough for the long-term control of glioblastom. We recently revealed the combined efficacy of temozolomide and OTS964 (T&O) in the long-term control of U87- and U251-GSC populations, where T&O more efficiently eliminated self-renewing GS clones than OTS964 alone and sufficiently disturbed their re-growth; further revealed that T&O quickly reduced the size of self-renewed/expanded GS populations via efficient elimination of GS clones. Thus, T&O reduced the initial size of GS populations and suppressed their later regrowth. We currently tested whether T&O is effective in a T98G-GSC population, which is known to resist TMZ. T&O eliminated T98G-GS clones, however, did not suppress the further re-growth of T&O-survived T98-GS clones. The phenotypes of T&O-suffered T98G-GS populations are similar to the OTS964 alone in the clone elimination and in the re-growth, suggesting that sufficient growth suppression is necessary for the long-term control of GSC population size. Thus, our quantitative GS system is enough valid for testing GSCs' sensitivity and resistance to anti-glioma drugs; a combination therapy of OTS964 and another growth suppressor is to be tested in the detailed quantitative system. The valid combination paradigm could control the TMZ-resistant glioblastoma via immediate and sustained shrinkage of power-law coded GSC populations.

Keywords: glioma stem cell, OTS964, temozolomide

MDD329

INVESTIGATION OF THE EFFECTS OF ORGANOPHOSPHATES ON IPSC-DERIVED NEURONS

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The need for a human neuro-muscular test model to find new potential nerve agent antidotes is highlighted by the increasing global political uncertainty, such as the use of nerve agents especially in Syria in recent years. By the generation and differentiation of human induced pluripotent stem cells (hiPSCs) into neurons, in particular motoneurons, a high throughput screening is made possible. Thus avoiding the use of ethically highly discussed embryonic stem cells. For the generation of hiPSCs, the pluripotency-inducing Yamanaka factors (Oct3/4, Sox2, c-Myc, Klf4) were inserted into human fibroblasts by plasmid nucleotransfection. Since the efficiency of hiPSC-differentiation approaches to motoneurons varies greatly a naive hiPSC-Line was taken along for comparison. During their differentiation into neurons continuous morphological documentation, identification and characterization by means of immunohistochemical staining, PCR and flow cytometry was performed. The derived neurons were exposed to 400 µM, 600 µM, 800 µM and 1 mM VX and were incubated for 5 days. During incubation time, morphologic changes were captured by the IncuCyte, a real-time imaging tool. Subsequently the cells were collected and analyzed by PCR. The neuronal precursor structures showed a positive staining for PAX6 and ß3-Tubulin. In the further course of cultivation Synapsin- and Peripherin-positive neuronal networks evolved which could also be identified as motoneurons by staining with MNX, Islet-1 and SMI-32. During exposure to VX a loss of neurites and cell shrinkage was observed. PCR-Analysis showed that apoptosis-inducing genes as FAS, CIDEA, CDKN1A and BIK were upregulated. These results suggest that both the intrinsic pathway as well as the extrinsic pathway of apoptosis are activated after VX exposure. Further investigations, e.g. on the expression of caspase 3, 8 and caspase 9, are necessary for clarification. This will provide new insights into the pathomechanism of nerve agents at the molecular level and might help to identify new therapeutic approaches.

Keywords: Organophosphates, Apoptosis, iPSC-derived Neurons

MDD335

MODELLING X-LINKED CHARCOT-MARIE-TOOTH (CMTX6): ENERGY METABOLISM AND MITOCHONDRIAL DEFECTS IN PATIENT IPSC-DERIVED MOTOR NEURONS WITH THE R158H PDK3 MUTATION.

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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. The p.R158H missense mutation in the pyruvate dehydrogenase kinase 3 gene (PDK3) has been identified as the genetic cause for an X-linked form of CMT (CMTX6). PDK3 is one of four PDK isoenzymes that negatively regulate the activity of the pyruvate dehydrogenase complex (PDC). The balance between kinases (PDKs) and phosphatases (PDPs) determines the extent of oxidative decarboxylation of pyruvate by PDC to generate acetyl CoA, critically linking glycolysis and the energy producing Krebs cycle. In vitro studies demonstrated the p.R158H mutation causes hyperactivity of PDK3. Our experiments have shown that increased kinase activity of the mutant PDK3 leads to hyperphosphorylation of the E1 subunit of PDC in patient fibroblasts, thereby providing a phenotypic signature specific for CMTX6. To model CMTX6 in vitro, we have generated a line of induced pluripotent stem cells (iPSCs) by re-programming CMTX6 patient fibroblasts (iPSCCMTX6). We have also engineered an isogenic control by correcting iPSCCMTX6 using the CRISPR/Cas9 system (iPSCisogenic). The iPSCCMTX6 and iPSCisogenic lines have been differentiated into spinal cord motor neurons. Patient-derived motor neurons (MNCMTX6) show increased phosphorylation of the PDC, energy metabolism defects and mitochondrial abnormalities. Treatment of the CMTX6 motor neurons with a pan PDK inhibitor, dichloroacetic acid (DCA), reverses E1 hyperphosphorylation to levels found in the MNisogenic control cells. This demonstrates that the MNCMTX6 and MNisogenic motor neurons provide an excellent neuronal cell system for compound screening approaches to identify effective drugs for the treatment of CMTX6 neuropathy.

Keywords: Charcot-Marie-Tooth neuropathy, Motor neurons, Mitochondria.

MDD336

AN IN VITRO MODEL OF MOTOR NEUROPATHY USING IPSC-DERIVED MOTOR NEURONS: A USEFUL PARADIGM TO INVESTIGATE GENOMIC ORGANISATION AND GENE REGULATION

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Distal hereditary motor neuropathies (dHMN) are a group of neurodegenerative diseases with length-dependent axonal degeneration of the lower motor neurons leading to chronic disability. Our group has mapped an autosomal dominant form of dHMN to chromosome 7q34-q36 (DHMN1: OMIM %182960) in a large Australian family (Family-54). Using whole genome sequencing we identified a novel 1.35 Mb complex insertion within the DHMN1 locus after having excluded all coding mutations in the linkage region. We hypothesise the DHMN1insertion is likely to cause peripheral neuropathy through gene(s) dysregulation which leads to axonal degeneration. We have utilised induced pluripotent stem cell derived spinal motor neurons (sMN) from patients (sMNDHMN1) and controls (sMNCTRL) to study gene dysregulation in an appropriate tissue with the DHMN1 genetic background. Motor neuron identity was confirmed by staining

with NF68, TUJ1 as well as the pan-neuronal markers ISLET1 and HB9. RNA-seq and NanoString analyses comparing gene expression in DHMN1 patient and control sMN demonstrated gene dysregulation due to the DHMN1 complex insertion. A custom designed NanoString panel targeting 65 genes within a 6 Mb interval flanking either side of the DHMN1 insertion site has shown significant dysregulation of 9 genes. Most notably SHH (distal to the insertion site) and the duplicated transcript MNX1, which have critical roles in motor neuron development have been identified as DHMN1-causative candidate genes using this approach. Time-lapse imaging shows that sMNDHMN1 display disorganised and delayed axonal out-growth when compared to sMNCTRL. The iPSC-derived sMN model of DHMN1 is a useful paradigm for further investigating how structural variation impacts genomic organization and gene regulation and is an ideal model for developing personalised treatment therapies for DHMN1 patients. *Authors contributed equally to this work.

Keywords: Motor Neurons, Development, Gene Regulation

MDD355

DEVELOPMENT OF MATURE FOREBRAIN ORGANOIDS BY SPATIALLY AND TEMPORALLY CONTROLLING NEURODEVELOPMENTAL CUES FOR SCHIZOPHRENIA MODELING

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The biggest challenge to study human brain is the lack of experimental systems that can precisely represent the complexity of human brain. Current brain organoid technology is limited in that it fails to recapitulate cell maturation in late fetal stages of brain development. Here, we generate human forebrain organoids that reproduce structural and functional maturity of human fetal brain, by spatially and temporally controlling patterning molecules. Firstly, the pathway activities of Hedgehog and Wnt signaling are pharmacologically increased to expand the neural progenitor population at the later stage, leading to increased number of functional neurons. Secondly, correct organization of developing cortex such as accurate positioning of neuronal layers could be finally achieved by reconstituting brain organoids with tetracycline-inducible, Reelin-expressing cells. Here, we apply our mature forebrain organoid system to model schizophrenia. Our organoid system revealed that schizophrenia is accompanied by massive alterations in enhancer activity, which is caused by SETD1A mutation. Taken together, our work shows the effective strategies to develop mature human forebrain organoids, and will provide the strong foundation to understand various neurological disorders for the development of better therapeutic options.

Keywords: Forebrain organoids, Neuronal development, Neurodevelopmental disorders

MDD359

AMYOTROPHIC LATERAL SCLEROSIS IPSC-DERIVED MOTOR NEURONS IDENTIFY A NOVEL ROLE FOR THE UBIQUITIN PROTEASOME SYSTEM IN CONTROLLING NEURONAL EXCITABILITY CHANGES IN ALS / MND

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One of the earliest changes observed in ALS patients is an increased excitability of motor neurons of the motor cortex and spinal cord, which can be detected even prior to the deterioration of motor function. The underlying mechanisms leading to changes in excitability are not yet fully understood, although it is hypothesised that intrinsic changes to ion channel function or expression in motor neurons contributes to the hyperexcitability phenotype. A recently identified mutation in cyclin F (CCNFS621G), an enzyme involved in the ubiquitin proteasome system (UPS), has been shown to cause ALS. Using iPSC-derived motor neurons from ALS-patients carrying the CCNFS621G/WT mutation compared to wildtype or biallelic isogenic controls, the aim of this study was to assess the contribution of this mutation to alterations in the electrophysiological function of motor neurons and to identify mechanisms of ALS pathogenesis. Motor neurons derived from the CCNFS621G/WT ALS lines showed an increase in neuronal excitability compared to isogenic wildtype controls, which was further exacerbated in the biallelic CCNF mutant. Increased repetitive firing and increased K+ and Na+ currents were identified in iPSC-derived motor neurons from CCNFS621G and the double CCNF mutant, consistent with a hyperexcitability phenotype. Mass spectrometry was used to quantify changes to the proteome and ubiquitome caused by CCNF mutations and modulators of the UPS. Pathway analysis identified the specific signaling mechanisms dysregulated due to CCNF mutation and subsequent alterations in UPS function. Inhibitors of specific UPS enzymes confirmed the pathways involved in ALS pathogenesis. Together our results show that impairments in the UPS control ion channel function in ALS motor neurons leading to alterations in neuronal excitability and increased motor neuron death, whilst pharmacological targeting of these signalling pathways affords neuroprotection.

Keywords: neurodegeneration, amyotrophic lateral sclerosis, ubiquitin proteasome system

MDD484

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PSYCHIATRIC DONORS OF THE NETHERLANDS BRIAN BANK

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Psychiatric disorders present an enormous personal, social and economic burden, accounting for 32.4% of all years lived with disability worldwide. There is a currently unmet need for better treatment strategies, which requires a better understanding of the underlying etiology and pathophysiology of psychiatric disorders. The use of human brain tissue provides the most direct strategy to develop and test new hypotheses about the molecular and cellular basis of psychiatric symptoms and recent years have seen great advances in our knowledge of molecular changes in psychiatric disorders. In order to explain psychiatric symptoms, it is crucial to focus on the cells implicated in the pathology of the disease and the development of experimental models for investigation, as current experimental animal models and modern brain imaging only provide a limited view. With the use of induced pluripotent stem cells (iPSC's) derived from skin tissue generously donated by psychiatric and control donors from the Netherlands Brain Bank (NBB), a unique insight in the pathological cellular mechanisms can be obtained. To this goal, the NBB established a resource of brain and skin tissue of seven major psychiatric disorders (depression, schizophrenia, bipolar disorder, obsessive-compulsive disorder, post-traumatic stress disorder, autistic spectrum disorder, attention-deficit hyperactivity disorder) and controls. Patients and controls who have consented to register as donors will undergo standardized and detailed phenotyping. State-of-the-art neuropathological diagnoses will be performed. cDNA banks of brains from subjects with psychiatric disorders and generation of primary and stable (stem)cell lines from fibroblasts and glia cells will further enrich this resource and provide the research community with powerful methods to study associated neuronal abnormalities. By making these resources publicly available to the national and international research community, we will contribute to research uncovering cellular mechanisms underlying psychiatric pathologies. Interfering in these mechanisms could enable therapeutic targeting to alleviate or prevent psychiatric symptoms in patients.

Funding source: The Dutch Research Council (NWO)

Keywords: Induced Pluripotent Stem Cells, Psychiatric disorders, The Netherlands Brain Bank

MDD501

FUNCTIONAL CHARACTERIZATION OF HUMAN IPSC-DERIVED NEURONAL NETWORKS USING HIGH-DENSITY MICROELECTRODE ARRAYS

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The advent of iPSC technology paved the way to studying and characterizing human cells in vitro. The technology enabled the generation of human neurons, which can be functionally characterized by microelectrode array technology. High-density microelectrode array (HD-MEA) technology allows for investigating neuronal electrophysiology at high spatial and temporal resolution. The main goal of this work was to combine HD-MEA and iPSC technologies, in order to assess and compare electrophysiological properties and functional phenotypes of human iPSC-derived neuronal lines (motor neurons and dopaminergic neurons) and related disease-model lines (Parkinson's disease and amyotrophic lateral sclerosis). Using a CMOS-based 26'400-electrode HD-MEA, we investigated standard metrics (e.g., spike rate) and network electrophysiology metrics (e.g., burst rate) by recording from hundreds of neurons

simultaneously. The aim was to distinguish functional network phenotypes of the corresponding human iPSC-derived neuronal cell lines. Comparing the isogenic dopaminergic neuron lines, we observed that control iCell® DopaNeurons and the disease line A53T MyCell® DopaNeurons could be discriminated by standard HD-MEA metrics. Specifically, the two dopaminergic neuronal lines showed different spike rates, spike amplitudes and inter-spike intervals. Furthermore, the two dopaminergic neuronal lines largely differed in burst durations and inter-burst intervals. Similar results were found with the motor neuron isogenic lines iCell® Motor Neurons and MyCell® Motor Neurons, which could be differentiated by their spike rates and inter-spike intervals. The healthy cells had a higher spike rate than the disease line at DIV 14. The two motor neuronal cell lines showed also significantly different burst durations and inter-burst intervals. Lastly, we analyzed the network burst shapes and found that this metric could be used to differentiate all healthy and disease lines at any day in vitro. In this work we demonstrated that by analyzing MEA metrics, it was possible to characterize and distinguish healthy and diseased dopaminergic and motor neuronal cell lines. In particular, metrics, such as spike rate and burst duration, hold great potential to assess cell excitability or network synchronicity in case of disease.

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Keywords: HD-MEA, Electrophysiology, iPSC-derived neurons

NEW TECHNOLOGIES

MDD413

THREE-DIMENSIONAL RECONSTITUTION OF BLADDER ASSEMBLOIDS THAT STRUCTURALLY AND FUNCTIONALLY RECAPITULATE IN VIVO TISSUE REGENERATION AND CANCER

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Current organoid models are limited by their inability to mimic mature organ architecture and associated tissue microenvironment. Here, we create multi-layered bladder assembloids by reconstituting tissue stem cells with various stromal components to represent an organized architecture with an epithelium surrounding stroma and an outer muscle layer. These assembloids exhibit the characteristics of mature adult bladders in the context of cell compositions at the single-cell transcriptome level, and recapitulate the in vivo tissue dynamics of the regenerative response to injury. As a malignant counterpart, tumor assembloids are also developed to recapitulate the in vivo pathophysiological features of patient-derived urothelial carcinomas. Using the genetically manipulated tumor assembloid platform, we identify tumoral FOXA1, induced by stromal BMP, as a master pioneering factor driving enhancer reprogramming for the determination of tumor phenotype, suggesting the importance of the FOXA1–BMP–HH signaling feedback axis between tumor and stroma in the control of tumor plasticity.

Keywords: Bladder organoid/assembloid, Tissue stroma/tumor microenvironment, Tumor plasticity

MDD429

N-ACETYLCYSTEINE PREVENTS BLADDER TISSUE FIBROSIS IN A LIPOPOLYSACCHARIDE-INDUCED CYSTITIS RAT MODEL

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Therapeutic options for non-Hunner type interstitial cystitis (IC), which is histologically characterized by fibrosis and mast cell infiltration, are limited. We developed a rat model that replicates chronic inflammation and fibrosis and evaluated the therapeutic effect of N-acetylcysteine (NAC), a well-known anti-fibrotic agent, on the model. Intravesical instillation of lipopolysaccharide (LPS, 750 μg) after protamine sulfate (10mg) was conducted twice per week for five consecutive weeks. One week after final instillation, 200mg/kg NAC (n=10, IC+NAC group) or phosphate-buffered saline (n=10, IC group) was daily injected intraperitoneally once daily for 5 days. LPS instillation induced bladder fibrosis, mast cell infiltration, and apoptotic tissue damage. Functionally, LPS insult led to irregular micturition, decreased inter-contraction intervals, and decreased micturition volume. NAC significantly improved most of the voiding parameters and reversed histological damages including fibrosis. NAC inhibited the induction and nuclear localization of phospho-Smad2 protein in bladder tissues and the upregulation of genes related to fibrosis, such as Tgf2, Tgf3, Smad2, Smad3, Cxcl10, and Card10. This is

the first study to demonstrate the beneficial effects on NAC in restoring voiding function, relieving tissue fibrosis and related bladder injuries, in the LPS-induced cystitis rat model.

Keywords: lipopolysaccharide-induced cystitis rat model, Interstitial cystitis/bladder pain syndrome, N-acetylcysteine

MDD433

GENERATION AND CHARACTERIZATION OF BOVINE LGR5+ INTESTINE STEM CELLS TOWARD THREE-DIMENSIONAL (3D) ORGANOID

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Intestine stem cells and three-Dimensional (3D) organoid technology offer great promise for disease modeling based host-pathogen interactions, pesticide toxicity assessment and feed efficiency measurement in livestock as well as regenerative medicine for therapeutic purposes. However, characterization and long-term cultivation of intestine stem cells in bovine require further investigation in details. Recently, significant efforts have been made to establish an in vitro system for 3D organoid culture of bovine intestine stem cells for replacement of animal experiments and practical applications, including stem cell transplantation and fundamental research. In this study, Lgr5+ intestine stem cells from different site of small intestine in adult bovine were characterized with several specific markers in vivo, and sequentially isolated. Furthermore, Lgr5+ intestine stem cells were long-term cultivated and evaluated the expression of several specific markers representing crypt-villus structure. In addition, they showed the key functionality with regard to paracellular permeability and nutrient transport was maintained during several passages of culture. Collectively, these results first time demonstrate the efficient cultivation and characterization of bovine Lgr5+ intestine stem cells based on 3D organoid culture. Finally, culturing derived bovine Lgr5+ intestine stem cells in 3D will facilitate the potential use for the exploration of nutrition physiology, pesticide toxicity assessment and the system development of fatal disease modeling approaches in the fields of animal biotechnology and biomedicine.

Funding source: This work was carried out with the support of the National Institute of Animal Sciences (Grant No. PJ01422201), Rural Development Administration (RDA), Republic of Korea

Keywords: Bovine, Intestinal Stem Cells, Organoid

MDD442

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF IPSC-DERIVED NEURONS (IONEURONS/GLUT) IN SUPPORT OF DRUG DISCOVERY APPLICATIONS

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The advent of stem cell technologies has opened new opportunities in drug discovery and development by providing new tools for drug screening, target identification and toxicity testing. The discovery of reprogramming patient derived somatic cells into induced pluripotent stem cells (iPSCs) has offered a promising alternative as they can be used to generate large numbers of cells and varied tissue types including human neurons which are relevant to CNS related diseases. Although methods for iPSCs generation have been well established, protocols for a highly consistent and scalable iPSCs neuronal differentiation still need further optimization to produce cost effective and reproducible neuronal cells. Charles River has performed molecular and functional characterization of human glutamatergic cortical neurons (ioNeuron/glut) differentiated from iPSCs using the forward reprogramming technology developed by Bit Bio. The advantage of forward reprogramming technology, consisting of forced expression of transcription factors, provides an alternative to conventional differentiation protocols as it accelerates lineage conversion and stem cell specification. Here we have tested iPSCs derived neurons (ioNeuron/glut) from Bit Bio for the expression of markers of the neuronal lineage, functional readouts, and their ability to be used for high through put screening (HTS). Preliminary immunocytochemistry and branched DNA data showed expression of pan neuronal markers after only 2 days in culture. Moreover, functional multi electrode array (MEA) data showed the presence of spontaneous activity after 3 weeks of differentiation when ioNEURONS/glut were cultured in Brain Phys media. Finally, when applying to HTS applications (TR-FRET assay) including a cytotoxicity assay (Cell Titer Glo), ioNEURONS/glut plated in 384 microplates and treated with tool compound showed good assay statistics indicating high suitability for high through put screenings.

Keywords: Forward reprogramming, Neurons, High through put screening

Theme: Tissue Stem Cells and Regeneration

ADIPOSE AND CONNECTIVE TISSUE

TSC101

PROSTAGLANDIN E2 REDUCES STEMNESS BY E-PROSTANOID 1 IN DERMAL STEM/ PROGENITOR CELLS

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Prostaglandin E2, a lipid-derived signaling molecule produced from arachidonic acid by cyclo-oxygenase, inhibits Sox2 expression, and induces differentiated marker expression in vitro. PGE2-induced differentiated marker expression and sox2 imhibition are aging phenomenon. This study investigated the role of E-prostanoid 1 (EP1) in PGE2 signaling in human dermal stem/progenitor cells (hDSPCs). When EP1 expression was inhibited by EP1 small interfering RNA (siRNA), there were no significant changes in messenger RNA levels of sox2/ differnetiated markers between siRNA-transfected hDSPCs and siRNA-transfected hDSPCs with PGE2. This result showed that EP1 is a PGE2 receptor. Extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation after PGE2 treatment significantly increased. In addition, PGE2 treatment increased the intracellular Ca2+ concentration in hDSPCs. These results indicated that PGE2 is directly associated with EP1 pathway-regulated ERK1/2 and inositol trisphosphate (IP3) signaling in hDSPCs.

Funding source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 201700280003).

Keywords: hDSPCs, PGE2, Sox2

TSC107

GOLGIN-84 IS ONE OF THE PERIODONTAL LIGAMENT PROGENITOR-SPECIFIC MARKERS AND MODULATES MEMBRANE EXPRESSION OF LIGAMENTOGENIC GLYCOPROTEINS.

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Ligamento-fibrogenic differentiation was highly induced by treatment with a low dosage of TGF-β1 in human periodontal ligament stem cells (hPDLSCs). Through a decoy immunization, we have developed hPDL progenitor-specific cell surface antibodies and focused on an α-LG11 antibody as a specific differentiation marker for periodontal ligament progenitor. The LG11 antigen was up-regulated upon the ligamento-fibrogenic differentiation in hPDLSCs. When an immunoprecipitant of the α-LG11 antibody was identified by proteomic analysis, the LG11 antigen turned out to be Golgin-84. In depletion of Golgin-84 in hPDLSCs, mRNA levels of ligamentogenic markers such as PLAP-1, scleraxis, and osteopontin were decreased, but osteogenic/cementogenic markers such as CAP and CEMP-1 were increased. Golgin-84 has been known as one of the trafficking proteins that tether COP1 in the Golgi. When TGF-β1 signaling was inhibited by treatment with a TGF-β1 receptor inhibitor, the surface expression of PDL-specific membrane glycoproteins such as SPARC and LRRC15 as well as Golgin-84 were dramatically decreased. Although the expression of these glycoproteins was also decreased in depletion of endogenous Golgin-84, in contrast, their expressions were increased under the overexpression of Golgin-84. Finally, we showed that Golgin-84 was a possible ligamentogenic marker of hPDLSCs and played an important role in the induction of ligamentogenic differentiation and suppression of cementogenic differentiation. In addition, Golgin-84 might up-regulated the expression and surface localization of ligamentogenic membrane glycoproteins, which has been known as an essential factor for collagen formation on the surface of periodontal ligaments.

Funding source: This research was supported by NRF-2019R1A2C1084524 of the NRF funded by MSIT.

Keywords: periodontal ligament, PDL progenitor, trafficking protein

TSC390

NORADRENALINE SENSITIVITY IMPAIRED IN IMMORTALIZED ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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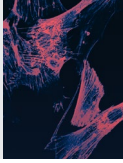
Mesenchymal stem cells (MSCs) are identified in the stromal-vascular compartment within the most of adult tissues, such as bone marrow and fat. MSCs mediate many physiological processes, including regeneration and homeostasis regulation. Hormones and neurotransmitters change the functional activity of these cells, and noradrenaline is their key regulator. Previously, we demonstrated on adipose-derived MSCs (adMSCs) the phenomenon of “heterologous sensitization” of adrenoceptors, in which intracellular signaling switches from beta-adrenoceptor/ cAMP to alpha1A-adrenoceptor/Ca2+ pathways, which causes an increase in sensitivity to catecholamines. Also, we showed that the ratio of adMSCs subpopulations expressing different adrenergic receptors vary depending on donor of cells. To further study the signaling mechanisms of adrenergic regulation of MSCs, we used immortalized adMSCs (hTERT-MSCs) with more uniform expression of adrenoceptors. In this study, we compared ability to respond to noradrenaline and the mechanisms of hormonal regulation on hTERT-MSCs and primary adMSCs.



Using immunofluorescent staining we showed that hTERT-MSCs, as well as adMSC, expressed all types of adrenergic receptors. Flow cytometry analysis demonstrated that the percentage of the hTERT-MSCs containing adrenoreceptor isoforms was stable, but lower compared to adMSCs. Using analysis of calcium signaling in single cells, we showed that responses to noradrenaline were not homogeneous in hTERT-MSC, and the percentage of noradrenaline-sensitive cells was 4 times lower compared to adMSCs. The responses variability of hTERT-MSCs was similar to primary cultures. Previously, we showed that Ca2+ release in adMSCs mediated by alpha1- and alpha2-adrenoreceptors. However, hTERT-MSCs respond predominantly using alpha1-adrenoceptors. Analyzing heterologous sensitization in hTERT-MSCs we showed that this phenomenon is severely impaired in these cells. Thus, we demonstrated that the immortalized adipose-derived mesenchymal stem cell line shows impaired ability to respond to noradrenaline and to regulate hormone sensitivity compared to primary adMSCs.

Funding source: This research was supported by Russian Foundation for Basic Research grant number 19-315-80018.

Keywords: MSC, calcium signaling, adrenoceptors

TSC391

SEROTONIN INDUCED HETEROLOGOUS SENSITIZATION OF ALPHA1A-ADRENERGIC RECEPTORS IN MESENCHYMAL STEM/STROMAL CELLS

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Mesenchymal stem/stromal cells (MSC) are identified in many tissues of adult organism. MSC play an important role in reparation, regeneration and homeostasis. MSC themselves are regulated by hormones and neurotransmitters and noradrenaline is one of their key regulators. Recently, we showed that, all isoforms of adrenergic receptors are expressed in MSC, most of them are not coupled with calcium-dependent system of intracellular signaling. Noradrenaline increases this coupling and induces heterologous sensitization of alpha1a-adrenergic receptors on MSCs by acting on beta-adrenergic receptors and adenylyl cyclase. In this work we investigated alternative to noradrenaline adenylyl cyclase inducers that are capable of increasing MSC sensitivity to catecholamines. We selected hormones and neurotransmitters whose receptors activate adenylyl cyclase, such as dopamine (DRD1, DRD5), histamine (HRH2), serotonin (HTR4, HTR6, HTR7) and adenosine (A2B, A2A). Using PCR we showed that MSC express mRNA of receptors A2A, A2B, DRD1, DRD5, HRH2, HTR6, and HTR7. We analyzed how these neurotransmitters affect the functional activity of MSC. We stimulated MSC with one of these neurotransmitters and 6 hours later we registered Ca2+-dependent responses to noradrenaline. We found that serotonin increases sensitivity of MSC to noradrenaline. Histamine, adenosine and dopamine do not change it. In order to establish mechanism of this phenomenon we showed that stimulation of MSC with serotonin leads to an increase in alpha1a-adrenergic receptors protein level. Using an inhibitor analysis, we investigated signaling pathways of serotonin regulated sensitivity of MSC to noradrenaline. We showed that serotonin activated signaling pathway of adenylyl cyclase/cAMP/proteinkinase A/CREB and EPAC leads to increase of noradrenaline sensitivity. Thus, we defined that serotonin regulated coupling of adrenergic receptors and calcium signaling in MSC by elevation of alpha1a-adrenergic receptors.

Funding source: The reported study was funded by RFBR according to the research project N° 18-015-00421.

Keywords: MSC, Serotonin, Alpha1a-adrenergic receptors

TSC392

AGED MESENCHYMAL STROMAL CELLS: DIFFERETIATION, PROLIFERATION AND HORMONAL REGULATION

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Nowadays mesenchymal stromal cells (MSCs) become the subject of many researches due to their regenerative properties, which find numerous applications, such as the autologous transplantation, stimulation of endogenous mechanisms of regeneration of damaged tissues. As the stem cells, they are capable of self-renew and differentiation, which are the processes that affect the size of the MSCs population and its regenerative potential throughout a person's life. The aging of stem cell population has negative effect on both proliferative properties of MSCs and their ability to differentiate. The main aim of our research was to clarify the effect of aging on the hormonal regulation of differentiation and proliferation of MSCs. We have implemented the measurements with two general models for aging of MSCs, namely the in vitro and in vivo aging. In first case, we used prolonged passaging in culture, thereby achieving replicative aging. As for second model, we isolated cells from donors of different ages. Mitotic activity, adipo-differentiation and hormonal sensitivity of cells were observed in real time at the single-cell level. We have demonstrated that MSCs of both young and elderly donors, planted with a higher density showed the more efficient adipo-differentiation. The most probable explanation of this effect involves the paracrine factors secreted by MSCs. Senescent cells ceded in both high and low density have less potential for differentiation and lose hormonal regulation of this process. Thus, MSCs isolated from young donors in the early passages are more effective in their adipo- differentiation after the serotonin

stimulation and less effective after the norepinephrine stimulation, though these effects are not inherent to senescent cells. Next we studied the change of character of hormone responses. First, we observed that senescent MCSs have worse calcium response to norepinephrine and serotonin. And second, we found a better calcium response to insulin, which is not typical for MSCs. Moreover, this response was found to show oscillatory behavior. To sum up, numerous patterns in the aging of MSCs have been discovered, as well as the influence of aging process on properties of MSCs, which is a crucial step towards deeper understanding of age-associated metabolic and other disturbances.

Funding source: We acknowledge financial support of the RFBR Grant Nº 19-315-80018.

Keywords: differentiation potential, hormonal regulation, aging

CARDIAC

TSC122

IMPROVED DRUG RESPONSE OF CHEMICALLY INDUCED CARDIAC SPHEROIDS BY ELECTRICAL STIMULUS

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The cardiotoxicity of drugs is one of the main reasons for failure in drug development. Conventional models such as 2D cells and animal models that have been used to assess drug cardiotoxicity often do not provide precise drug response and toxicity because they do not mimic accurately human heart tissue. Thus, the development of new models which are able to evaluate cardiac drug toxicity more precisely is urgently required. In this study, we created induced cardiac spheroids that show improved response to the drugs using direct chemical reprogramming and electrical stimulation. Mouse embryonic fibroblasts were converted to cardiomyocytes using small molecules, and 3D spheroids were prepared with induced cardiomyocytes using microwells. 3D cardiac spheroids show enhanced differentiation due to enhanced cell-cell and cell-matrix interactions. Moreover, when electrical stimulus that mimics the pulse of heartbeat was applied to the spheroids through the micropillar array, the cardiac differentiation was further enhanced. Importantly, the response of electrically stimulated cardiac spheroids to the drugs that affect the heart rate was increased. The engineered cardiac models would be useful for drug screening and development.

Funding source: This research was supported by a grant (19172mfds168) from Ministry of Food and Drug Safety in 2020.

Keywords: Direct reprogramming, cardiac spheroid, biphasic electrical pulse

TSC125

MESENCHYMAL STEM CELL-DERIVED ANGPTL4 IMPROVES ENDOTHELIAL PERMEABILITY AND RESOLUTION OF INFLAMMATION IN ATHEROSCLEROSIS.

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Atherosclerosis, a primary cause of cardiovascular complications, is driven by chronic inflammation and endothelial dysfunction. We recently showed that mesenchymal stem cells (MSCs) released high level of angiopoietin-like 4 (ANGPTL4) to suppress pathological inflammation. Given the fundamental contribution of inflammation to atherosclerosis, we determined the role of ANGPTL4 in regulating vascular inflammation and atherosclerotic lesion. ANGPTL4 (2 microgram per mouse) was injected three times a week to ApoE-/- mice. On Western diet, these mice exhibited marked decrease in lesion formation, lipid deposition, inflammatory cell content, and vascular permeability. ANGPTL4 suppressed inflammatory macrophage phenotype, attenuated foam cell formation and monocyte adhesion on endothelial cell. Moreover, ANGPTL4 increased anti-inflammatory macrophage phenotype in bone marrow-derived macrophages. We further showed that ANGPTL4 remarkably reduced the adhesion of monocytes in TNF-alpha-stimulated endothelial cells. Moreover, Kruppel-like factor 2 (KLF2) and VE-cadherin, markers of vascular integrity, were upregulated, while inflammatory markers such as ICAM-1 and VCAM-1 were inhibited by ANGPTL4 in endothelial cells. We are currently elucidating clinical significance of ANGPTL4 by cadaver analysis and ELISA using blood samples from patients with acute myocardial infarction. In conclusion, stem cell-derived ANGPTL4 attenuated atherogenesis by limiting pro-inflammatory monocyte adhesion and protecting endothelial stability, implicating that targeting vascular stability and inflammation may serve as a novel therapeutic strategy to prevent and treat atherosclerosis.

Keywords: Atherosclerosis, Endothelial permeability, Inflammation

EARLY EMBRYO

TSC131

THR177 PHOSPHORYLATION OF TFCP2L1 BY CDK1 IS REQUIRED FOR STEM CELL PLURIPOTENCY AND BLADDER CARCINOGENESIS

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Molecular programs involved in embryogenesis are frequently upregulated in oncogenic dedifferentiation and metastasis. However, their precise roles and regulatory mechanisms remain elusive. Here, we showed that CDK1 phosphorylation of transcription factor CP2-like protein 1 (TFCP2L1), a pluripotency-associated transcription factor, orchestrated pluripotency and cell-cycling in embryonic stem cells (ESCs) and was aberrantly activated in aggressive bladder cancers (BCs). In murine ESCs, the protein interactome and transcription targets of Tfcp2l1 indicated its involvement in cell cycle regulation. Tfcp2l1 was phosphorylated at Thr177 by Cdk1, which affected ESC cell cycle progression, pluripotency, and differentiation. The physical and functional interaction between TFCP2L1 and CDK1 is conserved in human BC cells and modulates their proliferation and stemness features. Employing in vitro culture models, the CDK1-TFCP2L1 pathway was activated in human BC cells with different molecular subtypes, stimulating their proliferation, self-renewal, and invasion. Lack of TFCP2L1 phosphorylation impaired the tumorigenic potency of BC cells in an orthotropic xenograft model. In patients with BC, high co-expression of TFCP2L1 and CDK1 was associated with unfavorable clinical characteristics including tumor grade, lymphovascular and muscularis propria invasion, and distant metastasis and was an independent prognostic factor for cancer-specific survival. These findings demonstrate the molecular and clinical significance of CDK1-mediated TFCP2L1 phosphorylation in stem cell pluripotency and in the tumorigenic stemness features associated with BC progression, advancing our understanding of how transcription regulation related to normal stemness can also act as a key player for modulating pathogenic stemness features.

Funding source: National Research Foundation of Korea (NRF-2018R1A2B2001392), by a NRF MRC grant funded by the Korean government (MSIP) (2018R1A5A2020732), and by the Ministry of Education (2018R1D1A1B07047450 and 2017R1D1A1B03031379).

Keywords: Bladder cancer, CDK1, embryonic stem-cell

TSC132

POST-TRANSLATIONAL MODIFICATIONS OF TRANSCRIPTION FACTOR CP2-LIKE 1 REGULATE THE PLURIPOTENCY OF MURINE EMBRYONIC STEM CELLS

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Transcription-factors and chromatin regulatory proteins regulate core functions of stem cells by maintaining their specific gene-expression patterns. Unlike differentiated somatic cells, embryonic stem cells (ESCs) commonly express the pluripotency core transcription-factors such as Oct-4, Nanog, and Sox2. These transcription-factors form the pluripotent core circuitry by reinforcing the expression of genes, which are involved to keep ESCs undifferentiated status but repressing the differentiation inducing transcription. Transcription factor CP2-like protein 1 (Tfcp2l1), a member of the CP2 family of TFs, is identified a Wnt-responsive gene in mouse embryos. Tfcp2l1 is implicated in establishment and maintenance of pluripotency through formation of complex transcriptional networks with the other transcription factors, Oct4, Sox2, and Nanog as well as regulating their transcription. However, the detail mechanism how of Tfcp2l1 regulation in ESCs remains to be determined. Our aim was to study the regulation of expression and post-translational modification of Tfcp2l1 in relation to protein activity and to the properties of ESCs. By analyzing Tfcp2l1 protein interactome in murine ESCs, we found that Tfcp2l physically interacted with Cdk1 protein. Tfcp2l1 was phosphorylated at Thr177 by Cdk1, thereby regulating its DNA-binding activity. Lack of phosphorylation of Tfcp2l1 disrupted proper cell-cycle progress, pluripotency, and differentiation of ESCs as well as naïve pluripotency or somatic cell reprogramming. Mechanically, Tfcp2l1 Thr177 phosphorylation regulates cell-cycle-related genes by direct targeting. Considering together, these results demonstrate that phosphorylation of TFCP2L1 by cyclin-dependent kinase 1 (CDK1) is a novel molecular circuitry that is necessary for pluripotency in ESCs, not only advancing our understanding the precise molecular nature of ESCs, but also accelerating the clinical application of ESC based stem cell therapy.

Keywords: Tfcp2l1, CDK1, Pluripotency

TSC374

DUX4 REGULATES TRANSCRIPTIONAL DYNAMICS DURING EARLY HUMAN EMBRYONIC DEVELOPMENT

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In the oocyte-to-embryo transition, fertilized oocyte undergoes final maturation and embryonic genome is gradually activated during the first three cell divisions. In human, how this transition is coordinated and which factors drive this process are still largely unknown. Here we studied the role of the double homeodomain transcription factor DUX4 during human early development. We found significant upregulation of DUX4 in zygotes and discovered that DUX4 plays an important role in regulating the human oocyte-to-embryo transition at transcriptome and epigenome level. Although DUX4 knockdown (siDUX4) human zygotes did not arrest during the two-day culture after microinjection, significant number of maternal transcripts which should be degraded during the oocyte-to-embryo transition were retained in the siDUX4 zygotes. When DUX4 was overexpressed in human embryonic stem cells (hESCs), 23 out of the 32 genes typically activated during the transition from oocyte to 4-cell stage were significantly upregulated. Interestingly, regions that gained chromatin accessibility by DUX4 overexpression were overrepresented with ERVL-MaLR retrotransposon long terminal repeat (LTR) elements, which were significantly enriched with DUX4 binding sites. We further identified ~10,000 novel enhancers in DUX4-overexpressed hESCs, including several enhancers targeting early embryonic genes. These observations indicate that DUX4 modulates the degradation of maternal transcripts and works as a powerful activator of early embryonic genes, LTR elements, and enhancers during the oocyte-to-embryo transition. These results provide us better understanding of regulatory mechanism during the first days of human embryonic development and new insights into the stem cell technologies.

Keywords: Early human embryo, Transcriptome, Enhancers

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

TSC140

SUSTAINED EXPRESSION OF STEM CELL TRANSCRIPTION FACTOR OCT 4 INHIBITS DIFFERENTIATION TO HEPATOCYTE-LIKE CELLS

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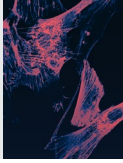
Many cell therapy studies have explored the use of stem cells for liver failure. For efficient generation of hepatocyte-like cells, we selected known liver transcription factors (hHnf1A, hHnf4A, and hFox4) and tested whether additional treatment with Oct4 as key marker of pluripotency of embryonic stem cells and A83-01 as an inhibitor of epithelial-to-mesenchymal transition could reinforce the induction of hepatocyte stem cells. To generate mouse hepatocyte-like cells (miHeps), mouse embryonic fibroblasts (MEFs) were co-transfected with three (hHNF1A/hHNF4A/hFOXA4, 3F) or four (hHNF1A/hHNF4A/hFOXA3/hOCT4, 4F) lentiviral vectors (MOI=1). The MEFs were cultured in hepatocyte induction medium alone or medium containing A83-01. After one month, clusters of hepatocyte-like cells with polygonal cytoplasm and bipolar nucleic acid were clearly observed, and there was a continual increase in the number of the clusters of the hepatocyte-like cells with an increase in time. miHeps were evaluated with respect to the activation of both exo/endo-genic liver transcription factors and genes related to hepatocyte function, using real time PCR. Compared with 3F, 4F did not enhance the expression of genes for endo/exogenic transcription factors in the absence and presence of A83-01, except for hFOXA3. In groups treated with A83-01, 4F decreased the levels of hHNF1A, hHNF4A, mHNF1A, and mHnf4a more than 3F. In the 3F groups, A83-01 accelerated the expression of hFOXA3, mHnf1a, and mFoxa3. Expression of the Alb and Trf genes related to hepatocyte function was highest in 3F treated with A83-01, whereas expression of the Vim fibroblast marker was lowest in 4F not treated with A83-01. Cyp3a11 expression was increased and decreased in 3F untreated or treated with A83-01, respectively. The collective findings indicate that the sustained expression of Oct4 could inhibit differentiation



to hepatocyte-like cells, whereas A83-01 could accelerate generation of hepatocyte-like cells. The data could help improve the generation efficiency of hepatocyte-like cells.

Funding source: This work was conducted with the support of "Cooperative Research Program for Agriculture Science &Technology Development" carried out with the support of Program (no. PJ01100203).

Keywords: Hepatocyte like cells, Mouse, Oct4

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

TSC422

METABOLIC REGULATION OF LINEAGE SPECIFICATION DURING ENDOTHELIAL TO HEMATOPOIETIC TRANSITION

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During early development, the first hematopoietic stem cells (HSCs) emerge and derive from hemogenic endothelial (HE) cells, through a process called endothelial to hematopoietic transition (EHT). Adult HSC quiescence, maintenance and differentiation are tightly regulated by changes in metabolism. However, the role of metabolic cues during the first emergence of HSCs from HE cells is still unclear. Using human iPS-derived HE cells, we showed that during EHT, there is a gradual and simultaneous increase in glycolysis and oxidative phosphorylation. Interestingly, blocking glycolysis or glutaminolysis severely impaired hematopoietic differentiation. While the rescue of glutamine deprivation by α-ketoglutarate boosted myeloid/lymphoid cell formation, we found that a combination of α-ketoglutarate and nucleotides was required for erythropoietic commitment of HE cells. Both in vitro and in vivo, differentially modulating pyruvate catabolism directed HE cell fate toward either erythroid or myeloid/lymphoid outcomes. Thus, our findings uncover that metabolism plays a crucial role during human EHT and nutrients modulate lineage specification during hematopoietic emergence.

Keywords: Hematopoiesis, Endothelial to hematopoietic transition, Metabolism

EPITHELIAL

TSC396

HUMAN SALIVARY GLAND STEM CELL-DERIVED EXTRACELLULAR MATRIX ENHANCES FORMATION AND MATURATION OF SALIVARY GLAND ORGANOIDS

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The purpose of this study was to culture human salivary gland organoids using salivary gland stem cell(SGSC)-derived extracellular matrix (ECM) instead of the basement membrane-derived ECM (Matrigel) to obtain transplantable organoids from human resources. Decelluarized ECM was prepared from primary human SGSCs of parotid glands. Human salivary cells were then embedded into Matrigel, human adipose mesenchymal stem cell (MSC)- derived ECM, or human SGSC-derived ECM. The number, size, and growth of organoids were measured and compared among three groups. The cell types in generated organoids were identified by immunofluorescent staining. The secretory salivary function was determined by measuring amylase activity. Salivary gland organoids were generated in all the ECM cultures and growth rate of organoids was similar in three conditions. However, the expression level of ductal cell markers, K5 and K7 at the basal and luminal position, respectively, was not identical. Although AQP5, a pro-acinar marker, was identified in three ECM cultures, Amy1 was only present in the cytosol in SGSC-derived ECM culture group. The activity of Amy1 per unit cell was significantly higher in SGSC-derived ECM than those of MSC-derived ECM or Matrigel. Salivary gland organoid using SGSC-derived ECM successfully generated salivary gland organoids to recapitulate the specific characteristics of human salivary glands. We propose that salivary gland organoid culture in SGSC-derived ECM may offer opportunities to broaden insights on tissue-mimicking salivary gland organoids.

Keywords: Salivary gland, Tissue stem cells, Extracellular matrix

TSC407

A COMPARATIVE STUDY OF TIMP OVEREXPRESSION IN HUMAN OVARIAN CANCER CELLS: A STEP TOWARDS CONTROLLING METASTASIS BY MMP INHIBITION

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Ovarian cancer is the most lethal of all the gynaecological cancers, with the highest case to fatality ratio. Since most cases are detected at later stages, the then highly metastatic cells are very hard to treat and require improved therapeutic regimes. The microenvironment of healthy and cancerous tissues have very different concentrations of matrix metallo-proteinases (MMPs), which play an important role in the invasion process of metastasis. Likewise, tissue inhibitor of matrix metalloproteinases, TIMPs, the endogenous inhibitors of these MMPs, also differ in their concentration. Normally, there is a balance between MMPs and TIMPs, which when disturbed contributes to cancer. Therefore, overexpressing each of the TIMPs in ovarian cancer cells, and assessing the resultant effects, gives an insight into the probable role of the TIMPs, individually and in combination. The overall aim of this project is to select a TIMP or set of TIMPs to be delivered therapeutically by mesenchymal stromal cells to the tumour site, reducing the incidence of metastasis. Each of TIMPs 1-4 were over-expressed in SKOV-3 cells (epithelial ovarian cancer cells) and in KGN cells (granulosa cell tumour cells). Each TIMP was cloned into the pLVX-IRES-tdTomato vector and transfected using lipofectamine 3000. The effects of the TIMP overexpression were assessed using viability, adhesion and migration assays. For both cell types, adhesion to extracellular matrix proteins was reduced by around 20 % through overexpression of each of the four TIMPs. Effects on viability and migration levels varied by TIMP and by cell line. In SKOV-3 cells, TIMP-4 decreased viability, whereas TIMP-2 increased it by 40 %. TIMP-2 also increased the number of migrating cells by 25 % for SKOV-3 cells and TIMP-1 transfection showed the lowest level of migration. For KGN cells, no TIMP increased viability or migration, and TIMP-4 lowered both to the greatest extent. Thus, the results show a mixed effect of TIMPs on the metastatic potential of ovarian cancer cells. This knowledge will be further explored. Mesenchymal stem cells, which possess an innate ability to home towards the tumour site and have been proven to be safe in many therapeutic studies, could be then used as a vehicle to deliver the best TIMP or combination of TIMPs to the tumour site.

Funding source: Funded by the Otago Medical Research Foundation, the Granulosa Cell Tumor Research Foundation and a University of Otago Research Grant and Dean's Bequest Award.

Keywords: Mesenchymal stromal cells, Cancer, Protease

TSC411

OXYGEN REGULATES DENTAL EPITHELIAL STEM CELL PROLIFERATION VIA RHOA-ACTOMYOSIN-YAP/TAZ SIGNAL

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Molecular oxygen (O2) has been proposed to be an important component of the cellular microenvironment serving as signalling molecule to regulate stem cell behaviour. However, little is known about the role of O2 in epithelial stem cells. In this study, we investigated the impact of microenvironmental oxygen level on the cell proliferation of dental epithelial stem cells (DESCs) using continuously growing mouse incisors. First, we found that the slow cycling DESCs are localized out of touch with blood vessels. Scanning electron microscopy analysis revealed that, in DESCs, the number of mitochondria was fewer and the size was smaller, compared to actively proliferating transit amplifying cells (TACs). Further, the expression of hypoxia-inducible factor 1 alpha (Hif1a) was higher in DESCs than that in TACs. In contrast, the expression of oxidative phosphorylation markers in DESCs were lower than those in TACs. These results suggested that DESCs were maintained in relatively more hypoxic environment. Next, when mouse incisors were cultured under hypoxic condition, the number of Ki67 and YAP/TAZ positive cells in dental epithelial cells decreased. Additionally, hypoxia induced RhoA activation in DESCs. Conversely, inhibition of Rho signal using ROCK inhibitor promoted cell proliferation and translocation of YAP/TAZ into nucleus concomitantly with loss of adherence junction and cortical actomyosin. Furthermore, inhibition of myosin light chain and knock down of adherence junction protein, Merlin, also induced cell proliferation and nuclear translocation of YAP / TAZ. These results suggested that hypoxic condition maintained slow cycling DESCs via RhoA-cytoskeleton-YAP/TAZ signal.

Funding source: This work was financially supported by JSPS KAKENHI, Grant Numbers 18K09526, 26462794, JSPS and NRF under the Japan-Korea Basic Scientific Cooperation Program (2018-2020)

Keywords: Oxygen, epithelial stem cells, RhoA signaling

TSC417

DISTINCT DIFFERENCE OF RADIATION RESPONSE BETWEEN LUMINAL AND BASAL CELLS IN RAT MAMMARY GLANDS

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Breasts are very susceptible to radiation-induced carcinogenesis, and mammary stem/progenitor cells are potentially important targets of this. The mammary epithelium is maintained as two mostly independent lineages of luminal and basal cells. To reveal their immediate radiation responses, we analyzed the mammary glands of female Sprague-Dawley rats, a radiation carcinogenesis model, using colony formation, flow cytometry (FCM), and immunofluorescence (IF). The results revealed that FCM successfully fractionates rat mammary cells into CD49fhi CD24lo basal, CD49fmed CD24hi luminal progenitor, and CD49flo CD24hi mature luminal populations, resembling human breast, rather than mouse tissues. The colony-forming ability of the basal cells was more radiosensitive than the luminal progenitor cells. FCM and IF showed more efficient cell cycle arrest, γH2AX responses, and apoptosis in the irradiated luminal progenitor and mature luminal cells, than the basal cells. These results indicate distinct difference of radiation response between luminal and basal cells in rat mammary epithelium, which will provide important insights into the early phase of radiation-induced breast cancer.

Funding source: This work was supported mostly by MEXT grant no. JPMX 16S16816001 and in part by MOE and JSPS grant nos. JP15H02824 and JP19K20455, and conducted partly in association with QST President's Strategic Fund (Exploratory Research).

Keywords: Mammary epithelial cells, Radiation Responses, mammary organoid

EYE AND RETINA

TSC192

APPLICATION OF IPSC DERIVED ENDOTHELIAL CELLS TO REPAIR ABNORMAL RETINAL VASCULATURE IN ZEBRAFISH ANIMAL MODEL

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Human endothelial cells (ECs) are essential for proper vascular human development and their dysfunction can cause a wide range of severe diseases. A number of these diseases, such as diabetic retinopathy (DR), familial exudative vitreoretinopathy (FEVR) and retinopathy of prematurity (ROP) affect the retinal vasculature and are on the rise within the human population. Our research focuses on the potential treatment of abnormal retinal vasculature using iPSC differentiated ECs. This approach is very appealing as the use of patient-specific iPSCs offers unlimited source of stem cells with limited risk of rejection. The human eye also provides an ease of administration due to its accessibility and being an immune-privileged organ. iPSCs were differentiated with high efficiency into fully functional ECs with the ability to form tubules in vitro, and characterized by several functional assays. A zebrafish animal model of defective vasculature, fzd4-/- fli1:EGFP, is used to model the human FEVR disease. The injection of the differentiated ECs into the subretinal space of a 5dpf and adult fzd4-/- fli1:EGFP mutant zebrafish is currently underway. Tracking of ECs over the course of several weeks followed by visualisation and quantification of their integration into the host vasculature, and their ability to rescue the abnormal phenotype is now in progress. As the number of people affected by vascular eye diseases is increasing, the need for effective treatment with ease of application and long-lasting effects is crucial, and with our current research we hope to begin to achieve this.

Keywords: retina, retinal vasculature, Endothelial cells (EC), zebrafish, animal model

GERMLINE

TSC205

DEVELOPMENT OF A NOVEL HUMAN ENDOMETRIAL STEM CELL-LADEN 3D ARTIFICIAL ENDOMETRIUM

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Serious injuries of endometrium in women of reproductive age are often followed by uterine scar formation and a lack of functional endometrium predisposing to infertility or miscarriage 1. In this context, extensive research efforts have been devoted

to regenerate endometrium and thus subsequently improve pregnancy rates. However, in fertility treatment, regenerating the endometrium is very challenging. Therefore, we have engineered collagen and hyaluronic acid interpenetrating polymer network matrix with various endometrium consisting cell types, including vascular endothelial cells, stromal cells, endometrial stem cells, as a 3D artificial endometrium in an attempt to devise novel endometrial regeneration therapies. The endometrial stem cell-laden 3D endometrium complex constitutes a triple-line endometrium reflecting the separation of the stratum basalis and functionalis layers to recapitulate the physiological feature of human endometrium. Collagen and hyaluronic acid complex provides 3-dimmensional architecture for the attachment, survival and differentiation of human endometrial stem cells and maintain their own characteristics and functions. We then evaluated the therapeutic effect of cell-laden 3D endometrium complex in endometrial ablation mice. At two weeks after the transplantation of endometrium complex, the complex was properly incorporated into the basal membrane of regenerative endometrium. Consistently, severe degenerative changes with the loss of the endometrial functional layer were significantly relieved by endometrium complex transplantation. Of particular importance was the successful subsequent pregnancy and live-birth after transplantation of 3D endometrium complex into endometrial ablation mice. The work not only provides a novel therapeutic approach to generate human endometrium but also demonstrates a new paradigm in tissue engineering via successful integration of stem cells into multiple types of natural polymers.

Keywords: Human endometrial stem cell, 3D artificial endometrium, successful pregnancy and live-birth

TSC207

MESENCHYMAL STROMAL CELLS CAN POTENTIALLY COORDINATE STEM CELL NICHE RECOVERY

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Function of adult stem cells is indispensable for tissue renewal or restoration after injures. Their microenvironment, a stem cell niche, maintains stem cell pools and regulates cell behavior in accordance with neighboring and distant cues. Thus, to guarantee sufficient tissue regeneration, coordinated niche restoration is a critical issue. Until now, the mechanisms regulating this process remain elusive. Mesenchymal stromal cells (MSC) might be considered as coordinators of stem cell niche restoration due to their ability to maintain viability and regulate the function of resident cells due to the secretion of various paracrine factors and extracellular vesicles. In some cases, to maintain stem cells and promote tissue regeneration, MSC might mimic the paracrine function of damaged niche components. However, the mechanisms of stem cell niche restoration and involvement of MSC in this process remain poorly understood. This research was devoted to the study of the participation of MSC in the restoration of a stem cell niche. The spermatogonial stem cell (SSC) niche was chosen as a model, since its composition was well-characterized, and the mechanisms of paracrine regulation of stem cell behavior and their microenvironment were described. In a model of abdominal cryptorchidism in rats SSC niche was injured. As a result it was found that subtunical injections of MSC or MSC secretome were sufficient to restore spermatogenesis and subsequently male rat fertility. That was also accompanied by an increase in the number of Sertoli cells as well as restoration of the secretory function of Leydig cells. Thus, the study showed that MSC stimulated the restoration of the SSC niche maintaining the key components of the niche. We suggest that the observed effect is mediated by the paracrine function of MSC. The data obtained indicate that MSC can temporarily mimic the functions of lost supporting cells providing paracrine stimuli for target cells and thereby supporting tissue regeneration processes after damage.

Funding source: The study was supported by the RSF (project no. 19-75-30007, investigation of MSC role in SSC niche restoration) and RFBR (no. 18-315-00403, experiments on isolated Leydig and Sertoli cells in vitro).

Keywords: Stem cell niche, Mesenchymal stromal cells, Spermatogenesis

HEMATOPOIETIC SYSTEM

TSC214

TRACING THE ENDOTHELIAL-TO-HAEMATOPOIETIC LINEAGE TRAJECTORY IN DIFFERENTIATING HUMAN EMBRYONIC STEM CELLS BY SINGLE CELL RNA SEQUENCING

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It has been established that haematopoietic stem and progenitor cells (HSPC) arise from specialized haematogenic endothelial cells in a process known as endothelial to haematopoietic transition (EHT). However, it is less clear the molecular processes involved. Using 10x sequencing technology and 65,000 cells collected at 4 different points during the differentiation of human Embryonic Stem cells towards HSPC, we have been able to map the lineage trajectory during EHT. We did observe a downregulation of endothelial genes, CDH5 and KDR, and upregulation of haematopoietic genes, RUNX1, ANGPT1, SPN and PTPRC, during EHT across our timepoints. Furthermore, our data reveal an increase in the expression of progenitor markers as differentiation progresses. Taken together, our lineage map reveals the transcriptional landscape towards HSPC generation. An understanding of the molecular program involved in EHT in current differentiation protocol, will shed light on its improvement towards generating Haematopoietic Stem Cells in the dish.

Funding source: Presidential Special Scholarship for Innovation and Development (PRESSID)

Keywords: Lineage tracjectory, Transcriptional landscape, Single Cell RNA Sequencing

TSC226

ABLATION OF SIRTUIN1 IN THE NON-HEMATOPOIETIC BONE MARROW MICROENVIRONMENT IS NOT REQUIRED FOR HEMATOPOIETIC STEM CELL FUNCTION IN THE ADULT MICE

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SIRTUIN1 known as a histone deacetylase is relevant to stem cell homeostasis. It involves various cellular responses including cell proliferation, apoptosis, and inflammatory responses. The previous study showed that loss of Sirtuin1 in the hematopoietic stem and progenitor system promotes the expansion of hematopoietic stem and progenitor cell population under stress conditions. Also, hematopoietic stem and progenitor cell population are regulated by SIRTUIN1 activators in the bone marrow. The role of SIRTUIN1 in the non-hematopoietic bone marrow microenvironment was examined in this study. Ablation of Sirtuin1 in the non-hematopoietic bone marrow microenvironment demonstrated that the production of mature blood cells and frequencies of hematopoietic stem and progenitor cell population attained similar results to those of controls. In addition, the ablation of Sirtuin1 in the non-hematopoietic bone marrow microenvironment had no influence on stem cell function under stress conditions. Thus, SIRTUIN1 is dispensable for a physiological role for non-cell-autonomous function in the maintenance of the adult hematopoietic stem cell compartment in the adult mice.

Keywords: SIRTUIN1, hematopoietic stem and progenitor cell, bone marrow microenvironment

MUSCULOSKELETAL

TSC380

DEER ANTLER AS A MODEL OF NEURAL CREST DERIVED STEM CELL ORGAN REGENERATION

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Deer antler is the only mammalian organ that can annually regenerate to produce a complex tissue containing dermis, blood vessels, nerves, cartilage and bone. The neural crest derived stem cells that drive this process result in tissue growth of up to 2 cm/day. The underlying mechanisms of this growth are not fully understood and this study aimed to examine the location of stem cells during antler growth, the proteomic profile of different pools of stem cells at different states of activation, and the role of the pleiotrophin (PTN) family in stem cell regulation. Mesenchymal stem cell (MSC) markers CD73, CD90 and CD105, along with PTN and its receptors, were examined in antler tissues using immunohistochemistry. Label-free mass spectrometry was used to detect the protein expression profiles of: dormant pedicle periosteum (DPP), growth centre (GC), post-active stem cells from mid-beam periosteum (MAP), and deer facial periosteum (FP) as a control (N=3, biological replication). PEAKS and Ingenuity Pathway Analysis software were used to analyze the proteomics data. Our research confirmed the central role of stem cells in the development of this mammalian organ by localizing the MSC markers within the antler. The high expression levels of PTN and its key receptors within the growth centre indicated its importance during rapid antler regeneration. Label-free proteomics identified unique markers of dormant (6), active (87) and post-active (3) antler stem cells. Activation of antler stem cells was associated with up-regulation of a number of canonical pathways and molecular/cellular functions such as PI3K/AKT signalling. This work identifies biomarkers for mammalian stem cells, as well as the key pathways and proteins involved in stem cell activation and control during rapid regeneration.

Funding source: University of Otago, Velvet Antler Research New Zealand

Keywords: Organ regeneration, Stem cells, Proteomics

NEURAL

TSC304

THE EFFICACY OF C5A RECEPTOR ANTAGONIST FOR HUMAN IPSC-DERIVED NEURAL STEM/ PROGENITOR CELL TRANSPLANTATION IN THE INJURED SPINAL CORD OF MICE

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We previously reported the efficacy of human-iPS derived neural stem/progenitor cell (hiPSC-NS/PC) transplantation for spinal cord injury (SCI) in the subacute phase. However, this procedure is not effective in the acute phase due to the inflammatory response occurring immediately after SCI, which weakens transplanted cell survival and differentiation. C5a, which is one of the complement components, is a powerful chemoattractant and recruits inflammatory cells through binding C5a receptor. Therefore, the purpose of this study is to suppress the inflammatory response immediately after SCI using C5a receptor antagonist (C5aRA) as an immunosuppressant, thus improving the efficacy of hiPSC-NS/PC transplantation for SCI in acute phase. We used immuodeficient SCID-Beige mice that lack lymphocytes and NK cells. First, to evaluate the influence of C5aRA on the inflammatory response post-SCI, we induced a thoracic spinal contusion injury in mice. We quantified inflammatory cytokines and inflammatory cells in injured spinal cord tissues using qPCR and flow-cytometry. Next, we divided the SCI mice into 4 groups (PBS only, C5aRA only, PBS + transplantation (PBS+TP), C5aRA + transplantation (C5aRA+TP)). Immediately after SCI, C5aRA or PBS was injected once a day for 4 consecutive days, and then, 5.0 × 105 hiPSC-NS/PCs were transplanted into the lesion epicenter on day 4. We evaluated cell survival rate by Bioluminescent Imaging (BLI), hindlimb motor function by BMS score, and the differentiation profile of the graft hiPSC-NS/PCs by immunohistochemistry. C5aRA administration reduced IL-1b, IL-6 and TNFα at 12 hours and macrophages at 4 days after SCI. The C5aRA+TP group had better functional improvement as compared to the PBS only group. BLI revealed that the C5aRA+TP group had a significantly higher cell survival rate compared to the PBS +TP group. There was no significant difference in the differentiation profiles of the graft hiPSC-NS/PCs between C5aRA+TP group and PBS+TP group. The present study demonstrated that administration of C5aRA could suppress the inflammatory response during the acute phase of SCI, and also improve the survival rate of transplanted hiPSC-NS/PCs and enhance motor functional restoration. hiPSC-NS/PCs transplantated with C5aRA are a promising treatment for acute phase SCI patients.

Keywords: spinal cord injury

TSC308

SPATIOTEMPORAL CONTROL OF AXONAL OUTGROWTH USING MAGNETIC NANOPARTICLES

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While neural cell transplantation offers a promising therapy for neurodegenerative diseases, the formation of functional networks between transplanted cells and host neurons constitutes one of the challenging steps. Here, we represent a magnetic guidance methodology that regulates neurite growth signaling via magnetic nanoparticles (MNPs) conjugated with antibodies targeting the deleted in colorectal cancer (DCC) receptor (DCC-MNPs). Activation of the DCC receptors by clusterization and subsequent axonal growth of the induced neuronal (iN) cells were performed in a spatially controlled manner. The directionality of axon projections was controlled by manipulating the magnetic field and an improved axonal growth of iN cells resulted in formation of highly functional connections with pre-existing primary neurons. Our results suggest magnetic guidance as a potent strategy for improving neuronal connectivity by spatially guiding the axonal projections of transplanted neural cells for synaptic interactions with the host cells. This study was supported by the Bio & Medical Technology Development Program of Korea National Research Foundation (NRF) funded by the Korean government, the Ministry of Science and ICT (MSIT) (2018M3A9H1021382).

Funding source: This study was supported by the Bio & Medical Technology Development Program of Korea National Research Foundation (NRF) funded by the Korean government, the Ministry of Science and ICT (MSIT) (2018M3A9H1021382).

Keywords: Magnetic nanoparticles, Reprogrammed neuron, Deleted in colorectal cancer receptor

TSC316

RESVERATROL PROMOTES ENRICHMENT OF NEUROPROGENITOR CELLS INDUCTION OF HUMAN STEM CELLS FROM APICAL PAPILLA

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The neurological disorders of central nervous system (CNS) account for more than 10% of permanent disabilities and death. Following CNS damage, endogenous repairing of the affected tissue by neuroprogenitor cells (NPCs) is limited. Therefore, exogenous stem cells-based therapies are emerging as alternative approaches. Human stem cells from apical papilla (hSCAPs) have been as mesenchymal stem cells (MSCs) which

derived from migratory neural crest stem cells. Therefore, the hSCAPs should be a good candidate for exogenous stem cells-based therapies of neurological disorders. Resveratrol (RSV), a natural polyphenol compound, has been described as a sirtin 1 (SIRT1) activator which play important roles in neuronal differentiation and neuroprotection. Moreover, RSV could promote enrichment of NPCs induction derived from the hSCAPs. Taken together, this study aims to enhance NPCs induction of the hSCAPs which induced by resveratrol treatment for further transplantation of neurological disorders model. The hSCAPs were collected from Thai healthy donors and characterized as the MSCs through high expression of CD73, CD90, CD105, CD146, colony forming unit (CFU), osteogenic and adipogenic differentiation. The cellular toxicity of resveratrol treatment to the hSCAPs (RSV-hSCAPs) weren't observed for up 50 µM for 12 hours. Moreover, nestin which represent NPCs marker highly expressed at 10 µM of RSV for 12 hours. Also, the RSV-hSCAPs at 10 µM, 12 hours should be more effectively differentiate into neuronal cells. After neuronal induction medium treatment for 30 hours, the hSCAPs and RSV-hSCAPs were changed into differentiated cells (d-hSCAPs and RSV-dhSCAPs respectively) which exhibited neuronal-like shape. Additionally, the RSV-dhSCAPs were highly expressed nestin when compared to hSCAPs and d-hSCAPs. In the other hand, the expression of MAP-2 and Beta-III tubulin which represent late neurogenic gene markers weren't significantly different between hSCAPs, d-hSCAPs and RSV-dhSCAPs. These results demonstrated that the hSCAPs can differentiate into NPCs and treatment of RSV can enhance NPCs induction. Thus, these finding provide the alternative of using the hSCAPs and potential of RSV treatment as stem cells-based therapy for further transplantation of neurological disorders model.

Funding source: This work was supported by grants obtained from the Science Achievement Scholarship of Thailand (SAST), Central Instrument Facility (CIF) Faculty of Science, Mahidol University, Thailand.

Keywords: Human stem cells from apical papilla (hSCAPs), Neuroprogenitor cells (NPCs), Resveratrol

TSC319

JDP2 CONTROLS ROS-MEDIATED APOPTOSIS AND NEURONAL DIFFERENTIATION OF MOUSE CEREBELLAR PROGENITOR GRANULE CELLS

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Jun dimerization protein 2 (JDP2) was predominantly expressed in granule cells of the cerebellum. Jdp2-knockout (KO) mice exhibited impaired development of the tubular structure of the cerebellum. The cerebellar progenitor granule cells (PGCs) from Jdp2-KO mice were less proliferative but were more resistant to ROS-dependent apoptosis compared with PGCs from wild-type (WT) mice. In Jdp2-KO PGCs, we found an elevation of reduced glutathione through upregulation of xCT/Slc7a11 cystine-glutamate antiporter, and lower levels of reactive oxygen species (ROS) and higher level of antioxidant response element (ARE)-driven luciferase. Overexpression of nuclear factor-E2-related factor 2 (Nrf2) and musculoaponeurotic fibrosarcoma-K (MafK) did not rescue ARE-luciferase promoter activity, indicating an essential role of JDP2 in inducing ARE activity. Moreover, both the expression of cyclin-dependent kinase inhibitor 1 (p21Cip1) and the interaction between p21Cip1 and Nrf2 were increased in Jdp2-KO PGCs. Knockdown of p21Cip1 induced higher levels of ROS and apoptosis in PGCs from Jdp2-KO PGCs than in those from WT mice, demonstrating the pivotal role of p21Cip1 in controlling oxidative stress and apoptosis of PGCs in the absence of JDP2. This indicated the retarded maturation of Jdp2-KO PGCs to form the altered lobes. We also isolated cerebellar granule stem-like cells and examined the neural differentiation. The differentiation into neuron was faster in Jdp2-KO PGC-stem like cells of than WT PGC-stem like cells. The characterization of neuronal cell types and its mechanism to differentiation will be discussed.

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Keywords: cerebellar granule progenitor cells, Jun dimerization protein 2, reactive oxygen species

NEW TECHNOLOGIES

TSC344

MIMICKING THE TUMOR-MICROENVIRONMENT: A NOVEL 3D CO-CULTURE PLATFORM OF INDUCED PLURIPOTENT STEM CELL DERIVED ORGANOIDS AND PATIENT-DERIVED MICROTUMORS

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Tumors naturally develop in a three-dimensional environment and interact with infiltrating immune cells, the extracellular matrix and stromal cells. The complex structure of the cancer ecosystem and the heterogeneity between different tumor-types and tumor-patients is one of the major challenges in the development of effective anti-cancer drugs. As a consequence, the majority of cancer therapeutics fail in clinical trials. In the past, different 3D cell culture models have been established to mimic cancer behavior in response to compound treatment. However, the currently existing technologies suffer from various limitations including the non-physiological composition of 3D cell culture matrices. Therefore, it is a pressing task to establish an in-vivo-like and reliable model which considers the highly complex interaction of a tumor with its environment. Our aim is to provide a novel 3D cell culture platform encompassing patient-derived microtumors embedded into induced pluripotent stem cell (iPSC) derived organoids enabling tumor growth in a more physiological, i.e. organ-like, surrounding. We are aiming to reprogram human CD34+ cells from peripheral blood into iPSCs and to differentiate these into kidney-, colon- and mammary-like organoids, respectively. We were able to establish the isolation and culturing of patient-derived microtumors from respective cancer types, which will be embedded into corresponding organoids. Ultimately, this iPSC-based system aims at providing a better insight into cancer cell biology as well as immune cell behavior within the tumor microenvironment under physiological conditions and thus, will function as a more reliable pre-clinical test-platform for drug development and mode-of-action analyses.

Keywords: 3D Co-Culture, Organoids, Patient-Derived Microtumor

TSC429

MACHINE LEARNING-DRIVEN LABEL-FREE FLOW CYTOMETRY

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Flow cytometry is useful for analyzing phenotypic characteristics of cells and is used for measuring the purity of target cells in heterogeneous cell populations for clinical diagnosis, as well as for controlling the quality of therapeutic cell products and monitoring them in blood samples. One critical issue for analyzing the cell products by flow cytometry is, however, that it requires multiple cell labeling using specific antibodies/reagents under GMP compliance, and the process is time-consuming and costly. Here we introduce label-free ghost cytometry (LFGC), a new machine learning-driven label-free flow cytometry that analyzes cell morphology based on cell-specific imaging waveform signals without image production. LFGC will become a simple, fast and cost-effective cell-typing method. In this study, we assessed the potential applicability of LFGC to analyze cell characteristics for the quality control of cell products. A model of supervised machine learning was developed from a data set of label-free GC waveform signals of T-cells that were labeled with markers of Annexin V plus PI. We then classified the T cells into live or dead cells by applying the trained model to the label-free GC waveforms without seeing the fluorescent labels. In a similar manner, we assessed the capability of LFGC to identify T cells in human white blood cells (WBC) by labeling them with CD3 markers. Moreover, we applied LFGC to discriminate in-vitro activated T cells from resting T cells by labeling them with CD25 markers. Our data showed the high accuracy of LFGC in classifying live and dead T cells with an area under the receiver operating characteristic curve (AUC) of 0.954. LFGC also discriminated the CD3 positive T cells from WBCs with AUC of 0.967 and also the activated T cells from the resting ones with AUC of 0.987. These and further results support that LFGC is able to accurately predict the viability, purity and possibly functions of T cells without any labeling and could be thus beneficial for the quality control of cell products.

Keywords: Cell therapy & regenerative medicine, Machine Learning, Cell Isolation

PLACENTA AND UMBILICAL CORD DERIVED CELLS

TSC366

AMNIOTIC FLUID MESENCHYMAL STEM CELLS PRECONDITIONED BY HYPEROXIC EXPOSURE BODY FLUID IMPROVE THERAPEUTIC EFFICACY IN THE HYPEROXIC ACUTE LUNG INJURY MOUSE MODEL

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Hyperoxic acute lung injury (HALI) is a common and serious side effects in patients requiring mechanical ventilation. Prolong exposure to hyperoxia in patients can exacerbate lung damage and even systemic injury, which associated with high mortality rate and no effective treatment currently available. Mesenchymal stem cells (MSCs) are widely considered for treatment of lung disease based on the anti-inflammatory, anti-fibrotic, anti-apoptotic and regenerative properties of the cells. However, elevated level of oxidative stress- and inflammation-related mediators in the body fluid, including bronchoalveolar lavage fluid and serum, of the HALI animal model and patients, which lead to a harsh microenvironment to influence the survival rate and therapeutic efficacy of engrafted MSCs for the repair of damaged lung. In this study, we aimed to prolong engrafted amniotic fluid mesenchymal stem cells (AFMSCs) survival and to enhance the effectiveness of HALI transplantation therapy by using body fluid-preconditioned AFMSCs. An optimal hyperoxia serum or BALF pretreatment condition has been established

in AFMSCs culture system. That resulted in upregulation of anti-apoptotic, anti-oxidant and anti-inflammatory genes. Intratracheal instillation of hyperoxia body fluid-preconditioned AFMSCs into hyperoxia-induced lung injury mice at 1h prior to hyperoxia exposure, the survival rate significantly improved and the expression level of inflammatory mediators, including IL-1beta and IL-6, and oxidative stress marker, iNOS, also remarkably reduced. Moreover, the histopathologic examination observed a significant amelioration in inflammatory cell infiltration, oxidative stress and DNA damage in alveolar cells in the body fluid-preconditioned AFMSCs transplanted groups. We conclude that transplantation of the body fluid-preconditioned AFMSCs showed better cytoprotective and regeneration efficacy than the general AFMSCs.

Keywords: Hyperoxia, Amniotic fluid mesenchymal stem cell, Preconditioned

TSC389

OSTEOGENIC DIFFERENTIATION POTENTIAL OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS CULTURED IN HYDROXYAPATITE SCAFFOLD

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Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into various cell types of mesodermal origin. MSCs play an important function in the bone regenerative process, therefore, they are the potential cell source for bone repair. However, transplantation of the cells for bone tissue repair is not much efficient due to hardly localize the cells. Recently, bone tissue repair has been focus on three-dimension (3D) culture. This study aims to explore the osteogenic differentiation potential of umbilical cord-derived MSCs during culture on hydroxyapatite (HA) scaffold. MSCs were isolated from the human umbilical cord (UC-MSCs) and cultured on the HA scaffold in the osteoinductive medium for 7, 14, 21, and 28 days. The osteogenic differentiation potential was determined by alkaline phosphatase (ALP) activity and the expression of the osteogenic genes including runt-related transcription factor 2 (RUNX2), osterix (OSX) and osteocalcin (OCN). The results showed that UC-MSCs could survive on the HA scaffold at least 28 days. ALP activity was increased during cultured on HA scaffold with osteogenic differentiation medium especially on day 21 and day 28 when compared to the growth medium. In addition, UC-MSCs cultured on HA scaffold had a higher osteogenic gene expression compared to the growth medium. The expression of RUNX2 was increased on day 14 and day 21 while the expression of OSX and OCN were increased on day 21 and day 28. The results demonstrated that UC-MSCs cultured on the HA scaffold could differentiate into osteoblasts, therefore, HA scaffold serves as compatible material for 3D cultured of UC-MSCs for application in bone tissue engineering.

Funding source: Thailand Graduate Institute of Science and Technology (TGIST)

Keywords: Hydroxyapatite, Mesenchymal Stem cell, Osteogenic differentiation

POSTER SESSION VI
11:00 – 13:00

Theme: Cellular Identity

ADIPOSE AND CONNECTIVE TISSUE

CI102

PROTEIN ARGININE METHYLTRANSFERASE 5 (PRMT5) IS A CO-ACTIVATOR AND NOVEL MEDIATOR OF HIGHER ORDER CHROMATIN STRUCTURE DURING ADIPOGENESIS

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Prmt5 catalyzes monomethylation and symmetric dimethylation of arginine residues of histone and other proteins. Prmt5 is required for embryonic development, regulates adult progenitor populations, transcriptionally represses growth control genes but also activates gene expression during myogenesis and adipogenesis. Previously, we found that Prmt5 co-activates Pparγ2 during adipogenesis by mediating a promoter-enhancer loop. Thus, we hypothesized that Prmt5 is a co-activator genome-wide by regulating higher order chromatin interactions during adipogenesis. To test this, we performed Prmt5 ChIP-Seq (chromatin immunoprecipitation paired with next generation sequencing) and Hi-C (high throughput chromosome conformation capture) of 3T3-L1 cells, a model for adipogenesis, harvested at day 0, 1, and 2 of differentiation (D0, D1, and D2 respectively). Prmt5 binds to chromatin that encodes regulators of DNA-templated transcription. 36% of Prmt5 peaks were at intergenic regions, 37% at intronic regions and 19% at promoters. Integration with public RNA- and ChIP-Seq data showed that Prmt5 binding correlates with incorporation of active histone marks and active transcription. Stable Prmt5 binding correlated with constant gene expression during adipogenesis. Inducible D1 Prmt5 binding was observed at the Pparγ2 promoter and 3,341 other intronic/intergenic regions, correlating with increased

transcription. 33,629 D0 peaks were lost by D1 and loss of Prmt5 binding lead to transcriptional repression. Prmt5-mediated co-activation was validated with Prmt5 pharmacological inhibitor EPZ015666, which prevented transcriptional activation of genes bound by Prmt5. Integration of D0 3T3-L1 Prmt5 ChIP and Hi-C data revealed that select Prmt5 binding sites were hubs for DNA looping. These hubs were contained within topological-associated domains (TADs) and were sensitive to Prmt5 knockdown (KD), while TAD boundaries remained unchanged. Aggregate peak analysis of 8,225 predicted chromatin loops bound by Prmt5 showed a loss of contact enrichment with Prmt5 KD while a gain in average contact enrichment was observed in another subset of 8,864 predicted loops. Together, these findings suggest that genome organization changes may be a key driver of Prmt5-mediated transcriptional changes during adipogenesis.

Funding source: Funding for this work provided by NIH Grant R01DK106162 and F32DK118846 (Imbalzano Lab) and HHMI grant and NIH Grants R01DK107980 and R01HG003143 (Dekker Lab)

Keywords: chromatin organization, adipogenesis, Prmt5

CARDIAC

CI305

PROFILING ATRIAL-VENTRICULAR SPECIFICATION DURING EARLY MOUSE DEVELOPMENT THROUGH SINGLE CELL RNA SEQUENCING

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The molecular mechanisms driving atrial and ventricular fate acquisition in vivo are incompletely understood. Others have sought to understand this fate specification by profiling transcriptional differences between the chambers through RNA sequencing, but much of this work is at stages after lineage specification. We have previously identified a cardiac progenitor population that transiently expresses Foxa2 during gastrulation and gives rise to progeny that form ventricular, but not atrial myocytes. Foxa2 lineage tracing thus allows us to track ventricular progenitors prior to and during the morphogenetic events that drive chamber formation. In this study, we sub-dissected the cardiac tissues and surrounding areas of Foxa2-Cre;mTmG embryos at the cardiac crescent (E8.25), primitive heart tube (E8.75), and late heart tube (E9.25) stages and performed single-cell RNA sequencing (scRNAseq) to profile the heterogeneity of cardiac precursors and identify mechanisms driving atrial and ventricular specification. We performed clustering and differential gene expression analysis on >10,000 cells at each stage. We identified several cardiac subpopulations corresponding to early progenitor populations, as well as other non-cardiac cell populations known to provide important signaling cues directing cardiac development. We find that Foxa2 lineage-traced cells can be identified on the basis of EGFP expression without the need for cell sorting, and that distribution of EGFP expression within cardiac lineages corresponds with clusters forming the developing ventricle. Next we will use computational lineage trajectory tools to track these progenitors over time to understand lineage hierarchies governing atrial/ventricular specification. In summary, combining genetic tools with scRNAseq provides resolution of atrial and ventricular precursors and highlights the sequential specification and differentiation trajectories in early heart development.

Funding source: Funding NIH/NHLBI R01HL134956-03

Keywords: cardiac development, atrioventricular specification, single-cell sequencing

EARLY EMBRYO

CI115

RNA POLYMERASE II PAUSING IS ESSENTIAL FOR LINEAGE DECISIONS IN THE EARLY MAMMALIAN EMBRYO

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Transcriptional control underlies the diversity of cell types within an organism. RNA Polymerase II pausing has been shown to serve as an intermediate step between transcriptional initiation and elongation at a subset of genes, yet the significance of this process remains unclear. Recent studies have suggested that pausing may be crucial to fine-tune cellular responses to signaling pathway activation in model organisms. However, the nature and relevance of this regulation have not been explored. To investigate whether pausing plays a role in signaling-mediated cell specification events in vivo, we utilized the early mammalian embryo, where FGF4 and NODAL signaling govern pluripotency establishment and progression. We deployed quantitative imaging methods to dissect the specification processes of the inner cell mass to pluripotent epiblast vs. primitive endoderm, and progression of epiblast from the naïve to primed state at single-cell resolution in embryos lacking the Negative elongation factor-b (Nelf-b), an obligate subunit for the pausing complex. Our results show that, while Nelf-b-/- embryos successfully specify primitive endoderm and epiblast lineages, the conserved primitive endoderm to epiblast ratio, ~1.5, observed in wild-type embryos is lost in Nelf-b-/- embryos and instead a divergent ratio of 0.7 – 2.5 appear due to accelerated lineage assignment. Following implantation, Nelf-b-/- embryos fail to initiate gastrulation and their epiblast fails to upregulate NANOG, a key marker of primed and posteriorly-localized pluripotent epiblast. The observed defects suggest a link between FGF and/or NODAL signaling and pausing and reveal RNA polymerase II pausing as a novel critical player in balancing and specifying cell fates during early mammalian development.

Funding source: NICHD: R01-HD094868 NIDDK: R01-DK084391 NCI: P30-CA008748 NIGMS: T32-GM007739

Keywords: RNA Pol II pausing, Developmental signaling, Early development

CI120

MICROENVIRONMENT- AND CELL TYPE-SPECIFIC FUNCTIONS OF CORE GENE REGULATORY NETWORK COMPONENT, FOXD3, IN HUMAN GERM LAYER DEVELOPMENT AND NEUROBLASTOMA

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Musculoskeletal conditions affect approximately 25% of the worldwide population, and neurocristopathies encompass at least 66 known disorders afflicting roughly 1 in 70 people globally. Production of relevant cell types via stem cell differentiation or direct cellular programming, both for therapies and modeling of human diseases, has progressed significantly. However, while it is well established that a common gene regulatory network (GRN) is deployed in multiple spatiotemporal instances during development to generate mesoderm-derived musculoskeletal and ectoderm-derived neural crest progenitors, it remains unclear how this core GRN produces both outcomes. Using high content analysis, we interrogated contextual determinants of effects generated by overexpression of a core GRN element, FOXD3, in human embryonic stem cells and a neuroblastoma cell line. This approach exposed interactions that separately regulate cell fate specification, apoptosis, and epithelial-mesenchymal character. Further, we identified a single developmental condition that promoted population-limiting FOXD3 activity similar to that observed in neuroblastoma and other cancer cell lines. RNA-seq comparison revealed that whereas 175 loci were found to be differentially regulated by FOXD3 overexpression in both conditions, only 3 loci were regulated in a way that correlated with phenotypic outcomes across multiple microenvironments and cell lines. Taken together, these results break down key GRN-microenvironment interactions in formation of progenitor populations of interest in musculoskeletal, craniofacial and peripheral nervous system disorders. Further, this study provides new insights that may lead to improved targeting or differentiation strategies for clinical interventions into the cell types of these systems, which are superficially similar but developmentally distinct.

Funding source: Funding for this project was provided by the New York Stem Cell Initiative (NYSTEM C30161GG).

Keywords: human embryonic stem cell, neuroblastoma, microenvironment

CI131

THE ROLE OF H3K79 METHYLATION AND DOT1L IN TRANSCRIPTION ELONGATION CONTROL AND CELL FATE TRANSITION

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Epigenetic landscapes of higher eukaryotes are implicated in transcription modulation, cell fate determination, and diseases. Lysine 79 methylation of histone H3 (H3K79) is the first core histone modification identified outside the histone tails, the distribution of which is highly correlated with actively transcribed genes. DOT1L, the only enzyme catalyzing mono-, di-, and tri-methylation of H3K79 in metazoans, plays a critical role in embryogenesis and leukemia transformation. Despite extensive studies, the molecular mechanisms underlying the role of DOT1L remain elusive. To dissect the relationship between H3K79 methylation, stem cell differentiation, and transcription regulation, we systematically examined DOT1L's functions in embryonic stem cells. Here, we find that DOT1L participates in regulating productive transcription elongation and cellular differentiation in a previously unreported fashion. Our study reveals an unforeseen role of DOT1L in modulating cell fate determination and transcription.

Funding source: K.C. is supported by the NIH Pathway to Independence Award from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant K99HD094906).

Keywords: H3K79 methylation, Neural differentiation, Transcription elongation

CI143

THE ROLE OF FGF PATHWAY DURING GASTRULATION IN HUMAN

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Several in vitro stem cell models have been developed to recapitulate early mammalian develop in vivo. In these model systems, human embryonic stem cells (hESCs) self-organize and differentiate into each germ layer that further patterns into spatial domains resembling embryonic patterning. By which we can study signaling mechanism underlying such pattern formation in a setting where experimental manipulation and observation are simpler to obtain quantitative data. A key question in pattern formation is how cells integrate multiple signals that are changing over time into cell fate decisions. Recent human gastrulation studies utilized micropattern technology with hESCs and revealed that Nodal and Wnt signaling do not form static gradients but rather continuously moving wave fronts where cells read rate of concentration change instead of absolute concentrations. FGF is one of the well known pathways required

for gastrulation in vivo but its precise roe is less well understood. Here we use high resolution live-cell imaging and computational analysis to shed light on the characteristics and role of FGF signaling in the micropatterned hESC model for gastrulation.

Keywords: FGF, embryo, patterning

CI154

THE DEVELOPING ODONTOBLAST: A NOVEL SINGLE CELL GENE EXPRESSION ATLAS OF HUMAN FETAL TOOTHGERM VIA SCI-RNA SEQ

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Tooth structure lost due to caries, trauma or genetic disorders such as amelogenesis imperfecta (AI) is currently restored by artificial prosthesis. To bioengineer natural tooth structure, increased knowledge of the signaling pattern responsible for tooth development is required. This project generates a novel single cell atlas to assess transcriptome heterogeneity and delineate cell lineage relationships in early development, differentiation, and fate determination in human fetal toothgerm via single-cell combinatorial indexing RNA sequencing (sci-RNA-seq). Toothgerms representing a 13-week period of gestation, ranging from early to late development, were analyzed via Monocle3. Resulting cell clustering, top gene expression and pseudotime trajectories identified signaling at important cell fate transitions and a unique odontoblast lineage including dental papilla (RUNX2, WNT5A, PAX9), odontoblast progenitor (MMP2, BMP4) and mature odontoblast (DSPP, SPARC, IBSP, DMP1) as well as a reserve cell population with elevated cell cycle and stem markers (FGF2, ITGB1, TWIST2). Moreover, mature odontoblasts express AI-associated genes WDR72 and MMP20, suggesting a disturbance in odontoblast-ameloblast communication can cause AI. Further, we observed ameloblast plasma membrane extensions towards the odontoblast. We dissect the signaling function of these morphological extensions between the two cell types and how the WDR72 and MMP20 signaling pathways are crucial in AI. The differential gene expression observed in the developing odontoblast highlights the transcriptional regulation of their differentiation and the need to identify the underlying regulatory mechanisms involved, both to characterize this highly specialized cell type and to develop therapeutic practices and optimize a novel differentiation protocol of human iPSC to odontoblasts to produce a tooth organoid.

Funding source: This work was supported by grants from the National Institute of Health T90DE021984 and the ARCS Foundation to S.H.D. and P01 GM 081619 and STEMFD for H.R-B.

Keywords: Odontogenesis, Regenerative Dentistry, Epithelial-Mesenchymal Signaling

CI304

ESSENTIAL AMNION SIGNALS FOR PRIMATE PRIMITIVE STREAK FORMATION RESOLVED BY SINGLE CELL RNA MAP

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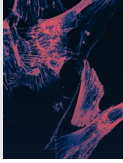
One of the milestone events during mammalian embryogenesis is the formation of the primitive streak, when cells of the columnar-shaped epiblast undergo epithelial-to-mesenchymal transition and migrate anteriorly to form mesoderm. This event, termed gastrulation, is well understood in mice but largely elusive in humans. Three recent studies on non-human primate (NHP) and human embryos have created a framework of post-implantation development in primates and characterized the major cell populations during gastrulation. However, the cellular



interplays and regulatory networks involved remain unknown. Here, we combined high-throughput single cell sequencing technology, in vitro embryo culture and CRISPR/Cas9-mediated gene editing to unravel signaling pathways governing this process in primates. We generated a serial single cell atlas of over 30 000 cells from in vitro cultured NHP embryos spanning peri-implantation to early primitive streak stages. We discovered that ISL1, a gene with a well-established role in cardiogenesis and is absent from mouse embryos at these early stages, is highly expressed in primate amniotic cells. To shed light on the functional role of ISL1 in amnion, we generated ISL1 hypomorphic mutants in NHP. Strikingly, in vitro cultured ISL1 hypomorphic mutant embryos failed to form primitive streak. BMP4 was identified as a key signaling pathway regulating this formation. These findings were confirmed in ISL1 loss-of-function human embryonic stem cell lines, suggesting a conserved role in humans. Notably, no viable ISL1 hypomorphic NHP mutants could be recovered after transferring mutated embryos into surrogate NHP recipients confirming the essential requirement of ISL1 in vivo and highlighting the importance of the amnion as a signaling center during primate embryogenesis. This might explain the drastic underrepresentation of ISL1 genetic variants in humans and demonstrates the importance and the potential of genetically modified primate embryos as in vitro systems to advance our understanding of human embryonic development and to identify new causes for congenital malformations, infertility and early pregnancy loss.

Funding source: This study was supported by grant from the National Key Research and Development Program of China, the Grant for Swedish-Chinese collaboration from the Swedish Research Council and a grant from the German research foundation.

Keywords: primitive streak formation in primate, primate amnion, ISL1 and BMP4 singling pathway

CI306

EMBRYONIC STEM CELL DIFFERENTIATION IS REGULATED BY SET THROUGH INTERACTIONS WITH P53 AND B-CATENIN

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The multifunctional histone chaperone, SET, is essential for embryonic development in mouse. Previously, we identified SET as a factor that rapidly downregulates during embryonic stem cell (ESC) differentiation, suggesting a possible role in the maintenance of pluripotency. Here, we further interrogate the function of SET in early differentiation. We perform RNA-seq and ChIP-seq experiments to detect SET-regulating genes and use liquid chromatography tandem mass spectrometry (LC-MS/MS) to find additional factors involved in SET-regulated lineage-specification. After observing a skewed differentiation phenotype in SET knockout (KO) ESCs, we identify P53 and β-catenin as binding partners of SET. shRNA-knockdown experiments of both factors in SET-KO ESCs reveal that P53 knockdown partially rescues lineage-marker misregulation during differentiation. β-catenin depletion, on the other hand, has a minimal effect on lineage skewing, but instead activates Wnt gene expression. Integration of ChIP-seq with RNA-seq datasets revealed a co-regulatory relationship between SET and TCF3, a downstream effector in the Wnt-signalling pathway. Overall, we indicate a role for both P53 and β-catenin in SET-regulated early differentiation and uncover a potential mechanism for SET function at the β-catenin-TCF regulatory axis.

Funding source: Patrick is a member of the Episystem Innovative Training Network with a grant provided by Marie Skłodowska-Curie actions within the HORIZON 2020 framework.

Keywords: SET, embryonic stem cells, wnt-signalling

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

CI189

TRANSIENT EXPRESSION OF YAMANKA FOUR FACTORS INDUCES CELLULAR REPROGRAMING AND ENHANCES CELL PLASTICITY IN THE MOUSE LIVER

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Mammals have a limited cellular regeneration capacity, both as adults and in neonatal stages. Vertebrates like the zebrafish and newt, however, have previously been identified to be capable of tissue regeneration via cell dedifferentiation. We hypothesized that experimentally induced dedifferentiation, in mouse liver cells, would have an increased level of cellular plasticity, therein resulting in more efficient cell transdifferentiation. Our work demonstrates that through short-term expression of Yamanaka 4 Factors (4F) in a lineage-traceable, hepatocyte-specific mouse model, dedifferentiation and proliferation is induced in adult, mouse liver cells. After induced 4F expression, global transcriptome analyses demonstrated reduced expression of hepatic-lineage markers alongside increased expression of several proliferation markers and epigenetic modifiers. In addition, global changes in DNA accessibility were also observed. Single-cell transcriptome analysis revealed that 4F-expression in hepatocytes corresponded with acquired stem/progenitor markers in these cells, suggesting the cells underwent partial reprogramming. We also observed enhanced MyoD-mediated transdifferentiation in 4F-induced hepatocytes, suggesting 4F expression may be responsible for increased cellular plasticity as well. Together, these results demonstrate that liver-specific 4F expression in mice induces dedifferentiation and promotes cellular transdifferentiation, something which is important to continue exploring in order to develop models and therapies for regeneration of mammalian tissue

Funding source: California Institute for Regenerative Medicine (CIRM)

Keywords: hepatocytes, reprogramming, dedifferentiation

EPITHELIAL

CI208

POTENTIAL COMPETITION BETWEEN THE BHLH TRANSCRIPTION FACTOR E2A AND THE STEM CELL DRIVER MYC IN PANCREATIC CANCER

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Pancreatic Ductal Adenocarcinoma (PDA) is an aggressive cancer, with a five-year survival rate of only seven percent. Currently, there are no effective treatments for PDA, underscoring the need for a better understanding of how pancreatic cancer develops to create new treatments. It has been shown that high levels of the potent oncogene MYC drive the stem cell phenotype associated with poor survival in PDA. MYC normally binds to a specific set of E-box sequences but it has been shown in some cancers that when MYC expression increases, its binding extends to additional low affinity E-box sequences, particularly in enhancer regions of the genome. Here we are pursuing the hypothesis that MYC invades specific E-box sequences used by the bHLH transcription factor E2A, a protein that we identified as a tumor suppressor in the pancreas. To test this theory, we are using several techniques, including ChIP-sequencing, qPCR, and Western blots in order to identify the exact chromatin sites bound by E2A and MYC, in non-cancer pancreatic cells versus pancreatic tumor cells. Moreover, we will test whether competition occurs primarily at enhancers. To this end, we have generated chromatin preparations from HPNE (preneoplastic) and PANC1 (PDA) cells. The chromatin has been precipitated with antibodies to: 1) MYC; 2) E2A; 3) H3K27Ac in order to identify core promoters and enhancers; 4) H3K4me1 to identify enhancers 5) H3K4me3 marking active promoters; and 6) H3K9me3 to identify repressed regions. Western blots demonstrate antibody specificity for MYC and E2A. qPCR of immunoprecipitated chromatin for known DNA binding sequences validates the ChIP samples prior to sequencing. At the conclusion of these studies, we anticipate to establish the complement of DNA sequences bound by E2A and MYC during progression to PDA, revealing for the first time whether E2A and MYC compete for binding to specific sites in the chromatin. We expect these studies to identify specific sequences of interest for subsequent functional studies with CRISPR. Together the data has the potential to reveal a new mode by which MYC drives the cancer stem cell phenotype-by blocking E2A tumor suppressor activity.

Funding source: California Institute for Regenerative Medicine

Keywords: Pancreatic ductal adenocarcinoma, bHLH transcription factor E2A, MYC

EYE AND RETINA

CI211

P53 INACTIVATION ENABLES MOUSE EMBRYONIC STEM CELLS TO MAINTAIN SELF-RENEWAL, BUT NOT RETINAL DIFFERENTIATION, WITHOUT WDR5 CHROMATIN FUNCTION

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p53 inactivation occurs during culture of mouse and human embryonic stem cells (ESCs). In fact, neural cells derived from p53-mutant human ESC lines have been transplanted into patients with retinal degenerative diseases and spinal cord injury. However, it is unclear how p53, and mutant p53, interact with ubiquitous chromatin-associated proteins during ESC self renewal and tissue specific differentiation. Here, we identify p53 as a target of WDR5 as ESCs differentiate. WDR5 is a broadly expressed chromatin-associated protein that is required for proper lysine 4 methylation on histone H3. Furthermore, WDR5 interacts with the ESC core transcriptional network to maintain ESC self renewal. Using RNA-seq, ChIP-seq, and ATAC-seq in conjunction with 3D retinal organoid differentiation of CRISPR/Cas9-mediated, gene-edited Wdr5 and p53-mutant ESCs, we have identified a novel WDR5-p53 cell fate determination pathway. Specifically, we find that WDR5 controls ESC fate-choice to Rx+ retinal neuroectoderm vs. cardiogenic mesoderm by triggering p53-dependent transcription within transiently-accessible chromatin. Our studies reveal a p53-dependent system of chromatin accessibility and lineage specification that responds differently to the same input (WDR5) at critical time windows. Further we found that during p53 inactivation, WDR5 promotes retinal differentiation in a chromatin-dependent manner, however ESC self-renewal and derepression of meiosis genes, is dependent on WDR5 but independent of its association with chromatin. Such a time-variant system offers a general model to describe how embryonically abundant transcription factors coordinate temporal inputs from ubiquitous epigenetic factors to control retinal specification during development. Finally, our work informs future translational studies that investigate potential off-target/teratogenic effects of WDR5 inhibitors used for WT and mutant p53-associated cancers, a field that has attracted a $1 billion investment by industry.

Funding source: National Eye Institute (K08EY026654, to R.C.R), the National Cancer Institute (P30CA046592, to the University of Michigan Comprehensive Cancer Center), and Research to Prevent Blindness (to the University of Michigan and R.C.R.).

Keywords: retina, WDR5, p53

HEMATOPOIETIC SYSTEM

CI216

TRANSCRIPTOME DYNAMICS OF HEMATOPOIETIC STEM CELL FORMATION REVEALED USING A COMBINATORIAL RUNX1 AND LY6A REPORTER SYSTEM

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Studies of hematopoietic stem cell (HSC) development from pre-HSC-producing hemogenic endothelial cells (HECs) are hampered by the rarity of these cells and the presence of other cell-types with overlapping marker expression profiles. We generated a Tg(Runx1-mKO2;Ly6a-GFP) dual reporter mouse to visualize hematopoietic commitment and study pre-HSC emergence and maturation. Runx1-mKO2 marked all intra-arterial HECs and hematopoietic cluster cells (HCCs) including pre-HSCs, myeloid- and lymphoid progenitors, and HSCs themselves. However, HSC and lymphoid potential were almost exclusively found in reporter double-positive (DP) cells. Robust HSC activity was first detected in DP cells of the placenta, reflecting the importance of this niche for (pre-)HSC maturation and expansion prior to the fetal liver stage. A time-course analysis by single cell RNA-Seq revealed that as pre-HSCs mature into fetal liver-stage HSCs, they show signs of interferon exposure, exhibit signatures of multi-lineage differentiation gene expression, and develop a prolonged cell cycle reminiscent of quiescent adult HSCs. We also generated embryonic stem cells from this Runx1-mKO2;Ly6a-GFP dual reporter mouse line. Time-lapse confocal imaging of ESC-derived endothelial cells confirmed that Runx1-mKO2-positivity HECs that undergo endothelial-to-hematopoietic transition (EHT). However, in contrast to their counterparts in the AGM, the HEC- and hematopoietic cluster cell compartments generated through in vitro differentiation of ESCs were devoid of Ly6a-GFP expression, mirroring the lack of HSC formation during conventional ESC in vitro differentiation.

Funding source: NIDDK (U54DK110805)

Keywords: Hematopoietic stem cells, Hemogenic endothelial cells, Reporter

CI219

YAP REGULATES HEMATOPOIETIC STEM CELL FORMATION IN RESPONSE TO THE BIOMECHANICAL FORCES OF BLOOD FLOW

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Hematopoietic stem cells (HSCs), which give rise to all differentiated blood lineages in vertebrate species, are born from arterial endothelial cells in the dorsal aorta that adopt hematopoietic potential and commitment during development. Physical forces, namely wall shear stress (WSS) and cyclic stretch (CS), produced by hemodynamic blood flow through this vessel are required to generate HSCs from arterial endothelium, but the mechanisms by which these forces are sensed and converted into a "stemness" regulatory program remain unknown. Using a novel "dorsal-aorta-on-a-chip" microfluidic platform, we demonstrate that exposure to WSS and CS increase expression of the hematopoietic transcription factor RUNX1 in human CD34+ hemogenic endothelial cells derived from induced pluripotent stem cells. Notably, CS specifically induced activity of the YAP transcription factor, a mechanically-activated regulator of organ size and pluripotency. We corroborate these findings in a zebrafish model, and demonstrate that loss or gain of YAP function in vivo can blunt or augment the production of HSCs, respectively, during definitive hematopoiesis. Importantly, through gene expression analysis and epistasis experiments modulating Notch signaling, we find that YAP is responsible for the maintenance, not initiation, of the hematopoietic program in newly specified hemogenic endothelial cells. Molecularly, we identify a stretch-induced, RhoGTPase-dependent mechanotransduction network controlling YAP nuclear availability to regulate RUNX1 expression, both in zebrafish and human cells. Moreover, we show that small molecule stimulation of RhoGTPases can rescue HSC production in zebrafish embryos with no flow, and enhance the hematopoietic potential of human iPSC-derived CD34+ endothelial cells grown in static culture. Together these findings uncover YAP as a transcriptional regulator of stem cell fate commitment in a transdifferentiating epithelial cell during tissue morphogenesis, and suggest a pharmacologically amenable mechanotransduction pathway that could be exploited to improve the in vitro derivation of HSCs from human cells for therapeutic purposes.

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Keywords: YAP, Mechanotransduction, Hematopoiesis

CI220

THE ROLE OF THE RNA EDITOR-EXONUCLEASE AXIS DURING ERYTHROPOIESIS: FROM MOUSE EMBRYO STUDIES TO HUMAN STEM CELL ENGINEERING

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Erythropoiesis is an intricate process by which lineage-committed erythroid progenitors become mature red blood cells. Reticulocytes are terminal-staged, immature red blood cells with residual RNA after enucleation. In the absence of pathology, reticulocytes are efficiently processed into mature red blood cells and typically represent a small percentage of cells in human peripheral blood. In contrast, when differentiated in vitro from pluripotent stem cells or CD34+ progenitor cells derived from the peripheral blood of donors, red blood cells tend to arrest at the reticulocyte stage. Recent studies have highlighted that uridylation by Terminal Uridylyl Transferases (TUTases) occurs on a broad spectrum of RNA classes in mammalian cells. Oligo-uridylated RNA is recognized by exoribonucleases and targeted for decay. We posited that the machinery behind RNA degradation that accompanies terminal erythropoiesis might involve RNA tail editors coupled to exonuclease activity. Utilizing constitutional murine knockout models, we observed that blood from the TUTase Zcchc6 RNA editor knockout embryos exhibited reticulocytosis and a terminal maturation defect, as documented by FACS, histology, and hematological profiling. Murine strains deficient in the downstream exonuclease Dis3l2 phenocopied the RNA decay defect of the Zcchc6 KO. Conditional knockout murine models of the TUTase-Dis3l2 axis driven by the red cell specific Erythropoietin Receptor-Cre exhibited comparable phenotypes, suggesting a cell intrinsic and niche-independent role for the TUTase-Dis3l2 axis in promoting red blood cell maturation. We are modulating the expression of this axis by various methods to optimize modeling of hemoglobinopathies such as sickle cell anemia.

Funding source: NHLBI Progenitor Cell Translational Consortium

Keywords: Erythropoiesis, RNA decay, In vitro red blood cell differentiation

CI311

ZBTB46 DEPENDENT ALTERED DEVELOPMENTAL PROGRAM IN MOUSE EMBRYONIC STEM CELL DERIVED BLOOD CELL PROGENITORS

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Zbtb46 is a recently identified dendritic cell (DC) specific transcription factor with poorly defined biology. Although Zbtb46 is highly expressed in conventional DCs, but evidence also points to its expression in erythroid progenitors and endothelial cells suggesting that this factor might influence the early hematopoietic development. Here we probe the effect of this transcription factor in embryonic stem cell (ESC) derived mesoderm and blood progenitors using chemically inducible mouse cell lines. Unexpectedly, forced expression of this protein elicited a broad repressive effect at the early stage of the embryonic stem cell (ESC) differentiation. Ectopic expression of Zbtb46 interfered with mesoderm formation and the cell proliferation was also negatively impacted. More importantly, reduced number of CD11b+ myeloid cells were generated from the ESC derived Flk1+ mesoderm cells in the presence of Zbtb46. On the other hand, ectopic expression of this transgene was associated with enhanced formation of erythroid progenitors at the early phase of the ESC differentiation. We also analyzed the genomic impact of this transcription factor in ESC derived mesoderm cells with RNA sequencing. Our results revealed that numerous myeloid and immune response related genes exhibited lower expression in the Zbtb46 primed cells. On the other hand, multiple genes were upregulated in the Zbtb46 primed cells. In summary, our results support that Zbtb46 suppresses the ESC-derived myeloid development and diverts the Flk1+ mesoderm cells toward other cell lineages including erythroid progenitors. Moreover, our transcriptomic data provide a resource for exploration of the Zbtb46 regulatory network in ESC-derived progenitors.

Funding source: This work was supported by Hungarian Scientific Research Funds (NKFIH K124890 and GINOP-2.3.2-15-2016-00044 PHARMPROT)

Keywords: Zbtb46, dendritic cells, myeloid differentiation

IMMUNE SYSTEM

CI225

THE H-Y ANTIGEN IN EMBRYONIC STEM CELLS CAUSES REJECTION IN SYNGENEIC FEMALE RECIPIENTS

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Pluripotent stem cells (PSCs) are promising candidates for cell-based regenerative therapies. To avoid rejection of transplanted cells, several approaches are being pursued to reduce immunogenicity of the cells or modulate the recipient's immune response. These include gene editing to reduce the antigenicity of cell products, immunosuppression of the host, or using major histocompatibility complex-matched cells from cell banks. In this context, we have investigated the antigenicity of H-Y antigens, a class of minor histocompatibility antigens encoded by the Y chromosome, to assess whether the gender of the donor affects the cell's antigenicity. In a murine transplant model, we show that the H-Y antigen in undifferentiated embryonic stem cells (ESCs), provokes T- and B-cell responses in female recipients. Immune assays included CyTOF abalysis, ELISPOTs, flow cytometry and mixed lymphocyte reactions. Cells survived in all male recipients, but were rejected does-dependent in female recipients. Data were confirmed using ESC-derived endothelial cells (ESC-ECs): All syngeneic female recipients (n=4) rejected H-Y ESC-ECs within 55 days, whereas cells survived in all male recipients (n=5). These data are relevant not only for hematopoietic stem cell or solid organ transplantation, but also for regenerative medicine using pluripotent stem cells as therapeutics.

Keywords: pluripotent stem cells, male, rejection

MUSCULOSKELETAL

CI301

MOLECULAR SIGNATURES AND MULTI-LINEAGE POTENTIAL IDENTIFY IMMATURE MESENCHYMAL PROGENITORS IN EARLY MOUSE LIMB BUDS

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The key molecular interactions governing vertebrate limb bud development are a paradigm to study the mechanisms controlling progenitor cell proliferation and specification during vertebrate organogenesis. However, little is known about the cellular heterogeneity of the mesenchymal progenitors in early limb buds that ultimately contribute to the chondrogenic condensations prefiguring the skeleton. We combined flow cytometry, transcriptome analyses and fate-mapping to identify the molecular signatures of several distinct mesenchymal progenitor cell populations present in early mouse forelimb buds. In particular, JAGGED1 (JAG1)-positive cells located in the posterior-distal mesenchyme were identified as the most immature limb bud mesenchymal progenitors (LMPs), which critically depend on SHH and FGF signaling in culture. The analysis of Gremlin1 (Grem1)-deficient forelimb buds showed that JAG1-expressing LMPs are protected from apoptosis by GREM1-mediated BMP antagonism. At the same stage, the osteo-chondrogenic progenitors (OCPs) located in the core mesenchyme are already actively responding to BMP signaling. This analysis sheds light on the cellular heterogeneity of the early mouse limb bud mesenchyme and the distinct response of LMPs and OCPs to morphogen signaling.

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Keywords: early limb bud mesenchyme, chondrogenesis, multi-lineage potential

NEURAL

CI235

VARIATION OF HUMAN NEURAL STEM CELLS GENERATING ORGANIZER STATES IN VITRO BEFORE COMMITTING TO CORTICAL EXCITATORY OR INHIBITORY NEURONAL FATES

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Better understanding the progression of neural stem cells (NSCs) in the developing cerebral cortex is important for modeling neurogenesis and defining the pathogenesis of neuropsychiatric disorders. Here we used RNA-sequencing, cell imaging and lineage tracing of mouse and human in vitro NSCs to model the generation of cortical neuronal fates. We show that conserved signaling mechanisms regulate the acute transition from proliferative NSCs to committed glutamatergic excitatory neurons. As human telencephalic NSCs developed from pluripotency in vitro, they first transitioned through organizer states that spatially pattern the cortex before generating glutamatergic precursor fates. NSCs derived from multiple human pluripotent lines varied in these early patterning states leading differentially to dorsal or ventral telencephalic fates. This work furthers systematic analysis of the earliest patterning events that generate the major neuronal trajectories of the human telencephalon.

Keywords: human iPSC variation, patterning of the cortex, neural transcriptional dynamics and neuron trajectory variation

CI237

SYSTEMATIC ANALYSIS OF THE GENETIC DETERMINANTS OF HUMAN NEURAL LINEAGE COMMITMENT BY QUANTITATIVE SMALL MOLECULE-BASED TRANSCRIPTOMIC PROFILING

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Small molecules offer great advantages for directed differentiation of human pluripotent stem cells into defined lineages. Although small molecule combinations are used for neural lineage induction (e.g. dual SMAD inhibition), there is no systematic understanding of how these small molecules, either applied alone or in combination, affect quantitative gene expression. Here, we employed a high-throughput transcriptomics method (RASL-Seq) to profile dose-dependent transcriptional responses of induced pluripotent stem cells (iPSCs) along a 7-day neural induction experiment using small molecule inhibitors for bone morphogenetic protein (BMP) and/or transforming growth factor-beta (TGF-β) pathways. A total of 354 lineage-related genes and transcription factors were tested in single or combined drug treatments across 7 dosages. Gene expression was analyzed using a custom-built algorithm to obtain dose-dependent curve classification, maximum efficacy and critical concentrations. This strategy identified genes regulated in opposite directions upon BMP inhibitor (LDN 193189) or TGF-β inhibitor (A83-01) treatment such as SMOC1, GADD45A, SESN3 and WLS. We also discovered genes that exclusively responded to LDN 193189 (e.g. GAD2, HESX1, PAX3 and FOXG1) but not A83-01 and vice versa (e.g. GAP43, MT1X and ZFHX4). Next, drug combination effects were addressed by comparing single drug treatment versus combined drug treatments revealing antagonistic effects on GBX2, TNNT2, HESX1 and PAX3. Notably, the correlation of differential drug response on gene expression profiles identified the distinct contribution of BMP inhibition and TGF-β inhibition to fate determination to either the CNS or PNS lineages. Lastly, to establish our approach as a general chemical biology resource, we mapped transcriptomic profiles regulated by 16 compounds which are widely used in the stem cell field for manipulating developmental pathways. In summary, precisely defining the quantitative relationships between small molecules and gene expression changes represents a novel approach to better control and predict differentiation of stem cells.

Keywords: neural differentiation, quantitative gene expression, induced pluripotent stem cells

CI248

UNDERSTANDING THE MECHANISM OF WNT SIGNALING IN BMP-DEPENDENT SPINAL DORSAL INTERNEURONS

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The somatosensory system of the spinal cord receives and processes sensory information from the peripheral nervous system. By sending signals to the brain and spinal motor neurons, the somatosensory system lets us detect and react to environmental cues, like temperature and pain. Six classes of dorsal spinal sensory interneurons (dI1-6) mediate different sensory modalities; for example, dI1s relay proprioceptory signals while dI4 and dI5s relay pain and itch. The Butler lab is investigating the mechanisms that specify dIs to establish protocols for therapeutics for patients with spinal cord injuries. Our studies have shown the identities of two dI populations (dI1 and dI3) are dependent on the activity of the bone morphogenetic proteins (BMPs). We have established an embryonic stem cell (ESC) directed differentiation protocol where the addition of BMP4 along with retinoic acid (RA) robustly produces both dI1 and dI3 identities. However, how this activity is translated into distinct neuronal fates (dI1 v/s dI3s) is not understood. Using RNA-Seq on RA+BMP4 treated ESCs, we have identified Wnt signaling as an immediate response to BMP4 treatment, raising the possibility that the combination of both BMP and Wnt signaling dictates the specific dI outcome (dI1 v/s dI3s). To further assess the role of Wnt signaling pathways in the specification of stem cell-derived dIs, Wnt signaling will be inhibited in BMP4-treated ESCs and analyzed using qPCR and immunohistochemistry. This analysis will not only elucidates the mechanisms of dI differentiation but also will provide means to produce specific populations of dI neurons from stem cells for therapeutic purposes.

Funding source: CSUN-UCLA Stem Cell Scientist Training Program Grant ID (EDUC2-08411) Grants from the National Institute of Health (NIH) (NS085097), Ablon Scholars and the UCLA Broad Stem Cell Research Center

Keywords: Innovative, New perspective, Useful

CI302

INTEGRATION OF SINGLE CELL TRANSCRIPTOMES AND CHROMOSOME ACCESSIBILITY TO DETECT REGULATORY ELEMENTS CRITICAL TO MOUSE INTERNEURON DEVELOPMENT

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GABAergic interneurons are a heterogenous cell population and their dysfunction is associated with numerous brain disorders. Interneurons are born in the ganglionic eminences (MGE, LGE and CGE), with distinct subtypes arising from different GE domains. Despite substantial evidence suggesting that initial interneuron

subtypes are specified within these neurogenic niches, only a handful of fate-determining genes have been identified. One possible explanation is that progenitors contain epigenetic signatures directing cells towards a particular interneuron fate that are not yet apparent in the transcriptome. Ample evidence indicates that epigenetic mechanisms induce heritable changes in gene expression and are critical in mediating cell fate and differentiation in a variety of tissues. Yet we lack a comprehensive understanding of how epigenetic states influence interneuron cell fate in normal development and disease models.

To explore the relationship between interneuron cell fate and epigenetic mechanisms in neural progenitors, we performed single cell RNA (scRNA-Seq) and single cell assay for transposase-accessible chromatin (scATAC-Seq) sequencing to determine the transcriptome and chromatin accessibility in the embryonic MGE, LGE, CGE and cortex. This allows us to screen for candidate enhancers and other non-coding regions regulating expression of specific interneuron subtypes. We detected distinct genes and corresponding chromatin profiles that are restricted to specific progenitor zones and interneuron lineages. By examining ATAC peaks in lineage-specific genes across cell types, we detected putative enhancers associated with specific GEs and potentially distinct interneuron subtypes. To expand on these initial observations, we generated a conditional knockout of Enhancer of zeste homolog 2 (Ezh2) in MGE progenitors. Ezh2 regulates histone methylation and acts as a transcriptional repressor; thus loss of Ezh2 likely disrupts chromatin architecture and gene expression. These Ezh2 cKO mice displayed a significant decrease in PV+ interneurons in multiple brain regions. These insights increase our understanding of how epigenetic modifications regulate interneuron fate and could open new avenues for understanding disease-associated genes or genomic loci.

Funding source: NICHD Division of Intramural Research Program

Keywords: cell fate, epigenomic, single-cell

CI303

VASCULAR-DERIVED SPARC AND SERPINE1 REGULATE INTERNEURON TANGENTIAL MIGRATION AND MATURATION

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Cortical interneurons are a diverse population of inhibitory locally-projecting neurons that form distributed microcircuits throughout the cortex. They play key roles in numerous brain functions and their dysfunction has been implicated in psychiatric disease. Efforts to model human interneurons for disease have met with a fundamental challenge: the limited and protracted maturation of human interneurons, even when they are introduced to neonatal mouse cortex. We therefore sought to directly address this limitation by initiating a cross-species study: Embryonic mouse interneurons migrate robustly. Thus, if we can understand what regulates their tangential migration, we may be able to translate this knowledge to improve human interneuron migration. We found that interneuron migration in mice is regulated by secreted vascular cues. We proceeded to identify the cues involved and characterized their necessity and sufficiency to elicit tangential migration in mouse interneurons. We then tested the functional capacity of these cues to induce migration in human stem cell-derived interneurons in vitro and found a robust effect. Remarkably, we also demonstrate that pre-treatment with vascular cues also augments human stem cell-derived interneuron migration and functional maturation when transplanted into neonatal mouse cortex.

Keywords: Cortical interneurons, Vascular, Migration

NEW TECHNOLOGIES

CI261

SUPER-RESOLUTION IMAGING OF HEPARAN SULFATE IN HUMAN EMBRYONIC STEM CELL DIFFERENTIATION

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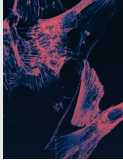

Heparan sulfate is a cell surface polysaccharide that influences cell fate during mammalian development. This highly polyanionic glycosaminoglycan may regulate embryonic development by binding critical growth factors in the extracellular matrix and modulating subsequent signaling pathways. A number of factors appear to impact heparan sulfate function, including its distribution and length, but tools to understand these parameters are lacking. Therefore, methods to image heparan sulfate features would be transformative. To this end, we have used high-resolution expansion microscopy (ExM) to visualize the dynamic changes in heparan sulfate structure during human embryonic stem (hES) cell differentiation. We discovered that undifferentiated hES cells are densely coated with elongated hair-like heparan sulfate, whereas the heparan sulfate structures of terminally differentiated hES cell-derived neurons are small, located in puncta, and highly dispersed. Quantitative mass spectrometry analysis of heparan sulfate indicated that the total content of this polysaccharide decreases as hES cells progress from undifferentiated to differentiated states. To link these changes to function, we assessed the ability of heparan sulfate chains to bind to the signaling growth factor FGF. Binding of FGF is decreased as cells differentiate toward the neural lineage. These data indicate that the dynamic changes in heparan sulfate structure have dramatic functional consequences, namely in their ability to regulate the heparan sulfate-FGF binding and potentiate critical signaling events in development. We anticipate that this study will be broadly applicable to others seeking to elucidate the roles of glycan structure in development.

Keywords: Expansion Microscopy, Heparan Sulfate, Neural differentiation

CI264

TRACKING AND PREDICTING OUTCOMES DURING HUMAN SOMATIC CELL REPROGRAMMING USING NUCLEAR CHARACTERISTICS

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Reprogramming of human somatic cells to induced pluripotent stem cells (iPSCs) generates valuable resources for disease modeling, cell therapy, and regenerative medicine. However, the reprogramming process can be stochastic and inefficient, creating many partially-reprogrammed intermediates and non-reprogrammed cells in addition to fully-reprogrammed iPSCs. Much of the work to identify, evaluate, and enrich for iPSCs during reprogramming relies on methods that fix, destroy, or singularize cell cultures, thereby disrupting each cell's microenvironment. To overcome these shortcomings, we developed a micropatterned substrate that allows for nondestructive dynamic live-cell microscopy of hundreds of microscale cell subpopulations undergoing reprogramming while also preserving many of the biophysical and biochemical cues within the cells' microenvironment. On this substrate, we were able to both watch and physically confine cells into discrete micron-sized islands during the reprogramming of human somatic cells from skin biopsies and blood draws obtained from healthy donors. Using high-content analysis, we identified a combination of eight nuclear morphometric characteristics that can be used to track the progression of reprogramming and distinguish partially-reprogrammed cells from those that are fully-reprogrammed. Non-cell autonomous characteristics, such as clustering of nuclei, were highly informative in classifying the progression of reprogramming, and were used to generate a predictive computational model of the process. This quantitative approach to track reprogramming in situ using micropatterned substrates could aid in biomanufacturing of therapeutically-relevant iPSCs, and be used to elucidate multiscale cellular that accompany human cell fate transitions.

Keywords: Reprogramming, Nucleus, Micropatterning

CI272

STREAMLINE ENGINEERING OF ISOGENIC IPSCS WITH CRISPR

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The ability to efficiently edit the genome of induced pluripotent stem cells to create isogenic cells hugely expands their utility in disease modeling and drug discovery. Isogenic iPSCs offer the opportunity to obtain a diverse progeny in sufficient quantities for the study of molecular mechanisms and cellular processes dysregulated in disease. We describe a streamlined method to efficiently 1) generate genetic knockouts mimicking relevant clinical phenotypes, 2) insert clinically relevant mutations allowing the study of a wide gamut of genetic diseases, and 3) insert sequences encoding fluorescent tags permitting the study of protein behavior in the normal and disease state. The approach includes the rational design of both single guide RNA and DNA repair oligos with asymmetrical arms using Bechhling, recombinant CRISPR/Cas9 system, and single-cell sorting using a microraft device or 96-well plates. We establish specific criteria for selecting gRNAs with high predictive targeting efficiency and donor DNA promoting high homologous recombination. Donor oligos and single-guide RNAs are chemically synthesized and electroporated along with recombinant Cas9 into iPSCs. After 72 hours, half of the cells are collected for Sanger sequencing, while the other half are cryopreserved for single-cell cloning. Targeting efficiency is calculated using the Interference for CRISPR Edits (ICE) tool from the Sanger sequencing of pooled

electroporated cells. Once targeting efficiency is known, then cells are thawed and plated on microraft devices or 96-well plates. After 7 days, single-cell clones are screened for specific modifications. This approach yields overall targeting efficiency of 100% for a gene knockout, 50% for single base change, and 10% for in-frame fluorescent tags. This approach is efficient, robust, and reliable, generating isogenic iPSCs that allow the study of specific disease mechanisms.

Keywords: CRISPR, ISOGENIC, IPSCS

CI274

VILLAGE-IN-A-DISH EXPERIMENTAL SYSTEMS: MAPPING THE GENETIC BASIS OF VARIATION IN DIVERSE NEURAL CELL TYPES TO TRANSLATE DISEASE-LINKED VARIATION INTO MECHANISTIC BIOLOGY

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The high polygenicity of neuropsychiatric disorders – shaped by thousands of common and rare variants – presents formidable challenges for biology. As these variants are often incompletely penetrant or of weak-effect, it would be valuable if cellular studies could learn from vast numbers of variants simultaneously. Biology today has powerful ways of measuring cellular phenotypes, complemented by the ability to differentiate previously intractable Human cell types from induced pluripotent stem cells (PSCs). And yet, efforts to ascertain effects of human genetic variation on cellular phenotypes encounter challenges; 1) Scale – how to assay enough cell lines to connect disease-linked variants to biological effects; and 2) Variance and reproducibility – how to ensure experiments on many different cell lines obtain highly comparable measurements in rigorously controlled conditions. We developed a novel strategy for population-scale cellular studies: pooling cell lines from hundreds of individuals to generate "villages" of cell lines that can be simultaneously perturbed and analyzed, then using sequencing-based strategies for deconvoluting cells' identities as part of a phenotypic readout. We established a repository of Human PSCs that allowed us to measure the population dynamics of ~250 cell lines in villages of increasing size and complexity. We leveraged the pluripotency of these villages to differentiate cells towards distinct neuronal fates – neural precursors, excitatory glutamatergic neurons and lower motor neurons. The scale and genetic diversity of these experimental systems allowed us to perform expression quantitative trait loci (eQTL) analyses where we link changes in direction and magnitude of gene expression directly to cis-regulatory variation. This approach yielded discovery of thousands of eQTLs in each cell type, with many demonstrating cell-type specificity. We overlap this repertoire of eQTLs with disease-linked variation identified from genome-wide association studies to help elucidate how alleles implicated in disease influence molecular pathways. These types of analyses will help find aspects of neural biology on which these many genetic influences converge, whether on specific neural cell types, biological processes, or molecular pathways linked to disease.

Keywords: Neural differentiation, genetic variation, eQTL

CI276

ROBUST IPS DIFFERENTIATION VIA REFINED COMBINATORIAL TRANSCRIPTION FACTOR SCREENS USING BULK RNA-SEQUENCING

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A key challenge for clinical cellular therapies is to establish robust protocols for creating large, sustainably cultured batches of cells of one type that can be differentiated into cell types that are less easily cultured or obtained. In recent years, iPSCs have proven to be ideal candidates for the former, and therefore the key challenge has become how to identify methods for converting iPSCs into other cell types. One highly effective approach to this problem is using transcription factor (TF) over-expressions to control differentiation of iPSCs into other cell types. While these methods have proven highly efficient, the task of identifying a subset of ≥ 1700 TFs to use to differentiate a new cell type is a daunting task that can take a very long time. Furthermore, while there exist software tools to identify TFs for conversion, they generally have the goal of identifying one specific combination of TFs for conversion, which might not hold up to all culturing conditions and still must be tested experimentally. To address this, we have developed a method with two major components: one, a method to prioritize TFs pools (as opposed to specific combinations) into a screen based on epigenetics and trascriptomics data using machine learning; and two, a method for using barcoded TFs and RNAseq to identify the TF-overexpression profiles of successfully differentiated cells from the screens to produce stable cell lines that differentiate at high speed and efficiency. We demonstrate successful creation of iPSC lines that differentiate to cell types from all three germ layers in ≤ 8 days at ≥ 50% efficiency (B cell/Mesoderm, Astrocyte/Ectoderm, Hepatocyte/Endoderm, Microglia/Unknown) using this method to demonstrate that it is applicable to the creation of iPSC lines that can differentiate into cells of any type needed for cell therapies.

Funding source: This work was supported by the FunGCAT program from the Office of the Director of National Intelligence, Intelligence Advanced Research Projects Activity, via the Army Research Office under Federal Award No. W911NF-17-2-0089"

Keywords: Computational biology, Trans-differentiation, Multiplexed screening

CI295

SPATIAL SINGLE CELL TRANSCRIPTOMICS AT MOUSE EMBRYO SCALE

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Spatial patterns of gene expression, which are established within and across tissues, underlie key embryonic developmental processes. Currently, many in situ methods for profiling gene expression are limited in the number of transcripts or region of interest they can measure, while other methods give local aggregate or averaged measurements. To provide a method for spatial transcriptomics with single cell resolution while simultaneously capturing heterogeneity across large sections of tissues or embryos, we have developed sci-Space. When we applied sci-Space to the developing mouse embryo, we were able to use spatially applied barcodes to approximate the spatial coordinates cells in conjunction with the measurement of their transcriptomic profiles. By mapping transcriptomes to the original embryo space we could produce “digital in situs” for individual genes and breakdown spatial patterns of gene expression by cell type. We identified genes including Hox-family transcription factors known to be expressed in an anatomically patterned manner. We resolved the spatial pattern of expression by excitatory neurons and demonstrated that the pattern spanned cell subtypes. We showed that variation in expression of these and other genes could be explained by spatial context but not cell state differences. We have also shown that sci-Space can resolve the differential contribution of cell types to localized signaling ligands’ gene expression. Finally, we use a new statistical approach for quantifying the contribution of spatial context to variation in gene expression within cell types. We then identify modules, or sets of genes, that vary spatially in different populations of tissue-specific and more universal endothelial cell subtypes. This work demonstrates that the sci-Space method for spatially resolving single cell transcriptomic data across large tissue sections can provide essential information regarding spatial state regulation during development. Future studies using this method have the potential to uncover key spatial regulators of cell fate specification and drivers of tissue morphogenesis.

Funding source: MR is funded by a Washington Research Foundation Postdoctoral Fellowship

Keywords: scRNAseq, spatial, embryo

CI314

HUMAN INDUCED PLURIPOTENT STEM CELL REPORTER LINES PROVIDE INSIGHT INTO STATES OF PLURIPOTENCY

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The Waddington landscape is frequently invoked as an illustration of how cells in an isogenic population can explore different phenotypic states. A population of cells often exhibits a range of phenotypes even when culture conditions are kept constant. We are developing induced pluripotent stem (iPS) cell lines to map the different states that individual pluripotent cells can occupy in the context of their relative expression of OCT4, SOX2 and Nanog. We are using CRISPR engineering to create dual-reporter iPS cell lines that produce a fluorescent reporter associated with each of 2 of the network factors simultaneously. The fluorescence of the reporters in individual cells will allow us to create a map - a 3-dimensional landscape - of how the relative expression of these three transcription factors influence the states of pluripotency across the population of iPS cells. This will allow us to assess how a cell’s state of pluripotency is affected by cell division, spatial location, morphology, etc. We will also use fluorescence microscopy to quantify the dynamics of the expression of these genes in individual cells. We have previously demonstrated the dynamic nature of gene expression in individual cells and have shown that phenotype expression in individual cells is stochastic and highly dynamic even while the population distribution of phenotypic expression is stationary. We have developed a statistical thermodynamics model that predicts that correlations in the fluctuations in expression of genes within individual cells is a measure of the interdependence between those components of the network. Together, the 3-D landscape and the correlations in dynamic interactions between network factors will allow prediction of the stability of different states of pluripotency, and the kinetics with which those states inter-convert.

Keywords: Induced pluripotent stem cells, Live cell imaging, Statistical thermodynamics model

CI333

SPATIALLY RESOLVED TRANSCRIPTOMICS AND PROTEOMIC CELL-TYPES IN THE HUMAN CENTRAL NERVOUS SYSTEM

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Understanding the detailed cellular composition and gene expression of the mammalian central nervous system (CNS) is critical for gaining insights into normal, developing, and diseased neuronal tissues. While single cell RNA-seq (scRNA-seq) makes possible high-resolution gene expression measurement in the CNS, the technique requires cells to be dissociated from tissue, thereby losing information about tissue structure and organization. Recently, the 10x Genomics Visium Spatial Gene Expression Solution was introduced to address this limitation by combining histological techniques and the massive throughput of RNA-seq. Visium incorporates ~5000 different molecularly barcoded, spatially encoded capture probes onto a slide over which tissue is placed, imaged, and permeabilized, allowing for the unbiased capture of native mRNA. RNAseq data is then mapped back to image coordinates placing gene expression into context within the tissue image. Here, we expanded on the technology by incorporating BioLegend's TotalSeq oligo-conjugated antibodies to spatially resolve cell-specific proteomic markers along with gene expression in the same tissue. We demonstrate this technique in serial sections of fresh frozen human spinal cord, cerebrum, and cerebellum. By aggregating proteomic and transcriptomic data from serial sections, we improved the resolution of cell-type identification. Results from these efforts suggest that this "multi-omics" approach can provide a powerful complement to traditional histopathology, enabling a greater understanding of cellular heterogeneity and organization within the mammalian CNS. This new, more detailed view of the human CNS anatomy as it varies across different regions, can provide essential insight into the cell type-specific nature of neurobiology and neurodegenerative diseases.

Keywords: Spatial Transcriptomics, Feature Barcoding, Multiomics

CI343

QUANTITATIVE ASSESSMENT OF CELL FATE ENGINEERING EXPERIMENTS WITH EMBRYONIC REFERENCES AT A SINGLE CELL RESOLUTION

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The advent of single cell RNA sequencing (scRNA-seq) has enabled an additional quantitative framework to assess the fidelity of cell fate engineering (CFE) studies, such as directed differentiation and direct conversion. Recently developed computational techniques to analyze scRNA-seq data have yielded new insights into CFE. For example, scRNA-seq has detected the emergence of myocyte cell fate during direct conversion experiment from MEFs to motor neurons using Ascl1 and additional neuron-specific factors. However, several technical challenges have prevented the field from addressing several pressing questions, including 1) what are the identities of the intermediates states during reprogramming 2) what are the differences between reprogramming trajectories and normal development in vivo. These technical challenges include the absence of a comprehensive reference data set of mammalian development, and the absence of sensitive metrics to allow direct comparison between studies across platforms and across species. Here, we address these issues by first integrating temporal scRNA-seq datasets across 3 studies, spanning blastocyst to early organogenesis resulting in cross-study reference data of 102908 cells and 47 cell types. Then we incorporated 5 distinct cell-typing methods to investigate 1) to what degree are those immediate cells hybrid in nature 2) how are reprogramming trajectories differ between CFE categories (e.g. direct conversion vs directed differentiation) 3) the fidelity of current CFE protocols. The cell typing methods that we used were gene expression-based scoring, expression correlation, support vector machine, random forest classification, and gene regulatory network establishment. To further evaluate the performance of the five assessment approaches we performed scRNA-seq on three major stages of mouse gastrulation. We found that most direct conversion studies did not exhibit distinct cell-type specific embryonic signatures. Most directed differentiated cells do not faithfully pass through the same developmental milestones observed in the in vivo embryonic differentiation. Ultimately, we anticipate that these quantitative tools will yield insights into the assessment and improvement of future cell fate engineering experiments.

Keywords: Cell Fate Engineering Assessment, single cell RNA sequencing, Cell typing

Theme: Clinical Applications

EPITHELIAL

CA129

ROLE OF MURINE SMOOTH MUSCLE CELL PIEZO1 IN BIOMECHANICAL STRETCHING OF THE SMALL BOWEL

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Biomechanical stretching of the small bowel has clinical implications for patients with small bowel obstruction (SBO) and intestinal failure. Little is understood of the mechanism by which biomechanical stretching results in thickening the muscularis and expansion of crypts and intestinal stem cells of the epithelium. Piezo1 is a stretch-induced ion channel involved in trophic effects in mechanosensitive tissues and is expressed in the gut. The aim of this study is to determine whether Piezo1 in smooth muscle cells (SMC) is necessary in stretch-induced intestinal growth. Using the Piezo1/Myh11-ERT2/Cre-LoxP system, we generated a mouse model with a tamoxifen (Tam) inducible-Piezo1 knockout (Piezo1ΔSMC) in the SMC of small bowel muscularis (SBM). Mice received Tam and distal SBO surgery to induce radial stretch. In vitro, SBM was isolated from mouse pups and developed onto 2D monolayers on a soft tissue scaffold composed of a thermoresponsive hydrogel to induce ~50% stretch by cooling from 37°C to 33°C. Confocal microscopy was used to record SBM contraction/Ca2+flux. The frequency and magnitude were quantified with MATLAB. In vivo: Mortality rates were similar between control (Piezo1WT) and Piezo1ΔSMC. Obstructed mice had a significant increase in crypt and SBM size compared to non-obstructed mice. However, there was significant attenuation in crypt elongation in Piezo1ΔSMC mice compared to Piezo1WT ($p=0.0006$). There were no statistical differences between both groups in obstructed SBM size. In vitro: At 37°C, Piezo1WT SBM cells have spontaneous and rhythmic contractions associated with Ca2+flux. At 33°C, Piezo1WT cells seeded on hydrogels doubled in frequency of contractility/Ca2+flux, compared to Piezo1WT cells on plastic, which had decreased contractility/Ca2+flux. When Yoda1, a Piezo1 agonist was added to Piezo1WT cells on plastic at 33°C, contractility/Ca2+flux robustly resumed. In contrast, Piezo1ΔSMC cells displayed decreased frequency and disorganized pattern of contractions/Ca2+flux, which diminished further on stretching at 33°C and only partially resumed when adding Yoda1. Our data suggests that Piezo1 in the SMC of the SBM partly mediates crypt expansion associated with SBO and is important for the maintenance of regular SBM contractions/Ca2+flux.

Funding source: CIRM CSUN/UCLA Bridges Grant NIH-NIDDKT32 DK007180 CURE/P30 DK041301

Keywords: Short bowel syndrome, Piezo1, regenerative medicine

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

CA134

UMBILICAL CORD BLOOD BANKING: KNOWLEDGE AND ATTITUDES OF BRAZILIAN PREGNANT WOMEN

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The umbilical cord blood (UCB) is rich in hematopoietic stem cells and has been used as an alternative source to bone marrow transplants to treat several malignant and non-malignant disorders. However, the lack of knowledge regarding UCB banking negatively affects the mother's decision whether to donate or store their infant's UCB. Higher donation rates would increase the chances of finding compatible donors for transplants. Thus, this study aimed to assess the Brazilian pregnant women's awareness, knowledge, beliefs, and opinions about UCB banking. A previously published questionnaire was adapted, validated and applied in 387 pregnant women who consented to participate in the study, in Florianopolis, Brazil, from Jun 2017 to Dec 2018. 61% of the responders were aware of UCB banking, and 86.9% of those considered to know a few or very few about it. The main source of information was the media: internet, TV or radio (71.6%), and only 13.6% answered health professionals (doctors and nurses). Being older than 35 years old, having a family income higher than 3 minimum wage or a college degree was associated with greater awareness of UCB banking (all $p=0.000$). Furthermore, knowing someone that received a bone marrow or organ transplant ($p < 0.01$) or being a blood donor ($p < 0.01$) also showed to have significant influence. When asked specific questions about the UCB banking, such as whether there are two types of banks, private and public, almost

50% answered “I don’t know” to all the questions. 83% of the women correctly knew the UCB is used to treat leukemia and 50% that is used to treat serious anemias, but 63% incorrectly thought spinal injury is currently treated by the UCB. Only 14% of pregnant women were asked by someone whether they would like to store or donate the UCB. Of them, 65% were asked by private bank sellers and 5% were asked by a nurse or doctor. Just 13% have made a decision by the time of the survey and more than half of those decided not to donate or store it. However, 55.9% would consider donating the UCB and 91.2% would allow its use for medical research. Finally, 94% think women should be told about UCB banking by their doctor before the last stage of pregnancy. In conclusion, this study demonstrated that Brazilian pregnant women have poor knowledge about UCB banking and wish to be better informed about it by health professionals.

Funding source: Ministério da Ciência, Tecnologia e Inovação/Conselho Nacional de Desenvolvimento Científico e Tecnológico (MCTI/CNPq/Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) and Pro-bolsas – UFSC.

Keywords: Hematopoietic stem cells, Umbilical cord blood storage, Survey

EYE AND RETINA

CA262

FIBROBLASTS PROMOTE THE FORMATION OF SELF-ORGANISED CAPILLARY-LIKE NETWORKS BY HESC-DERIVED CD34+ ENDOTHELIAL CELLS IN A LAYERED COLLAGEN HYDROGEL SYSTEM

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Pre-vascularisation of tissue engineered constructs for transplantation is essential for the advancement of regenerative medicine. A substantial amount of research has therefore focussed on generating tissue engineered vascular networks. Recently, fibroblasts have been recognised for their ability to promote self-organisation of endothelial cells into vascular networks. However, most of these studies have used human umbilical vein endothelial cells, and there has been little in the way of investigating the potential of human embryonic stem cell (hESC) derived endothelial cells in forming vascular networks in the presence of fibroblasts. Previous work in our lab has led to the successful treatment of 2 patients with age related macular degeneration (AMD), by transplanting a patch of hESC-derived retinal pigment epithelial (RPE) cells into the macula. The capillaries that supply the RPE with nutrients lie immediately adjacent to the RPE and are planar in geometry. We therefore wanted to investigate the potential of hESC-derived CD34+ endothelial cells to self-organise into capillary like networks (CLN). In order to encourage the formation of a planar vascular structure within a 3-dimentional construct, hESC-ECs were cultured between 2 layers of collagen hydrogel, with or without fibroblasts, for 4 days. Whilst hESC-ECs were capable of self-organising into CLNs in layered collagen alone, there was a significant improvement in network formation when collagen hydrogels were populated with fibroblasts. CD31 staining revealed a more continuous network in the presence of fibroblasts and the overall capillary diameter was significantly smaller in fibroblast populated constructs (26.3 +/- 2.2um) compared to collagen alone (41.7 +/- 1.5um). The presence of fibroblasts also significantly enhanced 13 out of 19 angiogenic parameters measured using ImageJ. Confocal microscopy

revealed apparent lumen formation in microvessels in the presence of fibroblasts, however these structures were absent in microvessels cultured without fibroblasts. These data show that successful self-organisation of CLNs by CD34+ hESC-EC in a planar geometry is enhanced by fibroblasts. Furthermore, this method of assembly may be beneficial for generating pre-vascularised RPE tissue grafts for the treatment of AMD.

Funding source: This work was supported by The Michael Uren Foundation.

Keywords: Tissue engineered vasculature, Retinal Pigment Epithelium, Fibroblast

IMMUNE SYSTEM

CA245

REPROGRAMMING OF CD4+ OR CD8+ T CELLS TO INDUCED PLURIPOTENT STEM CELLS

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Recent advancements in T cell immunotherapies have led to clinical successes in the treatment of blood cancers, melanoma, and viral infections. Currently most T cell therapy manufacturing utilizes autologous processes which uses a patients' own cells which can often be too dysfunctional and exhibits decreased proliferation. When cells with specific cancer identifying T cell receptors (TCRs) are isolated and reprogrammed they have been observed to retain their original TCR. T cell derived induced pluripotent stem cells (T-iPSCs) can thus provide an endless supply of cells specific to a given cancer or virus. We have previously reported on the optimization of workflows to provide successful pan CD3+ T cell reprogramming. However, many T cell therapies are based on a specific subset of CD4+ or CD8+ tumor infiltrating lymphocytes (TILs). Here we look to apply the pan CD3+ reprogramming methods to CD4+ or CD8+ T cells. We have successfully generated iPSCs from CD4+ or CD8+ T cells, though at a lower efficiency than previously observed for reprogramming of CD3+ pan T-cells. Resulting CD4+ and CD8+ derived iPSC clones were also analyzed for immune repertoire, and were confirmed to possess a TCR. Post transduction clonal characterization of T-iPSCs ensures iPSC pluripotency and retention of intact TCRs. By reprogramming T cells that retain their TCR we have generated a robust reprogramming method that can help develop future T-iPSC antigen specific immunotherapies.

Funding source: California Institute for Regenerative Medicine

NEURAL

CA179

PARACRINE MODULATION OF MICROGLIAL ACTIVATION AS A POTENTIAL THERAPEUTIC MECHANISM OF MESENCHYMAL STEM CELL-DERIVED NEURAL PROGENITOR CELLULAR THERAPY IN MULTIPLE SCLEROSIS

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Patients with progressive multiple sclerosis (MS) experience a steady worsening of neurologic function attributed to chronic demyelination and axonal loss. A novel regenerative therapy utilizing autologous bone marrow-derived MSC-NP cells is currently under clinical investigation as a treatment for patients with progressive MS. Pre-clinical studies suggest that the mechanism of action of MSC-NPs occurs through the paracrine release of trophic and immunomodulatory factors. Recent results from a phase I clinical trial demonstrated reversal of established disability after repeated intrathecal MSC-NP injections. CSF biomarker analysis showed that MSC-NP injections correlated with reduced levels of CCL2, a chemokine marker of activated microglia. The objective of this study was to investigate the mechanisms by which MSC-NP cell therapy can influence human microglial cell activity that contributes to the pathogenesis and progression of MS. MSC-NPs were derived from bone marrow of 10 individual donors with MS according to a previously described protocol. Two in vitro models, human iPSC-derived microglia and the human immortalized microglia cell line HMC3, were used to test paracrine effects of MSC-NPs on microglial activity. IL1β, INFγ and TNFα-stimulated human microglia were cultured with MSC-NP conditioned media, and activation markers were tested by quantitative PCR. We found culturing microglia with conditioned media from MSC-NP cells resulted in a decrease in activated microglia markers, including a reduction in inflammatory signaling molecules IL-8, IL-6 and Nos2. These effects were not observed with unconditioned media controls. The degree of inhibition correlated with MSC-NP cell concentration, suggesting that MSC-NPs secrete specific factors capable of paracrine signaling to microglia. These results demonstrate that MSC-NPs reduce the pro-inflammatory activity of microglia, suggesting a possible therapeutic target of MSC-NP cell therapy in MS.

Keywords: mesenchymal stem cells, multiple sclerosis, microglia

CA181

SPINAL CORD INJURY TRANSPLANTABLE NEURAL CIRCUITRY

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Human stem cell-derived neuronal and glial cells have the potential to restore damaged circuitry of the injured spinal cord. We are generating encapsulated homotypic cervical spinal cord cells and circuitry for transplantation into the damaged spinal cord to enhance region-specific integration, provide anisotropic placement of cells, and protect against initial waves of cell death from the complex injury microenvironment. The induced pluripotent stem cell (hiPSC) line F3.5.2 derived from the African American fibroblasts is used to generate spinal cord neural stem cells, scNSCs, with cervical regional identity and their differentiated spinal motor neurons (SMNs). Comprehensive multi-parameter analysis of SMNs in vitro demonstrates the formation of synaptic networks, innervation of rodent myotubes, active mitochondrial transport in neurites, action potential firing, and relevant bioinformatics profiles. Neural ribbon composition and parameters have been evaluated and optimized for the encapsulated SMNs to induce axon outgrowth perpendicular to ribbons or along the length of neural ribbons as customized for appropriate recovery needs. Neural circuits within the neural ribbons demonstrate synapse formation, spontaneous firing, and directed calcium signaling output. Our rat acute hemicontusion studies reveal striking advantages to this approach. Encapsulated transplanted scNSCs retain robust viability at the injury site along with neurite extension and synaptogenesis, even following overnight shipping at 37˚C (From Albany, NY to Houston TX). We are also evaluating homotypic SMNs, oligodendrocytes, and microenvironment modulators such as chondroitinase ABC (chABC) enzyme that we generate in house. Striking is the significant survival and integration obtainable with as few as 5,000 cells. Increasing precision in cell delivery numbers and ratios, improved cell survival, and host-cell integration that are priorities for therapeutic success. We are proceeding with long-term repair and behavioral studies. This work is funded by the New York State Spinal Cord Injury Review Board (SCIRB), “Healing the Contusion Injured Spinal Cord Microenvironment with Nanotechnology and Stem Cells”.

Funding source: NYSCIRB, SUNY Polytechnic SEED funding

Keywords: chondroitinase, cell therapy, organotypic

CA184

REGENERATIVE MEDICINE’S FIRST “BIOMARKER” FOR PREDICTING RESPONSIVENESS TO STEM CELL THERAPY BASED ON MECHANISM-OF-ACTION: EVIDENCE FROM HYPOXIC-ISCHEMIC CEREBRAL INJURY

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Regenerative medicine (RM) may offer therapies for heretofore untreatable conditions. However, RM still has no patient-specific non-invasive biomarkers for predicting responsiveness to treatment based on the mechanism-of-action (MOA) of a given stem cell for a given condition in a given individual. It is not yet possible to match a patient’s pathophysiological profile to a cell’s MOA. MOA-based patient selection and exclusion is critical for: ensuring safety and efficacy in a patient (Who should and should not undergo a procedure?); establishing standards-of-care for RM; rational resource allocation; improving clinical trial success; patient monitoring. As proof-of-concept, we developed a technique for stratifying cerebral damage in a rat model of

perinatal hypoxic-ischemic injury (HII), a condition for which stem cell therapy has been advocated. We used a magnetic resonance imaging (MRI)-derived algorithm, "hierarchal region splitting (HRS)", to segment the lesion into quantifiable necrotic core (irreversibly damaged) vs. salvageable penumbral volumes and, based on the penumbra:core ratio, categorize the HII as mild, moderate, or severe; the smaller the ratio, the greater the severity. Rats were transplanted with human neural stem cells (hNSCs) (the MOA of which is neuroprotection) and re-evaluated at 90d post-transplant for lesion volume (including the ratio), cell fate, motor and cognitive function. Only rats with a penumbra >core displayed favorable outcomes; those with predominantly core showed no improvement; pre-existing core remained unchanged; unrescued penumbra transitioned to core. We then showed that: an increased penumbra>core ratio on MRI corresponded with: regional upregulation of the surrogate molecular marker for repair (heat shock protein 27 [HSP27]) in host cells; maximal HSP27+ hNSC engraftment, integration, and differentiation; preserved neurological function. Identical MRI can be performed in humans. These preclinical data suggest that only patients with lesions containing a sufficiently-sized MRI-defined penumbra capable of responding to the neural network-preserving MOA of hNSCs should receive transplants.

Keywords: Novel Biomarker based off MRI, Predict Therapy Responsiveness, Allows for Patient Selection and Exclusion

NEW TECHNOLOGIES

CA227

NEGLIGIBLE-COST AND WEEKEND-FREE CHEMICALLY DEFINED HUMAN IPSC CULTURE

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Human induced pluripotent stem cell (hiPSC) culture has become routine, yet pluripotent cell media costs, frequent media changes, and reproducibility of differentiation have remained restrictive. Here, we describe the formulation of a hiPSC culture medium (B8) as a result of the exhaustive optimization of medium constituents and concentrations, establishing the necessity and relative contributions of each component to the pluripotent state and cell proliferation. The reagents in B8 represent only 3% of the costs of commercial media, made possible primarily by the in-lab generation of three E. coli-expressed, codon-optimized recombinant proteins: fibroblast growth factor 2 (FGF2-G3); transforming growth factor β3 (TGFβ3); and neuregulin 1 (NRG1). We demonstrated the derivation and culture of 34 hiPSC lines in B8 as well as maintenance of pluripotency long-term (over 100 passages). This formula also allows a weekend-free feeding schedule without sacrificing capacity for differentiation.

Keywords: human induced pluripotent stem cell, culture media, weekend-free

CA238

THE ROLE OF MITOCHONDRIA TRANSFER AND BIOENERGETICS IN STEM CELL THERAPIES

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Modulation of mitochondrial bioenergetics (MB), mitochondria transfer (MT), and extracellular vesicle (EV) release from stem cells (NSCs) have recently emerged as regenerative therapies. Spinal cord injury (SCI) is a debilitating neurodegenerative condition, and it is estimated that 288,000 people with SCI currently live in the United States. Mitochondria transfer from bioenergetically enhanced transplanted neural stem cells can help restore the loss of bioenergetics in the pathophysiology of SCI. In SCI, the initial insult is followed by a secondary injury cascade characterized by an ischemic oxidative microenvironment, which leads to further necrotic neuronal death, demyelinated axons, and mitochondrial dysfunction. Our lab has developed several fetal-derived human NSC lines and tested their efficacy in restoring functional recovery in SCI. Among these, the UCI161 line was shown to be significantly efficacious, whereas the UCI152 line was not. Transcriptomics analysis of mitochondrial-related genes revealed an upregulation of mitochondrial bioenergetics and biogenesis in the UCI161 line. Next, the lines were augmented with mitochondria-modulating compounds, leading to increased mitochondrial hyperfusion consistent with bioenergetic enhancement. Leflunomide treatment resulted in an increase in ATP production; however, the MTT mitochondrial reductase activity showed that the UCI152 line was sensitized to prolonged increased metabolic activity. We also identified a novel role for Leflunomide in enhancing the formation of tunneling nanotubes (TNTs), membranous tubes that mediate intercellular communication. A co-culture system showed that Leflunomide treatment induces the transfer of both mitochondria as well as CD63+-EVs. A wound-healing assay revealed that Leflunomide-treated UCI161s exhibit increased migratory behavior as early as 4 hours. Stem cells with enhanced bioenergetics and mitochondrial transfer capabilities show promise for translation to chronic traumatic injuries and a host of other neurodegenerative disorders characterized by loss of mitochondria function.

Funding source: UC President's Postdoctoral Training Fellowship

Keywords: Mitochondria Transfer, Bioenergetics, Neural Stem Cells

Theme: Modeling Development and Disease

ADIPOSE AND CONNECTIVE TISSUE

MDD104

RECAPITULATING BROWN ADIPOCYTE DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS.

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Brown adipose tissue (BAT) has a specialized thermogenic and mitochondrial program which allows dissipation of energy from substrate oxidation in the form of heat. Therefore, elevating BAT activity or mass presents a promising opportunity to enhance energy expenditure and weight loss in diabetic and obese patients. While a lot of effort has been made to understand brown fat physiology and function in mice, there is a lack of knowledge about BAT development in humans. One of the roadblocks in understanding brown fat development and its use for therapeutic purposes has been its inaccessibility. We have recently developed a method to differentiate murine and human pluripotent stem cells (hPSCs) into paraxial mesoderm cells which are precursors of skeletal muscle and brown adipocytes. Here, we report that we have developed this protocol further in order to generate brown adipocytes. By systematically recapitulating known developmental cues our method allows cells to differentiate into paraxial mesoderm state followed by PAX3 positive somite-like cell identity. The PAX3 expressing cells differentiate into the brown fat lineage, as also seen in vivo. Using an hPSC reporter line for the brown adipocyte-specific gene UCP1, we have been able to dynamically follow the progression of brown adipocyte differentiation in vitro. We also analyzed developing murine BAT using single-cell RNA sequencing to identify regulators of brown adipocyte differentiation. We identified several signaling pathways that were differentially activated or inactivated in differentiating adipocytes. Interestingly, manipulation of these pathways in vitro notably improved the brown adipocyte differentiation efficiency. We show that hPSC derived brown adipocytes undergo lipolysis and increase mitochondrial uncoupling in response to beta-adrenergic stimulus. In ongoing work, we are investigating the transcriptome of differentiating human brown adipocytes at single-cell level which will allow us to identify regulators of human brown fat differentiation. In conclusion, our method provides a model to study brown fat development and a source of brown adipocytes for potential cell therapy treatments.

Funding source: This work is supported by Harvard Stem Cell Institute (grant #DP-0146-15-00).

Keywords: Brown adipocytes, Differentiation, paraxial mesoderm

CARDIAC

MDD111

SINGLE-CELL TRANSCRIPTOMIC ANALYSIS AND PATIENT-SPECIFIC IPSCS REVEAL DYSREGULATED CELL CYCLE IN CORONARY ENDOTHELIAL CELL IN HYPOPLASTIC LEFT HEART SYNDROME

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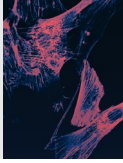
Hypoplastic left heart syndrome (HLHS) is a single ventricle congenital heart disease that results in severe underdevelopment of the left ventricle, mitral valve, aortic valve, and ascending aorta. Early serial postmortem examinations also revealed a high rate of coronary anomalies in HLHS, which included multiple ventriculo-coronary arterial connections as well as thick-walled and kinked coronary arteries. A previous study showed that fetal hypoplastic left hearts had a reduced endothelial cell (EC) population and lower capillary density compared with normal hearts. However, the mechanism underlying coronary abnormalities associated with HLHS is not fully understood. Thus, we generated induced pluripotent stem cells derived ECs (iPSC-ECs) from three HLHS patients and three age-matched controls. Single Cell RNA-Seq (scRNA-seq) profiling identified both endocardial (NPR3+/CDH5+) and coronary endothelial populations (APLN+/CDH5+) from the heterogeneous iPSC-ECs. Intriguingly, a subcluster of the coronary endothelial cells (CECs) were enriched in HLHS patients. Furthermore, cell cycle analysis showed that 30.6% of the HLHS cells were trapped in the G1 phase, while the majority of the control CECs entered cell cycle normally. Additionally, the cell cycle differences between control and HLHS was only seen in CECs, not in the endocardial population. To verify our transcriptomic analysis, we applied negative cell sorting (NPR3-/CDH5+) on iPSC-ECs to purify CECs (iCECs) and confirmed that HLHS iCECs showed a profound reduction of cell cycle/proliferative genes (KI67, PCNA, CCNA2, CCNB1) and abnormal induction of CCND2, which is the hallmark of G1 phase. BrdU assays also indicated suppressed proliferation in HLHS iCECs. Furthermore, we profiled the transcriptome from a human heart with an underdevelopment left ventricle (ULV) at single cell resolution. When compared to the normal human heart, pathway enrichment analysis of differentially expressed genes in ULV hearts demonstrated reduced cell proliferation in the CEC subpopulation. Here, we identified that CECs from HLHS patients exerted proliferative defects that can potentially impede the development of vascular/capillary structure and cause related functional deficiencies.

Funding source: Lei Tian was supported by AHA 20POST35210924



Keywords: Hypoplastic Left Heart Syndrome, Coronary Endothelial Cell, Cell Cycle

MDD119

SELF-ASSEMBLING HUMAN PLURIPOTENT STEM CELL-DERIVED HEART ORGANOIDS FOR THE STUDY OF CARDIAC DEVELOPMENT AND DISEASE

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Cardiovascular disorders, including cardiovascular disease (CVD) and congenital heart defects (CHD), constitute a significant worldwide health problem. CVD is the leading cause of death in developed countries, making up a third of the mortality rate in the US. CHD affects ~1% of all live births and under most circumstances the causes are unknown. Thus, there is a dire necessity to develop efficient organ-like platforms recapitulating complex in vivo phenotypes to study human development and disease in vitro. Recently, we developed a highly efficient, reproducible and high-throughput protocol to produce human PSC-derived heart organoids by self-assembly (hHOs). hHOs are created by step-wise manipulation of WNT signaling using chemical inhibitors and growth factors under completely defined culture conditions. hHOs were successfully derived from three independent hiPSC lines and one hESC line. Robust beating was observed as early as 6 days after differentiation. hHOs are spherical, grow up to 1 mm in diameter and develop sophisticated interconnected internal microchambers. Immunofluorescence staining and confocal analysis for cardiac markers revealed the presence of numerous cardiac lineages found in the heart, including cardiomyocytes (cTnT+), epicardial cells (WT1+, ZO1+), cardiac fibroblasts (CD90+, Vimentin+), endothelial cells (CD31+), and endocardial cells (NFAT2+). Morphologically, hHOs develop well-defined epicardial and adjacent myocardial tissue, present a distinct vascular plexus and endocardial-lined lumens. RNA-seq time-course analysis revealed hHOs recapitulate the complexity of fetal heart tissue better than previous monolayer-based differentiation protocols. In conclusion, hHOs allow higher-order interaction of distinct heart tissues and the presence of biologically relevant physical and topographical 3D cues that closely resemble the human fetal heart. hHOs might prove to be a valuable model to study cardiac development in vitro, as well as a large range of cardiovascular diseases upon further maturation.

Funding source: The Aguirre Lab is supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award number HL135464 and by the American Heart Association under award number 19IPLOI34660342.

Keywords: Heart Organoids, Cardiac Development, Cardiovascular Disease Modeling

MDD123

NANOSTRUCTURED BIOMIMETIC SURFACES PROMOTE THE STRUCTURAL MATURITY OF VARIOUS STEM CELL-DERIVED CARDIOMYOCYTE LINES

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Although induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) have great potential in the fields of therapy development, their phenotypic immaturity remains a critical roadblock towards translation. The heart is a highly organized, cell-dense organ, and its structural organization is critical to appropriate cardiac function. As a result, methods to physiologically structure engineered cardiac tissues without sacrificing cell density will contribute towards improving the translational utility of iPSC-CMs. Towards this, we demonstrate a highly scalable, cost-effective method of organizing and aligning iPSC-CMs in 2D using nanostructured, biomimetic culture surfaces mimicking the native cardiac extracellular matrix topography. Fabrication of nanostructured surfaces were successfully achieved in large-scale (e.g. bioreactor-compatible) as well as high-throughput (e.g. high-throughput screening compatible) formats with high pattern fidelity. The nanostructured surfaces were also compatible with a number of extracellular matrix preparation, including simple Fetal Bovine Serum incubation, to further promote the attachment of iPSC-CMs. Academic, commercial, and patient-derived iPSC-CMs were all successfully seeded onto the nanostructured surfaces and all formed confluent, aligned, and syncytial monolayers. Brightfield microscopy and video analysis of spontaneous contractions demonstrated anisotropic cellular morphology with anisotropic contractions. Immunofluorescent staining and imaging also demonstrated cytoskeletal organization of cardiac monolayers, including well-ordered sarcomere arrays of the iPSC-CMs. Finally, to demonstrate compatibility with standard drug development assays, iPSC-CMs demonstrated a dose-

dependent response to cytotoxic compound doxorubicin on our nanostructured surfaces in a microplate format. Based on these results, we demonstrate the use of a scalable and inexpensive technique which can organize iPSC-CMs into physiologic structures and is compatible with a variety of formats, culture protocols, and endpoint assays, demonstrating the versatility of our approach.

Keywords: Cardiomyocyte, Nanofabrication, Tissue engineering

MDD445

DUAL FUNCTION OF IPSC-DERIVED PERICYTE-LIKE CELLS IN VASCULARIZATION AND FIBROSIS-RELATED HUMAN CARDIAC TISSUE REMODELING IN VITRO

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Myocardial interstitial fibrosis (MIF) is characterized by excessive extracellular matrix (ECM) deposition, increased myocardial stiffness, functional weakening, and compensatory cardiomyocyte (CM) hypertrophy. Fibroblasts (Fbs) are considered the principal source of ECM, but contribution of perivascular cells, including pericytes (PCs), has gained attention since MIF develops primarily around small vessels. The pathogenesis of MIF is difficult to study in humans, because of the pleiotropy of mutually influencing pathomechanisms, unpredictable side effects and the lack of available patient samples. Pluripotent stem cells (PSCs) offer the unique opportunity for de novo formation of bioartificial cardiac tissue (BCT) using a variety of different cardiovascular cell types to model aspects of MIF pathogenesis in vitro. Here, with the aim of understanding the role of PCs in MIF, we present an optimized protocol for the derivation of PSC-derived PC-like cells (iPSC-PCs) showing typical PC transcriptional signature and function. A BCT in vitro model was developed to study the interplay of different cell types, i.e. PSC-derived CMs, endothelial cells (ECs) and iPSC-PCs or primary Fbs, respectively. While the addition of iPSC-PCs improved sarcomere structure and supported vascularization in a PC-like fashion, functional and histological parameters of BCTs revealed PC- and EC-mediated effects on fibrosis-related cardiac tissue remodeling. In the iPSC-PC containing tissue groups, we experienced enhanced deposition of ECM, increased collagen cross-linkage and tissue stiffness (CM+iPSC-PC+EC BCTs: 133.10±13.50 kPa vs. CM+Fb+EC BCTs: 25.77±3.73 kPa), secretion of brain natriuretic peptide, functional weakening - all typical in MIF - and the involvement of PC-like cells in the fibrotic process as it has been shown in other organs. In order to better understand the pathomechanism of fibrosis and develop new treatment options, this tissue model including the most abundant cardiovascular cell types provides us with a powerful tool to investigate the complex cellular interplay involved in fibrosis-related remodeling in vitro.

Funding source: German Research Foundation (GR 3993/2-1; MA 2331/15-1; Cluster of Excellence REBIRTH, EXC 62/2); Clinical Research Group (KFO) 311 (GR 3993/3-1); German Center for Lung Research (BREATH 82DZL002A1), and BMBF (iCARE, 01EK1601A).

Keywords: iPSC-derived cardiovascular cells and tissue, myocardial interstitial fibrosis, cardiac pericytes

MDD481

MODELING DRUG-INDUCED CARDIOTOXICITY UTILIZING HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES: TOWARDS PROTECTIVE THERAPY FOR THE HEART

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Advances in the development of anti-cancer agents have made the treatment of several cancers more effective, but unfortunately have also increased the risk for cardiotoxic side effects. Cardiotoxicity is one of the main adverse effects of cancer therapy and has primarily been assessed in different in vivo animal models. However, animal models fail to recapitulate human physiology because of species differences and therefore are not predictive for drug-induced cardiotoxicity. Human pluripotent stem cell-derived cardiomyocytes (hPSC-cardiomyocytes) provide a reliable source of human cardiomyocytes and have already proven valuable for cardiotoxicity studies. We utilized high throughput time-lapse imaging of cardiomyocytes differentiated from an NKX2.5-eGFP-α-actinin-mRuby2 hPSC cardiac reporter for assessment of cell viability and sarcomeric disarray after treatment with Doxorubicin, one of the most effective anti-cancer drugs. These findings were confirmed by immunostaining of live/dead, apoptotic and DNA damage markers. Moreover, we evaluated the cardiotoxic

effect of Doxorubicin on a functional level by evaluating calcium transients and calcium accumulation. To more closely resemble the human heart compared to simple 2D monolayer cultures, we have established a more advanced platform for creating 3D engineered heart tissues (EHTs). Combined culture of hPSC-cardiomyocytes and hPSC-epicardial-derived cardiac fibroblasts resulted in robust formation of contractile EHTs, which allowed us to measure the effect of different concentrations of Doxorubicin on contractile force. In summary, these human stem cell-based assays provide a versatile screening platform for assessment of cardiotoxicity of anticancer drugs, such as Doxorubicin analogues or other compounds, which may ultimately lead to a better and safer treatment of patients.

Funding source: This work was supported by ERA-CVD 2016T092 and the Dutch Heart Foundation.

Keywords: HPSC-derived cardiomyocytes, Cardiac tissue, Cardiotoxicity

EARLY EMBRYO

MDD146

TISSUE GEOMETRY AND MECHANICS ORGANIZE MORPHOGEN SIGNALING DURING MICROPATTERNING OF HUMAN PLURIPOTENT STEM CELLS.

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During embryogenesis, morphogen signals induce the differentiation of progenitor cells, patterning the embryo. This patterning is accompanied by rapid changes in the geometry and structure of the embryo, leading to its morphogenesis. Though much is known about how morphogen signals drive the differentiation of specialized cell types, less is known about how the changing physical properties of the embryo shape signaling during development. Here, we investigate how morphogen signaling is regulated by the geometry and mechanics of tissues using human embryonic stem cell (hESC) epithelia as a model system. We constructed “Tet-On” inducible hESC lines expressing secreted activators or inhibitors from the BMP, WNT, and ACTIVIN/NODAL signaling pathways, as well as fluorescently-tagged hESC lines to track the dynamics of regulatory transcription factors as cells differentiate. By co-culturing these cells on PDMS micropost arrays of tunable stiffness and porosity, we can rapidly assay how the structure of the tissue microenvironment regulates and integrates signals from multiple morphogen sources to pattern nearby cells. We show that the geometry of epithelial hESCs dramatically restricts BMP and NODAL signaling due to the basolateral localization of their receptors, while substrate mechanics regulate the effectiveness of WNT ligands to drive nuclear localization of β-catenin and mesendoderm differentiation. Furthermore, through precise 2D micropatterning of morphogen source hESCs, we can drive the differentiation of epithelial sheets of hESCs into reproducible patterns of mesoderm, endoderm, and ectoderm cell fates. We find that mesoderm and endoderm progenitors undergo EMT and break impermeable tight junctions with their neighboring cells, enabling apical BMP and NODAL ligands to reach previously inaccessible basolateral receptors and reshaping morphogen signaling within the epithelium. By combinatorially generating many different micropatterns, we investigate which arrangements of morphogen sources can give rise to germ layer patterns of stereotyped size and sequence as found in the human primitive streak. We anticipate that this engineering approach will provide novel insights into the physical constraints that regulate signaling and morphogenesis during gastrulation in vivo.

Funding source: Supported by NIH under award number [1R01GM131105-01].

Keywords: Micropatterning, Morphogen, Gastrulation

MDD471

MODELING POST-IMPLANTATION EMBRYONIC DEVELOPMENT AND SOMITOGENESIS IN VITRO WITH GASTRULOIDS (EMBRYONIC ORGANOIDS)

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Gastruloids are three-dimensional aggregates of embryonic stem cells that display key features of mammalian post-implantation development, including germ-layer specification and axial organization. Gastruloids can be used to study embryology in vitro, and have some key advantages over embryos: they can be grown in large quantities (allowing

screens), are easier to genetically modify, and provide new insights into embryonic development. The currently available versions of the gastruloids system are however not able to generate somites (the embryonic precursors of skeletal muscles and vertebrae) and can thus not be used to study somitogenesis in vitro. In addition, human versions of this model system have not yet been developed, and this system can therefore not yet be used to study development in a human context. Additionally, the expression pattern of only a small number of genes in gastruloids has been explored with microscopy, and the extent to which genome-wide expression patterns in gastruloids mimic those in embryos is therefore currently unclear. To address these shortcomings, we in this poster first compare mouse gastruloids with mouse embryos using single-cell RNA sequencing and spatial transcriptomics. We identify various embryonic cell types that were not previously known to be present in gastruloids, including endothelial and head mesenchymal cells, and show that key regulators of somitogenesis are expressed similarly between embryos and gastruloids. Using live imaging, we show that the somitogenesis clock is active in mouse gastruloids and has dynamics that resemble those in vivo. Next, we perform a small screen that reveals how reduced FGF signaling induces a short-tail phenotype in embryos. We then demonstrate that embedding in Matrigel induces mouse gastruloids to generate somites, which appear sequentially in an anterior-to-posterior direction over time and display correct rostral–caudal patterning. Finally, we develop a first human version of this model system, and use microscopy and spatial transcriptomics to demonstrate that this system can be used to study anterior-posterior axis establishment in a human context. Altogether, our work shows the power of gastruloids as a model system for exploring embryonic development and somitogenesis in vitro in a high-throughput manner.

Keywords: gastruloids (embryonic organoids), embryonic development, somitogenesis

EPITHELIAL

MDD196

PATIENT-SPECIFIC IPSCS CARRYING AN SFTPC MUTATION REVEAL THE INTRINSIC ALVEOLAR EPITHELIAL DYSFUNCTION AT THE INCEPTION OF INTERSTITIAL LUNG DISEASE

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The incompletely understood pathogenesis of pulmonary fibrosis (PF) and lack of reliable preclinical disease models have been major hurdles in developing effective therapies. An emerging literature now implicates alveolar epithelial type 2 cell (AEC2) dysfunction as an initiating pathogenic event in the onset of a variety of PF syndromes, including idiopathic pulmonary fibrosis (IPF). Mutations in surfactant protein C (SFTPC), an AEC2-restricted gene, have been associated with both sporadic and familial PF. Elucidating the molecular pathogenesis of AEC2 dysfunction caused by these mutations is likely to inform the broader mechanisms by which AEC2 dysfunction leads to IPF. As access to primary AEC2s is limited and these cells are difficult to maintain in culture, we sought to engineer an in vitro disease model utilizing patient-specific induced pluripotent stem cells (iPSCs). We generated iPSC lines expressing the most common SFTPC mutation (SFTPCI73T) and their gene-edited, corrected counterparts. Through directed differentiation to alveolar epithelium, we derived mutant and corrected AEC2s (iAEC2s) which were able to expand >10^20 fold in vitro. SFTPCI73T expressing iAEC2s accumulated large amounts of misprocessed pro-SFTPC protein which mistrafficked to the plasma membrane, similar to the donor patient's in vivo AEC2s. These changes resulted in a time-dependent late block in autophagic flux, accumulation of dysfunctional mitochondria with consequent metabolic reprograming of mutant iAEC2s from oxidative phosphorylation to glycolysis, and marked alterations in self-renewal capacity. Treatment of SFTPCI73T expressing iAEC2s with hydroxychloroquine, a medication commonly prescribed to these patients, resulted in aggravation of autophagy perturbations and metabolic reprogramming. Thus, PSC-

derived AEC2s provide a patient-specific preclinical platform for modeling the intrinsic epithelial dysfunction associated with the inception of pulmonary fibrosis.

Keywords: alveolar epithelial type 2 cell (AEC2), pulmonary fibrosis, autophagy

MDD197

SINGLE NUCLEI RNA-SEQUENCING OF HUMAN COLITIS ASSOCIATED CANCER IN VITRO CO-CULTUERS

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Colitis-associated cancer (CAC) is a feared consequence of ulcerative colitis. Recent studies elucidate significant genetic changes in non-dysplastic colitis epithelia even after an inflammatory event subsides. Evaluating patient-derived epithelial organoids and fibroblasts, with high-resolution single cell analysis, permits unprecedented dissection of transcriptional expression. We hypothesize that co-cultures of CAC epithelial organoids with matched proximal uninvolved fibroblast will result in a more proliferative transcriptional profile and increased stemness. Matched patient derived epithelial organoids were cultured alone, or co-cultured with fibroblasts from an area of CAC, or with an area of proximal colon that was histologically normal. After 7 days of culture, the diameter of individual colonies (N = 150/sample) were measured as an indication of proliferation. Colony diameters were found to be significantly increased in both the epithelial/CAC fibroblast co-culture and epithelial/normal fibroblast co-culture vs epithelia alone ($p<0.0001$). Single-nucleus RNA-seq (sn-RNA-Seq) was performed (10x Genomics) with secondary analysis using Seurat. Epithelial cells were filtered and underwent further analysis. Both co-culture conditions showed increased expression of CD44, a cancer stem cell marker, when compared to the organoid alone ($p< 0.0001$). The CAC and CAC fibroblast co-culture had increased expression of ALCAM, a putative cancer stem cell marker. The proximal fibroblast co-culture condition showed a more proliferative expression profile with increased MYC and CCND1 expression when compared to co-cultures with CAC fibroblasts or organoids alone. Likely, this more proliferative transcriptional profile arises from the regenerative micro-environment present in histologically normal colitis samples. Further use of organoid co-culture systems will allow for further deconvolution of the transcriptional changes seen throughout the course of ulcerative colitis.

Funding source: R01 CA237304 and CTSC Core Pilot Grant 2018 Case Western Reserve University

Keywords: RNA-Seq, Ulcerative Colitis, Colon Cancer

MDD211

ORGANOIDS MODEL TRANSCRIPTIONAL HALLMARKS OF ONCOGENIC KRAS ACTIVATION IN MOUSE LUNG EPITHELIAL PROGENITOR CELLS

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Mutant KRAS is the most common oncogenic driver of epithelial cancers. Despite numerous studies of oncogenic RAS in cell lines and in vivo, the first molecular changes induced by RAS activation in primary epithelial cells remain unknown. Here, we took an unbiased approach to determine transcriptional changes at single-cell resolution after KRAS activation in distal lung epithelial cell populations in vivo. We then developed an organoid system to define the oncogenic KRAS transcriptional program and model lung cancer in vitro. In both approaches, lung alveolar epithelial progenitor cells expressing oncogenic KRAS lost their mature identity and acquired a program more similar to lung development and progenitor cells. While these transcriptional changes have previously been observed in advanced lung cancers in mice and humans, our studies revealed that they also occurred in early stage lung cancer in vivo. Thus, loss of alveolar differentiation is an early event in RAS-mediated tumorigenesis that has not been appreciated. This work provides a new organoid tool to rapidly recapitulate lung cancer progression in vitro and a window into the transcriptional changes that immediately follow oncogenic KRAS expression in epithelial cells, revealing candidate targets for early intervention of lung cancer and other KRAS-driven cancers.

Keywords: Organoids, Lung cancer, KRAS

EYE AND RETINA

MDD234

PROBING THE DIFFERENTIATION OF RETINAL GANGLION CELLS FROM HUMAN EMBRYONIC STEM CELLS WITH INDUCIBLE CRISPR INTERFERENCE

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Human embryonic stem cell (hESC)-derived retinal ganglion cells (hRGCs) are valuable in facilitating developmental studies, retinal disease modeling, and drug discovery efforts. However, the processes of how hESCs differentiate into hRGCs are not fully characterized. To understand the role of transcription and other factors in in vitro RGC differentiation, we utilized an Inducible CRISPR interference (CRISPRi) system to suppress their expression during differentiation. Based upon our previously described H9-BRX BRN3B/TdTomato hESC reporter line, we generated an inducible CRISPRi system consisting of an inducible dCas9-KRAB and a continuously expressed sgRNA (CRISPRi-hESC). We have used the system to target the endogenous ATOH7 and SOX11 genes. We generated H9-BRX cells with inducible expression of dCas9-KRAB, a catalytically dead Cas9 (dCas9) fused to the transcriptional repressor Krüppel-associated box (KRAB), under control of the Tet Response Element (TRE). Exogenously supplied doxycycline (dox) binds to the reverse tetracycline-controlled transactivator (rtTA) and drives expression of dCas9-KRAB by binding to its TRE promoter. We then infected these cells with lentiviral vectors containing sgRNAs complementary to a region proximal to the transcription start site (TSS) of the ATOH7 or SOX11 genes, under the control of a U6 promoter, and performed puromycin selection. We thus established cell lines ATOH7-CRISPRi-hESC and SOX11-CRISPRi-hESC. The addition of dox to culture media led to the suppression of endogenous ATOH7 or SOX11 gene expression during differentiation. In the absence of dox, ATOH7-CRISPRi-hESCs and SOX11-CRISPRi-hESCs differentiate efficiently into hRGCs which can be visualized by the expression of the tdTomato reporter under control of the RGC marker gene BRN3B. While adding dox to control CRISPRi-hESCs had no effect on RGC differentiation, adding dox to ATOH7-CRISPRi-hESCs and SOX11-CRISPRi- hESCs starting on day 6 of differentiation greatly reduced the generation of tdTomato+ RGCs. These results confirm the importance of ATOH7 and SOX11 in the in vitro differentiation of human embryonic stem cells into RGCs and provide a system to interrogate the gene regulatory networks governing RGC differentiation as well as the role of disease genes that affect human RGC development.

Keywords: hESC, RGC, differentiation

HEMATOPOIETIC SYSTEM

MDD242

ONTOGENY IS A CONSERVED, CRITICAL DETERMINANT OF NATURAL KILLER CELL POTENTIAL AND FUNCTION

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Human pluripotent stem cell (hPSC)-derived natural killer (NK) cells are an exciting “off the shelf” adoptive immunotherapy strategy, as they have been demonstrated to be potently cytotoxic in comparison to donor-derived NK cells. As the differentiation of hPSCs mimics early embryonic development, this raises the possibility that hPSC-derived NK cells are ontogenically distinct from adult NK cells. We have developed a stage-specific hPSC differentiation method that allows for exclusive specification of either WNT-independent (WNTi) HOXAneg CD34+ hematopoietic progenitors that harbor extra-embryonic-like hematopoietic potential, or WNT-dependent (WNTd) HOXA+ CD34+ multi-lineage definitive hematopoietic progenitors. Using this system, we find that CD34+ cells from both populations harbor NK cell potential. NK cells from WNTi progenitors (WNTi-NK cells) mature rapidly, are significantly more granular, and express very high levels of CD16 in comparison to their WNTd counterparts (WNTd-NK cells) and cord blood-derived NK (CB-NK) cells. Further, WNTi CD34+ progenitors always gave rise to a granulocyte population alongside NK cells, suggesting they may be derived from a myeloid progenitor. To assess their function, each population was stimulated by tumor targets, antibody-dependent cell-mediated cytotoxicity (ADCC), or PMA/ionomycin. In all cases, WNTi-NK cells exhibited a strong bias for cytolytic degranulation over cytokine production, while WNTd- and CB-NK cells were biased for IFNg secretion. Similarly, WNTi-NK cells exhibit superior killing of Raji cells in comparison to WNTd- and CB-NK cells. Critically, this was a conserved population that could be identified in the murine embryo. Using temporal-specific Csf1r-CreER labeling, we surprisingly identified that the yolk sac erythro-myeloid progenitor (EMP) harbors robust NK cell potential, and that these NK cells were functionally similar to WNTi-NK cells, as they exhibit a potent degranulation response in comparison to HSC-derived NK cells. Collectively, these studies suggest that ontogenic origin is an unexpectedly important consideration in the design of hPSC-derived NK cell-based therapeutics, and raise new questions regarding the potential of early hematopoietic progenitors in the mammalian embryo.

Keywords: natural killer cells, human pluripotent stem cell differentiation, hematopoiesis

MDD248

NOTCH ACTIVATION DURING MESODERMAL INDUCTION MODULATES EMERGENCE OF THE T/NK CELL LINEAGE FROM HUMAN IPSC

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A robust method of producing mature T cells from iPSCs is needed to realize their therapeutic potential. Notch 1 is known to be required for the production of definitive hematopoietic stem cells in vivo, leading us to hypothesize that activation of the Notch pathway during differentiation of iPSCs into hematopoietic progenitors would drive access to the T/NK cell lineage. Autonomous control of the Notch 1 receptor was simulated by inserting a Tet-On:NICD1 construct into the AAVS1 locus of four different iPSC lines. Using an optimized hematopoietic progenitor differentiation protocol followed by co-culture with OP9:hDLL4:hMHCII feeder cells, we found Notch activation from day 0-2 of differentiation yielded a robust increase in CD7+ cells by day 19 of differentiation (1 week of co-culture). The kinetics of Notch activation during differentiation suggested the improvement in T/NK lineage capacity is driven by Notch activation before it is normally induced during hematopoietic differentiation. T cell development was followed during differentiation with flow cytometry, leading to a CD7+/CD5+/CD8+/CD3+ population by 5-6 weeks of co-culture. We confirmed T cell identity with the expected upregulation of T cell genes such as BCL11b and robust proliferation of tetramer and PMA/ionomycin stimulated cultures at week 5 of co-culture. Our hematopoietic progenitors were also capable of differentiating into a natural-killer-like cell based on CD56 and NKp46 surface expression and the lysis of the OP9 feeder cells. The T/NK cell lineage decision was influenced by the plating density of hematopoietic progenitors into the co-culture system with low density favoring T cells. Single cell RNA sequencing at 5 time points from day 12 to 42 showed a clear developmental trajectory toward the T cell lineage, ending with both a T cell and NK cell cluster. The T cell cluster showed a highly similar transcriptional profile with human primary thymocytes. Comparing day 12 progenitors from dox treated and un-treated cultures showed that early Notch activation yielded a group of progenitors that were more homogeneous and expressed fewer lineage defining transcripts. We conclude that very early Notch activation during mesoderm induction yields hematopoietic progenitors with robust access to the T/NK cell lineage.

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Keywords: T cells, Human iPSC, Notch

IMMUNE SYSTEM

MDD254

MODELING HUMAN NATURAL KILLER CELL DEFICIENCY WITH PATIENT-DERIVED IPSCS

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Human natural killer (NK) cells are large granular innate lymphocytes that mediate anti-tumor and anti-viral responses. Patients with primary immunodeficiencies that primarily affect NK cell development or function have severe and recurrent viral infections and increased incidence of malignancy. These NK deficiencies (NKD) can be linked to specific genetic abnormalities and can impact NK cell differentiation and maturation. Unexpectedly, one pathway that has been demonstrated as important for NK cell development through the study of NKDs is the function of the DNA replisome. Our aim is to study the impact of mutations in DNA helicase complex members on the kinetics of NK cell development and maturation with single cell spatial and phenotypic resolution. NK cells are derived from common lymphoid precursors that are generated in the bone marrow but traffic to peripheral sites for further differentiation to functionally mature NK cells via discrete stages of developmental intermediates. Here, we model NK cell differentiation using iPSCs from NKD patients with helicase mutations, their family members, and healthy donors. We demonstrate that patient-derived iPSCs have delayed commitment to the hematopoietic lineage, and impaired maturation of NK cell developmental intermediates and terminally mature effector cells. Impaired development includes decreased expression of granzyme and perforin, which are associated with NK cell maturation. This poorly matured phenotype is reflected by decreased cytotoxic function of patient-derived NK cells compared to NK cells differentiated from healthy donor derived iPSCs. Our work highlights the potential of this method for studying impact of NK deficiency causing mutations on NK cell development in vitro. It additionally uncovers novel mechanisms by which perturbation of the replisome can impact human NK cell development.

Funding source: R01AI137275 to EMM

Keywords: Natural killer cells, Primary Immunodeficiencies, iPSCs

MDD256

NEUROINFLAMMATION AND INTEGRATED STRESS RESPONSE SIGNALING PERSIST IN A HUMAN IPSC TRI-CULTURE MODEL OF HIV INFECTION DESPITE ANTIRETROVIRAL TREATMENT

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HIV-Associated Neurocognitive Disorders (HAND) affect over half of HIV-infected individuals worldwide. While antiretroviral therapy (ART) has reduced the severity of HAND, the prevalence has increased due to increased life expectancy. Therapeutically targetable mechanisms underlying HAND remain elusive due to a lack of tools to study the direct interactions among HIV-infected microglia, neurons, and astrocytes. We developed a human-induced pluripotent stem cell (HiPSC) based model; whereby, we independently differentiate HiPSCs into neurons, astrocytes, and microglia and systematically combine to generate a tri-culture with or without HIV-infection and ART. scRNAseq analysis on tri-cultures including HIV infected iMg revealed inflammatory signatures and integrated stress response, specifically EIF2 signaling, in all three cell types; although, the microglia were most affected. ART mostly resolved these signatures but did not completely quell inflammation in the microglia and neurons. Remarkably, ART alone induced a similar response to infection. scRNA analysis also revealed activation of Fcγ receptor mediated phagocytosis pathway in infected iMg. However, there was a reduction in synaptic phagocytosis by infected microglia, suggesting that enhanced phagocytosis by HIV-infected microglia may not account for the synaptodendritic damage observed in HIV-infected patients. Pathway analysis also revealed increased RhoGDI and CD40 signaling in the HIV-infected microglia exposed to ART. This activation was associated with a persistent increase in TNFa expression. This work establishes an all human tri-culture that recapitulates key features of HIV infection in the CNS and provides a new model to examine the effects of HIV infection and its treatment with antiretroviral compounds in a multicellular context.

Keywords: HIV, iPSC, Anti-retroviral

MDD257

SNORA31 VARIATIONS IMPAIR HUMAN CORTICAL NEURON-INTRINSIC IMMUNITY TO HSV-1 AND UNDERLIE HERPES SIMPLEX ENCEPHALITIS

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Primary infection with herpes simplex virus type 1 (HSV-1) can precipitate herpes simplex encephalitis (HSE), the most common sporadic form of viral encephalitis in humans. Inborn errors of cell-intrinsic immunity in the central nervous system (CNS) can account for HSE, with defective TLR3- and DBR1-mediated immunity underlying forebrain and brainstem HSE, respectively. We report five unrelated patients with forebrain HSE, each carrying one of four rare heterozygous variants of SNORA31 (HSE cohort mutation enrichment p-value: 0.00029), which encodes a small nucleolar RNA (snoRNA) that is highly conserved in human populations. Two of these variants (including one found in two patients due to a hotspot) impair snoRNA31 production, whereas the other two impair its function

in another manner. SnoRNA31 is produced and functional in human CNS neurons, as a CRISPR/Cas9-introduced biallelic deletion in SNORA31 impairs pseudouridylation of the ribosomal RNA 18S uridine residue 218 in isogenic human ESC-derived CNS neurons. Moreover, snoRNA31 is a CNS neuron-intrinsic HSV-1 restriction factor, as CRISPR/Cas9-introduced SNORA31 biallelic and monoallelic deletions render neurons highly vulnerable to HSV-1. Accordingly, CNS cortical neurons derived from SNORA31-mutated patients' iPSCs are highly susceptible to HSV-1, like those from TLR3- or STAT1-deficient patients. Exogenous IFN-beta renders SNORA31- and TLR3- but not STAT1-mutated neurons resistant to HSV-1 infection. Finally, transcriptome analysis of the SNORA31-mutated hPSC-derived cortical neurons revealed normal responses to TLR3 and IFN-alpha/beta but abnormal response to HSV-1, suggesting that SNORA31 haplo-insufficiency impairs intrinsic immunity by a distinctive mechanism independent of known anti-viral signaling pathways. These experiments establish the essential role of human SNORA31 in CNS neuron-intrinsic immunity to HSV-1, thereby providing evidence that snoRNAs can be essential for host defense.

Keywords: Encephalitis, HSV-1, Cortical neuron-intrinsic immunity

MDD260

UNCOVERING THE CELL-TYPE SPECIFICITY IN HUMAN T1D IMMUNE ACTIVATION USING IPSC-β CELLS

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It is known that T1D involves a complex and destructive interaction between pancreatic β-cells and immune cells. Here, we show that exposing human induced pluripotent stem cells derived β cells (hiPSC-β cells) to autologous peripheral immune cells in vitro recapitulates aspects of T1D β cell rejection. We profiled innate and adaptive immune activated populations and identified relevant populations to T1D autoimmunity. After co-culture of donor-matched target and effector cells, we performed mass cytometry (CyTOF). We used an immunophenotyping CyTOF panel to identify activation in different immune populations. All experiments were performed with hiPSC-α cells and donor-matched peripheral immune cells, and we did not observe activation of immune cells in the hiPSC-α cells donor-matched coculture. Exposing T1D hiPSC-β cells to donor-matched peripheral immune cells in vitro revealed the activation of distinct immune cell populations that participate in T1D β cell rejection. We also observed differences between T1D patient samples suggesting the possible use of this approach to study T1D patient heterogeneity.

Funding source: Supported by American Diabetes Association

Keywords: Type 1 Diabetes, Autoimmunity, Autologous disease modeling

NEURAL

MDD292

PROBING THE ORIGINS OF LATE-ONSET ALZHEIMER'S DISEASE (LOAD) WITH THE IPSC PLATFORM: DO INHERENT CELLULAR METABOLIC ALTERATIONS DETERMINE RISK FOR LOAD?

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Alterations in bioenergetics and metabolism have been implicated both in the normal aging process and in the pathogenesis of late-onset Alzheimer's disease (LOAD). However, it is not known whether there is an inherent or only an acquired metabolic phenotype that predisposes to disease. The iPSC paradigm offers an opportunity to investigate mechanisms in LOAD pathology taking into account one's individual genotypic determinants in the context of cell development and functions. We used this cellular platform to investigate the bioenergetics and metabolic properties of LOAD patients' and healthy control individuals' iPSC-derived neural cell populations that play a role in brain development and metabolism, including neural progenitor cells (NPCs) and astrocytes. Our studies show that LOAD cells exhibit a number of key alterations in mitochondrial respiration and glycolysis, reduced levels of NAD/NADH, diminished glucose uptake and response rates to insulin (INS)/IGF-1 signaling, decreased INS receptor and glucose transporter 1 densities, changes in the metabolic transcriptome, deficiencies in activating the machineries to produce reducing agents, and shifts in using bioenergetic pathways in the glycolytic process or the mitochondrial respiratory chain. Altogether, these data suggest an inefficient cellular energy management in LOAD that is inherent and may already occur early in life and, thus, could be a predisposition to an altered aging process and a risk factor in disease development. The range of abnormalities is consistent with the known lack of single major inherited determinants for most cases of LOAD. In addition, our studies demonstrate the use of the iPSC paradigm as a platform to detect core mechanisms in aging and in the pathogenesis of LOAD and its potential as a "personalized cell system" to develop novel diagnostic and/or therapeutic strategies.

Funding source: Program for Neuropsychiatric Research, Steel Fund

Keywords: induced pluripotent stem cells iPSC, Alzheimer's disease and aging, Bioenergetics and Metabolism

MDD294

THE AUTISM RISK FACTOR CHD8 IS A CHROMATIN ACTIVATOR AND FUNCTIONALLY DEPENDENT ON THE ERK-MAPK PATHWAY EFFECTOR ELK1

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The chromodomain helicase DNA binding protein CHD8 is among the most frequently found de-novo mutations in autism [1-3]. Unlike other autism-risk genes, CHD8 mutations appear to be fully penetrant [4]. Despite this prominent disease involvement, relatively little is known about its molecular function. Based on sequence homology, CHD8 is believed to be a chromatin regulator but mechanisms for its genomic targeting and its function on chromatin are unclear. Here, we developed a human cell model carrying conditional CHD8 loss-of-function alleles. Remarkably, while undifferentiated human embryonic stem (ES) cells required CHD8 for survival, postmitotic neurons survived following CHD8 depletion. High-resolution nucleosome mapping revealed that CHD8 is a highly potent and general chromatin activator, enhancing transcription of its direct target genes. We further established that a subset of neuronal activity-induced genes requires CHD8 for activation. We also found that CHD8 genomic binding in human neurons was significantly enriched at Elk1 DNA binding motifs as previously found in other cell types [5]. Given its prominent role as effector molecule of the ERK-MAPK pathway, we decided to further explore its relationship with CHD8. Elk1 motif-containing CHD8 binding sites showed a higher degree of chromatin opening function of CHD8 than other CHD8 binding sites. Moreover, Elk1 was required for CHD8 binding to Elk1-containing sites, but not other sites. Finally, the anti-apoptotic function of CHD8 in human ES cells could be rescued by depletion of Elk1 and the enhancement of neurogenesis by Elk1 was dependent on the presence of CHD8. In summary, our results establish a clear role of CHD8 for chromatin opening and transcriptional activation and a molecular and functional interdependence of CHD8 and Elk1. These data imply the involvement of the ERK-MAPK pathway effector Elk1 in the pathogenesis of autism caused by CHD8 mutations.

Keywords: Disease modeling, Chromatin remodeling factor, Autism

MDD299

MULTIWELL MICROELECTRODE ARRAY (MEA) TECHNOLOGY FOR THE QUANTIFICATION OF NEURONAL, SYNAPTIC, AND NETWORK FUNCTION FOR IN VITRO STEM CELL DERIVED NEURONAL MODELS

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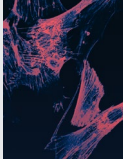
The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of neuron electrophysiology to be used in screening applications in drug discovery and safety. Furthermore, advanced cells preparations, such as spheroids or organoids, are under intense investigation with aims towards establishing mature human phenotypes in vitro. For the development and validation of in vitro neuronal models, it is critical to evaluate the function of neurons, synapses, and networks. Here, we present data supporting the use of multiwell microelectrode array (MEA) technology as an efficient and intuitive approach for the reliable quantification of neuronal function. Action potentials are the defining feature of neuron function, and were quantified as the Activity endpoint, with high levels of Activity reflecting frequent action potential firing and low levels of Activity indicating impaired neuronal function. Synchrony reflects the prevalence and strength of synaptic



connections, and thus how likely neurons are to generate action potentials simultaneously on millisecond time scales. Finally, Oscillations, defined by alternating periods of high and low activity, are a hallmark of functional networks with excitatory and inhibitory neurons. Oscillation is a measure of how the spikes from all of the neurons in a well are organized in time. High values indicate that the network exhibits bursts of action potentials interspersed with periods of relative quiescence. Low values indicate action potentials are not coordinated across neurons in the network. In this abstract, we present case studies illustrating how these three parameters can quantify neural circuit function across various culture model systems and applications, including the development, optimization, and validation of disease-in-a-dish models of neuronal disorders. These results support the continued development and use of human iPSC-derived neural assays on multiwell MEA technology for high throughput drug discovery and safety assessment.

Keywords: Electrophysiology, Disease-in-a-Dish, Oscillations

MDD303

ROLE OF THE TRANSCRIPTIONAL REPRESSOR, REST, IN HUMAN NEURONAL AGING

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Neurons, as post-mitotic cells, must find a way to survive and perform their functions throughout an organism's entire lifetime. Precise regulation of gene expression plays a critical role in maintaining neuronal function for a healthy brain. Accumulating evidence indicates very little overlap in gene expression patterns between the mouse and human brain, manifested during ageing, suggesting that mechanisms required for maintaining a healthy brain are fundamentally different in these species. Consistent with this idea, our lab has identified completely opposing age-dependent expression patterns of a unique neuronal transcription factor, RE1 Silencing Transcription Factor (REST) in mouse and human brain. REST represses hundreds of genes in both species by virtue of binding to a consensus sequence called Repressor Element 1 (RE1). Genes containing RE1 or related RE1 sequences encode proteins that as an ensemble are required for the terminally differentiated neuronal phenotype. We find that in mice, REST levels are highest in early adulthood and decline with age, whereas in humans REST levels are low during early adulthood, increase by around the end of the third decade of life and remain elevated even in nonagenarians, suggesting a specialized role for REST in the ageing human brain. Recently, it has been suggested in studies by others that REST is neuroprotective during human brain ageing, a compelling hypothesis that has not been independently tested. Here, I re-examine the role for REST in neuronal ageing using induced human neurons derived by direct cellular reprogramming of human fibroblasts that maintain age-associated epigenetic and cellular signatures. The findings from this study will provide new insights into how neuronal health is maintained in the adult human brain.

Keywords: Neuronal aging, Direct neuronal reprogramming, Epigenetics

MDD305

UNDERSTANDING THE IMPACT OF LOAD-ASSOCIATED AD GENETIC VARIANTS ON CLEARANCE OF Aβ USING IPSC-DERIVED ASTROCYTES

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized by extracellular amyloid-beta (Aβ) plaques and intracellular tau tangles. Late-onset AD (LOAD) accounts for more than 90% of AD cases and is thought to involve decreased Aβ clearance. The strongest genetic risk factor for LOAD is APOE, a gene that encodes apolipoprotein that interacts with Aβ and regulates its clearance. Despite the identification of multiple LOAD associated genes, the mechanism by which these genes are involved in Aβ clearance in human astrocytes is unclear. Here, we have utilized induced pluripotent stem cells (iPSCs) from individuals with diverse genetic backgrounds (with and without LOAD) to generate astrocytes and established an in vitro system to assess Aβ clearance in astrocytes. First, we generated astrocytes from three groups of iPSCs using two differentiation protocols (EB derived and induced astrocytes): iPSCs with different APOE isoforms, SORL1 (a LOAD risk gene) KO and WT iPSCs, and iPSCs derived from ROSMAP, a LOAD cohort with individuals with varying amyloid levels and cognition. To measure the Aβ clearance ability of the astrocytes, synthetic or cell-derived Aβ were applied to astrocytes, and both the extracellular and intracellular Aβ levels were monitored to assess the uptake and degradation of Aβ. Our results indicate that APOE KO astrocytes showed elevated Aβ level in the media compared to lines with different APOE genotype, suggesting a role of APOE in regulating Aβ clearance. Furthermore, SORL1 KO astrocytes also showed impaired Aβ clearance, which suggests a potential role of LOAD-associated genes in Aβ clearance. We have established an in vitro system to assess Aβ clearance in iPSC-derived astrocytes. Utilizing astrocytes from the LOAD cohort (ROSMAP), this will be a useful system that can reveal interactions between LOAD risk SNP profiles and Aβ clearance.

Funding source: NIH R01AG055909

Keywords: Alzheimer's disease, Astrocytes, APOE

MDD306

SINGLE CELL ANALYSIS REVEALS LINEAGE DIVERSITY IN INNER EAR ORGANOIDS GENERATED FROM HUMAN PLURIPOTENT STEM CELLS

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Inner ear development requires the complex interaction of numerous cell types arising from multiple lineages. Much of the current knowledge gained from research on inner ear development has been derived from animal studies; thus, the cellular and molecular idiosyncrasies of human inner ear development is a current gap in knowledge. Our group recently showed how to recapitulate human inner ear organogenesis in vitro using 3D self-organizing stem cell cultures. Our method promotes development of an entire sensorineural circuit, including hair cells, inner ear-like neurons, and Schwann cells. Although we have characterized many of these cell types, we have not fully defined the process by which individual cells commit to lineages represented in inner ear organoids. Our goal here is to construct a map of the cell lineage diversity during inner ear organoid development. We analyzed inner ear organoids using single-cell RNA sequencing at various time points during the first two weeks of differentiation. We identified expression patterns of inner ear organoids as they progress from pluripotent stem cells to surface ectoderm then to otic placode following treatment with FGF, BMP, and WNT signaling modulators. One major cell lineage we have analyzed is neural crest. In our datasets, we see development of a SOX10-positive neural crest cell population which then bifurcates into mesenchymal and glial lineages. Additionally, we have categorized the neuronal diversity contained in our organoids. There are distinct neuronal populations as well as a quantity of cells expressing transcripts consistent with neural tube. By elucidating organoid developmental lineages, we have created a platform upon which to perturb developmental signaling mechanisms. Further research will allow the development of a platform to model genetic inner ear disease using gene editing as well as test new therapeutics.

Funding source: NIH grants R01 DC017461 and F30 DC018715

Keywords: inner ear, organoid, lineage tracing

MDD328

POSTNATAL INTERNEURON MIGRATION CONTRIBUTES TO THE FINAL STAGES OF CORTICAL DEVELOPMENT IN THE PIGLET BRAIN

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Gabaergic inhibitory neuron (interneuron) migration persists in the human frontal lobe for several months after birth. Such extensive postnatal interneuron migration does not exist in species such as mus musculus. Thus, the molecular composition and cortical targets of these unique interneurons become important, as they mediate the development of higher-level cortical gyration and circuitry. Herein, we used the domestic pig (Sus scrofa domesticus) as a gyrencephalic model to study postnatal cortical development. As observed in the neonatal human cortex, migratory interneurons organized themselves into a four-tiered structure at the dorso-lateral wall of the lateral ventricle. Analysis of embryonic day 89 (E89), E100, and E115 (or postnatal day 0 (P0)) piglet brains revealed that the tiers were not present until E100 and this organization was more clearly observed at P0. This suggests that these migratory corridors are unique to the perinatal period, immediately before and after birth. We mapped doublecortin (DCX) positive neurons in P0 piglet brains and confirmed the presence of frontal migratory streams, as observed in human, which target the cingulate gyrus and superior frontal gyrus. Interestingly, we also found streams of DCX+ young migratory neurons in previously unidentified areas such as the insular gyrus and superior temporal lobe. Using region-specific transcription factors NKX2.1, LHX6, NR2F2, and SP8, we observed that the postnatal migratory interneurons can be medial or caudal ganglionic eminence (MGE or CGE) in origin, with CGE (NR2F2+ or Sp8+) origin being predominant. These findings suggest that postnatal migratory interneurons, particularly CGE-derived ones, play a significant role in the final stages of cortical development in gyrencephalic species. Our study will facilitate the understanding of higher species normal cortical development and its clinical ramification in neurological disease pathophysiology.

Funding source: K08 Ninds Clinical Training Grant NARSAD Young Investigator Award The Roberta and Oscar Gregory Endowment in Stroke and Brain Research

Keywords: Postnatal cortical development, Inhibitory neuron migration, Gyrencephalic model

MDD342

SYNTHETIC ANALYSES OF SINGLE-CELL TRANSCRIPTOMES FROM MULTIPLE BRAIN ORGANOIDS AND FETAL BRAIN

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Human brain organoid systems offer unprecedented opportunities to investigate both neurodevelopmental and neurological disease. Single cell-based transcriptomics or epigenomics have dissected the cellular and molecular heterogeneity in the brain organoids, revealing a complex organization. Similar but distinct protocols from different labs have been applied to generate brain organoids, providing a large resource to perform a comparative analysis of brain developmental processes. Here we take a systematic approach to compare the single cell transcriptomes of various human cortical brain organoids together with fetal brain to define the identity of specific cell types and differentiation routes in each method. Importantly, we identify unique developmental programs in each protocol, which will be a critical benchmark for the utility of human brain organoids in the future.

Funding source: NIH (GM111667-01, R01MH118344-01A1, R01AA025080-01, R01CA203011-2), CSCRF (14-SCC-YALE-01, 16-RMB-YALE-04), Kavli Foundation, Simons Foundation, and the Research Council of Norway (project number 262613).

Keywords: Brain organoid, Single-cell RNA-seq, Fetal Brain

MDD352

UNDERSTANDING SHIP1 EXPRESSION AND FUNCTION IN THE HUMAN BRAIN

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects memory and other cognitive functions. Recent studies suggest that the resident immune cells of the brain, microglia, may play a more active role in neurodegeneration either through microglia engulfing synapses or from the secretion of pro-inflammatory cytokines as a result of microglia responding to elevated Aß. Thus, understanding the regulation of microglial activation is critical towards illuminating the role of microglia in the adult human brain and in the disease state. SHIP1, or SH2-domain containing inositol polyphosphate 5-phosphatase 1, is a protein that has been identified in GWAS studies to be associated with late-onset forms of Alzheimer's disease. In peripheral immune cells, SHIP1 has been shown to dephosphorylate phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P3] to generate phosphatidylinositol-3,4-bisphosphate [PI(3,4)P2]. However, the expression and function of SHIP1 is largely unknown in the brain. Elucidating the role of SHIP1 in microglia would not only offer more insight into the dynamic regulation of microglia in the non-disease state, but also may advance our understanding of AD pathogenesis. Through RNA sequencing and Western blotting, we have demonstrated that SHIP1 expression is restricted to human microglia in both postmortem brain and human iPSC-derivatives. Further, immunostaining in human brain tissue has confirmed that SHIP1 expression is limited to human microglia. Finally, we have generated human iPSC-derived microglial models of SHIP1 loss-of-function, and are now characterizing how reduction in SHIP1 activity affects microglial cells.

Funding source: NIA NIH F31 AG063398 NIA NIH R01 AG055909

Keywords: Microglia, Alzheimer's Disease, SHIP1

MDD358

THE GROWTH DYNAMICS OF HUMAN NEURAL PRECURSOR CELLS REVEALED THROUGH TIMELAPSE IN VIVO IMAGING

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While the structural changes that human neurons undergo during development are key to understanding neural circuit function, there are currently limited ways to study these dynamic processes. To address this challenge, we performed timelapse in vivo two-photon imaging of GFP-labeled human neural precursor cells (hNPCs) transplanted into the visual cortex of a mouse to characterize the growth dynamics of living human neurons (N = 15 animals). Imaging was performed with either short time intervals between imaging sessions (~1 hour) to assess rapid dynamics occurring within a day or with much longer time intervals between sessions (~1 week) to examine long-term dynamics over the course of 2-4 months. When we imaged the hNPCs shortly after transplantation, we observed two general types of transplants: "distributed" transplants contained a large number of cells covering a large fraction of the imaging area while "clustered" transplants contained far fewer cells confined within a smaller area. In animals with distributed transplants, we observed extensive cell proliferation and migration. Migrating cells tended to travel towards and accumulate around blood vessels, with neurites surrounding and ultimately ensheathing these vessels. In contrast, clustered transplants displayed less cell proliferation and migration; in these animals, we tracked individual neurites over time and observed remarkable structural dynamics including neurites that rapidly extended and retracted, neurites that underwent slow degeneration over the course of days, and neurites that remained structurally stable. In general, neurites tended to radiate outwardly from the transplant site and travel horizontally within superficial cortical layers, often forming fascicles with other processes and sometimes growing along blood vessels. Additionally, we observed neurites that extended ventrally and travelled towards deep cortical layers. Taken together, our findings suggest that while transplant type may influence the growth dynamics of transplanted hNPCs, a common feature of all the transplants is the potential role of the host vasculature, which may serve as an energy source that supports the dynamics of developing neurons in need of metabolic support.

Funding source: NIH grant MH113924 (to KP).

Keywords: Neural precursor cells, Transplantation, In vivo imaging

MDD367

TREATING BIOENERGETIC DEFICIENCIES IN IPSCS DERIVED BRAIN CELLS FROM LATE-ONSET ALZHEIMER'S DISEASE PATIENTS USING NICOTINAMIDE RIBOSIDE AND CAFFEINE

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Bioenergetic and metabolic alterations have been associated with both the normal aging process and with late-onset Alzheimer's disease (LOAD). In a series of studies, we have consistently found that cells from LOAD patients, including dermal fibroblasts and iPSC-differentiated neural progenitor cells (NPCs) as well as astrocytes, all exhibit reduced levels of nicotinamide adenine dinucleotide (NAD), which constitutes the major oxidizing agent in cellular redox reactions. In addition, LOAD cells showed alterations in key bioenergetic functions, such as glycolysis and mitochondrial respiration, which suggests inefficient energy management in LOAD as a consequence of impairments in the production of key bioenergetics substrates, such as NAD. We, therefore, evaluated whether NAD could be a metabolic target for treatment and tested the effects of nicotinamide riboside (NR), which is a dietary precursor for NAD de novo synthesis, and caffeine, which has been shown to increase the expression of NMNAT2, an essential enzyme in NAD production, using the LOAD iPSC paradigm. Our results reveal that combined treatment with NR and caffeine boost NAD levels and functionally increase mitochondrial respiration but not glycolysis. These increases were transient, lasting for about 8 hours, after which the bioenergetic cell functions start to decline and eventually show reverse effects. The response rates to NR and caffeine were cell-type specific: astrocytes demonstrated the strongest dynamic responses, which were overall less pronounced in LOAD cells when compared to control cells. Altogether, our data demonstrate that cellular bioenergetic deficiencies as a consequence of diminished NAD substrate availability can temporarily be normalized with NR and caffeine substitution in a cell-type specific manner, in both healthy and LOAD-associated cells. While the consequences of such treatment on the aging process and as a therapeutic avenue in LOAD needs further investigation, our studies demonstrate the use of the iPSC paradigm as a platform to not only detect core mechanisms in aging and in the pathogenesis of LOAD but also as a potential "personalized cell system" to develop or test novel diagnostic and/or therapeutic interventions.

Keywords: Alzheimer's disease, NAD+/NADH, nicotinamide riboside

MDD368

USING BRAIN ORGANOIDS TO MODEL THE SCN8A MUTATION RELATED EARLY INFANTILE EPILEPTIC ENCEPHALOPATHY 13

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Early Infantile Epileptic Encephalopathy 13 (EIEE13) is a severe developmental epileptic encephalopathy that causes seizures, intellectual disabilities, and a heightened risk of sudden unexpected death in epilepsy. EIEE13 is caused by a gain of function mutation in the SCN8A sodium channel gene. SCN8A encodes for the Nav1.6 voltage-gated sodium channel subunit which is expressed highly in the central nervous system. Particularly in areas in the brain such as the hippocampus, cerebellum, and the cortex. The Nav1.6 subunit takes part in the excitability of neurons and in the initiation and propagation of action potentials. Therefore, a mutation in SCN8A can potentially cause changes in action potentials. Today, there is a limited understanding of the impact of the SCN8A mutation on neuronal network activity in distinct regions of the human brain. In this study, we aim to focus on the effects of neurons and their networking activity in brain regions that are caused by this mutation. Here, we sought to develop a brain organoid model for EIEE13 starting with iPSCs from a patient that harbored the SCN8A mutation. We generated hippocampus and cortex (dorsal forebrain) organoids. Results have shown that these structures do not have gross structural differences compared to controls. We are currently employing calcium indicator imaging and extracellular recordings of local field potentials to determine the effects of the SCN8A mutation on hippocampal and cortical physiological activity.

Keywords: Organoid, Neurology, Epilepsy

MDD369

SCALABLE DIFFERENTIATION OF HUMAN IPSC-DERIVED PSEUDO-UNIPOLAR NOCICEPTORS WITH IN VIVO-LIKE PAIN INITIATING PROPERTIES AND TRANSLATIONAL APPLICABILITY

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Development of new non-addictive pain medications requires advanced strategies to differentiate human induced pluripotent stem cells (iPSCs) into relevant cell types amenable for disease modeling and drug discovery. Here, we devised a highly efficient and scalable protocol that differentiates iPSCs exclusively into nociceptors under chemically defined conditions. By manipulating developmental pathways using small molecules, iPSCs were first converted into SOX10+ neural crest cells followed by differentiation into bona fide pseudo-unipolar BRN3A+ nociceptors. Detailed molecular and cellular characterization and single-cell analysis confirmed that the differentiated nociceptors expressed the neuronal markers, transcription factors, neuropeptides and over 150 ion channels and receptors expressed by primary nociceptors. Focusing on pain-relevant receptors and channels expressed by iPSC-derived nociceptors (e.g. P2RX3, TRPV1, NAV1.7, NAV1.8), we demonstrated robust functional activities and differential response to noxious stimuli and specific drugs and suitability for phenotypic screens. Lastly, a robotic cell culture system was used to automate the production of billions of cryopreservable cells for high-throughput drug screening, urgently needed to develop new nociceptor-selective analgesics and help to tackle the opioid crisis.

Funding source: NIH Common Fund, NIH HEAL Initiative

Keywords: nociceptors, pain, opioid crisis

MDD377

STEM CELL MODELS OF PRIMARY TAUOPATHIES REVEALS DEFECTS IN SYNAPTIC FUNCTION

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Primary tauopathies are characterized neuropathologically by inclusions containing abnormal forms of the microtubule-associated protein tau (MAPT) and clinically by diverse neuropsychiatric, cognitive, and motor impairments. Autosomal dominant mutations in the MAPT gene cause heterogeneous forms of frontotemporal lobar degeneration with tauopathy

(FTLD-Tau). Common and rare variants in the MAPT gene increase the risk for sporadic FTLD-Tau, including progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). We generated a collection of fibroblasts from 140 MAPT mutation/risk variant carriers, PSP, CBD, and cognitively normal controls; 31 induced pluripotent stem cell (iPSC) lines from MAPT mutation carriers, non-carrier family members, and autopsy-confirmed PSP patients; 33 genome engineered iPSCs that were corrected or mutagenized; and forebrain neural progenitor cells (NPCs). To begin to identify the genes and pathways that are dysregulated in primary tauopathies, we performed transcriptomic analyses in induced pluripotent stem cell (iPSC)–derived neurons carrying MAPT p.R406W and CRISPR/Cas9-corrected isogenic controls. We found that the expression of the MAPT p.R406W mutation was sufficient to create a significantly different transcriptomic profile compared with that of the isogeneic controls and to cause the differential expression of 328 genes. Sixty-one of these genes were also differentially expressed between MAPT p.R406W carriers and control brains. Twelve of these genes are also differentially expressed between PSP and control brains. Together, these genes are enriched for pathways involved in GABA-mediated signaling and synaptic function, which may contribute to the pathogenesis of FTLD-tau and other primary tauopathies. Here, we present a resource of fibroblasts, iPSCs, and NPCs with comprehensive clinical histories that can be accessed by the scientific community for disease modeling and development of novel therapeutics for tauopathies.

Funding source: Tau Consortium, National Institutes of Health

Keywords: neurodegeneration, stem cell models, genomics

MDD450

MECHANOSENSORY CHANNEL ACTIVATION IN THE PATHOGENESIS OF TRAUMATIC BRAIN INJURY USING HUMAN IPSC-DERIVED CORTICAL BRAIN ORGANOIDS

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Traumatic brain injury (TBI) represents a major environmental risk factor for dementia and its incidence is on the rise, with over 3.5 million annual cases in the United States alone. Pathologic responses following TBI have been well characterized and include neuronal aggregates of misfolded, hyperphosphorylated tau and TDP-43, axonal injury, astrogliosis, excitotoxicity, metabolic dysfunction and progressive neural degeneration. However, how acute mechanical injury contributes to long-term neurodegeneration and dementia is poorly understood, in part due to differences between current rodent and cellular TBI models with degenerative and biophysical properties of the human brain, respectively. As a result, available treatment options remain limited and are largely ineffective. To address these issues, we have developed a unique system of focused ultrasonic injury to mimic TBI in vitro using a 3-D cortical organoid culture system grown from human induced pluripotent stem cells (iPSCs) which retains key aspects of human cellular diversity and cytoarchitecture. Our results show that mechanically injured organoids recapitulate key hallmarks of TBI in vivo, including increased phosphorylated tau and TDP-43 aggregation, metabolic dysfunction, and neurodegeneration. We find that injury activates a gene expression program indicative of mechanosensory channel (MSC) activation, and that pharmacologic inhibition of MSCs and their downstream targets partially rescues injury-induced phenotypes. Moreover, we find that iPSC-organoids derived from frontotemporal dementia patients with a V337M disease-causing mutation in MAPT produce higher levels of phosphorylated tau and TDP-43 upon mechanical injury compared to CRISPR-corrected isogenic controls. This suggests that the MAPT mutation sensitizes neurons to degenerative mechanisms in TBI. Together, these studies enhance the current understanding of TBI pathogenesis by establishing MSC activation as a potential therapeutic target and provides a platform to integrate environmental and genetic contributions to neurodegeneration while preserving human-specific biology.

Keywords: Traumatic Brain Injury, Neurodegeneration, Organoids

MDD454

MOLECULAR SCREENING OF FDA-APPROVED DRUGS IN HUMAN PLURIPOTENT STEM CELLS FOR THE TREATMENT OF RARE MONOGENIC DISEASES

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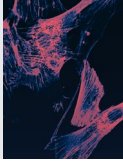
Given the high attrition rates, substantial costs and slow pace of new drug discovery and development, repurposing of 'old' drugs to treat both common and rare diseases is increasingly becoming an attractive proposition. In this study, we evaluated



the potential of using human pluripotent stem cells to identify repurposable drug candidates. For this purpose, we treated derivatives from human embryonic stem cells with 50 marketed drugs and we annotated the induced molecular changes by RNA deep sequencing. Focusing on genes previously involved in monogenic diseases, we identified drugs capable to modulate the expression of PLP1, PMP22, LMNB1 and p62/SQSTM1 genes known to be involved in Pelizaeus-Merzbacher disease (PMP), Hereditary neuropathy with liability to pressure palsies (HNPP), Adult-onset autosomal dominant leukodystrophy (ALDL) and Amyotrophic lateral sclerosis (ALS) respectively. We focused on ALS, a neurodegenerative disease characterized by progressive muscular paralysis reflecting degeneration of motor neurons in the primary motor cortex, corticospinal tracts, brainstem and spinal cord. Most ALS cases are sporadic but 5-10% of cases are familial, and involve a mutation in the SOD1, TARDBP, C9orf72, OPN or p62/SQSTM1 gene. We treated motor neurons differentiated from human embryonic stem cells and we showed that one of the drugs identified was capable to upregulate p62/SQSTM1, to promote its aggregation in puncta, appearing during autophagosme formation. To further explore this therapeutic potential, we have generated p62+/- and p62-/- human pluripotent stem cells by using CRISPR-Cas9 technology. Thanks to these cellular tools, we highlighted the impairment of autophagy in p62+/- and p62-/- motor neurons. Treatment of the cells with the drug was able to restore autophagy. We next tested the potential of the drug in a zebrafish model of ALS caused by p62 knockdown. In vivo treatment improved the swimming of fishes without the induction of toxicity. Altogether, these results identify a FDA-approved molecule capable to modulate the autophagy pathway in human motoneurons through the induction of p62/SQSTM1. These data raise the question of the therapeutic potential of this molecule both for ALS but also for other neuromuscular disease associated with abnormal protein aggregation.

Funding source: France Genomique. AFM Téléthon.

Keywords: Drug repurposing, Amyotorphic Lateral Sclerosis (ALS), Motor neuron

MDD488

GENERATION OF HUMAN ASTROCYTE CELL LINES TO MODEL FRAGILE X SYNDROME

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Fragile X syndrome (FXS) is the most common familial form of mental retardation and autism. FXS is caused by the lack of functional fragile X mental retardation protein (FMRP) due to epigenetic silencing of the Fmr1 gene leading to changes in the synaptic plasticity and unbalanced neuronal network function. Recently, an increasing number of studies has also linked astrocyte malfunction to FXS. Astrocytes form functionally and morphologically distinct populations of cells with both brain region- and species-specific properties. In here, we describe a differentiation method that can be used to generate iPSC-derived human astrocyte cultures with forebrain identity. The induction of neural differentiation was accomplished using dual SMAD inhibition combined with Dickkopf-Related Protein 1- and cyclopamine treatments to inhibit hindbrain and midbrain patterning, respectively. Generated progenitors were directed towards astrocyte lineage with ciliary neurotrophic factor. The cells were characterized for their gene expression profiles during the differentiation. Transcriptomic analysis confirmed that early forebrain patterning was successful and stayed active during the protocol. Both control and FXS cells followed similar pattern of differentiation and generated astrocytes clustered with previously published embryonic human astrocytes. The cells expressed selected astrocyte markers and calcium imaging analysis showed that in both control and FXS astrocyte lines 80 – 90 % of the cells were responsive to ATP. Though FXS phenotype did not disrupt with the astrocyte differentiation, FXS astrocytes had changes in their transcriptomic profile. Furthermore, astrocyte-conditioned medium from FXS cells was seen to have an effect neuronal activity. The differentiation protocol, therefore, allows the generation of functional patient-derived astrocyte cultures that can be used to study the mechanisms underlying impaired astrocyte function in FXS.

Keywords: Astrocyte, Differentiation, Fragile X syndrome

MDD490

IPSC-BASED MODELING OF THE CELLULAR AND MOLECULAR MECHANISMS UNDERLYING THE COMPLEX PHENOTYPE OF HEREDITARY SPASTIC PARAPLEGIA TYPE 11

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Hereditary spastic paraplegia (HSP) is a heterogeneous group of rare motor neuron disorders characterized by progressive weakness (paraplegia) and spasticity of the lower limbs. HSP type 11 (SPG11-HSP), the most common form of complex autosomal recessive HSP, is accompanied by thin corpus callosum, white matter abnormalities, and progressive cognitive decline. To characterize the complex, disease-associated impairments and to study the involved mechanisms, we differentiated patients' induced pluripotent stem cells (iPSC) into 2D and 3D neural models. Our results reveal that premature neurogenesis underlies

the pathology. Namely, SPG11-HSP-derived neural progenitor cells exhibit an increased rate of asymmetric divisions, which in turn leads to a higher neuroblast number. The neuroblasts differentiate into defective neurons characterized by increased cell death and reduced neurite complexity. The mechanism is linked to increased activity of GSK3β. Correspondingly, GSK3 inhibitors rescued the majority of the observed impairments. To elucidate further the mechanistic aspects at the core of the pathology, and due to a lack of specific antibodies, we have employed the CRISPR-Cas9 genome editing technique to generate endogenously tagged SPG11-neural reporter lines. These new lines provide an indispensable model for future studies of the SPG11 linked pathology.

Keywords: Cerebral Organoids, CRISPR-Cas9, Motor Neuron Disorder

NEW TECHNOLOGIES

MDD390

OSTEOSARCOMA MODELING USING INDUCED PLURIPOTENT STEM CELLS

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Osteosarcoma (OS) is the most common bone cancer in adolescents and young adults. Long term survival is 70% for localized disease, but only 30% if distal metastases are present at the time of diagnosis. Despite progress in our understanding of OS biology, current treatments have changed little in decades and continue to rely on tumor resection and combination chemotherapy. Only prototypical cancer genes, such as TP53, RB1, and MYC have been definitely implicated in OS; with TP53 being dysregulated in >90% of all OS patients. Additional candidate driver genes have been nominated, however definitive validation is hindered by a lack of accurate human OS models. To address this challenge, we have employed human induced pluripotent stem cells (iPSC) and genome engineering to model OS development. Using CRISPR-Cas9, we installed foundational TP53 and RB1 mutations in human iPSC and differentiated these cells along mesenchymal (MSC) and osteoblast (OB) lineages. Functional assays were conducted at intermediate stages of development to determine the contribution of genotype and developmental status on transformation. Compared to isogenic WT controls, TP53-/- and TP53-/-/RB1-/- OB exhibited increased proliferation that was magnified by overexpression of cMYC and hRAS. In colony formation assays, the capacity for anchorage independent growth was observed in TP53-/-/RB1-/-, but not TP53-/- or WT OB. Overexpression of cMYC and hRAS significantly increased colony formation by TP53-/-/RB1-/- OB. In contrast to OB, TP53-/-/RB1-/- MSC did not form colonies without cMYC and hRAS overexpression. To evaluate this phenomenon in vivo, engineered MSC and OB were injected into the calcaneal bones of immunodeficient mice. Both TP53-/-/RB1-/- and TP53-/-/RB1-/-+cMYC/hRAS OB formed tumors, with the latter showing accelerated tumor formation and growth. Consistent with our in vitro observations, only TP53-/-/RB1-/-+cMYC/hRAS MSC gave rise to tumors. Studies are underway to determine how closely these engineered iPSC-derived tumors mimic patient-derived OS tumors. Collectively, our results illustrate the feasibility of modeling pediatric sarcomas using human iPSC and demonstrate the utility of this strategy by uncovering developmental-stage specific differences in permissiveness to transformation.

Keywords: Genetic engineering, induced pluripotent stem cells, cancer modeling

MDD407

THE NINDS CELL AND HUMAN DATA REPOSITORY - A STEM CELL RESOURCE FOR THE GLOBAL COMMUNITY

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Since its inception in 1998, RUCDR Infinite Biologics (RUCDR, www.rucdr.org) has provided the global scientific community with the highest quality biomaterials, technical consultation, and logistical support. In 2011, with rising interest in induced pluripotent stem cells (iPSC) models of human development and disease progression and for drug screening and toxicology testing RUCDR began offering a wide range of stem cell services. These include source cell and iPSC banking, iPSC generation, iPSC gene editing and source cell and iPSC distribution. RUCDR maintains the NINDS Cell and Human Data Repository (NHCDR), which houses fibroblasts, iPSC and clinical data nearly 400 subjects. These cell lines are available to academic and for-profit researchers worldwide and can be viewed on the NHCDR website (https://bioq.nindsgenetics.org/). After completing an MTA (also available online) and a simple statement of research intent, cell lines can be ordered via a cart-based checkout system. The NHCDR functions via a cost recovery model and iPSC are available to not-for-profit researchers for $500 a vial and to for-profit researchers for $1500 per vial. Not-for-profit researchers can obtain fibroblast lines for $350 per vial and $500 per vial for for-profit researchers. We have distributed more than 2300 vials of cells from the NHCDR and other collaborative projects such as the NIH Regenerative Medicine Program (NIH RMP), Target ALS, the Children's A-T project and the Myotonic Dystrophy Foundation. The NHCDR is also distributing 10 isogenic pairs of iPSC developed through CRISPR gene editing and a GMP grade iPSC line as well as a non-GMP grade cell line for pre-clinical research from the same subject. All cell lines are distributed with a certificate of assurance, guidelines for culturing the cells and any technical support required to help a client meet their research goals.

Keywords: iPSC, repository, Neurological disease models

MDD408

CRISPR/CAS9 GENE EDITING FOR GENERATING IPSC MODELS OF HUMAN DISEASES AND DEVELOPMENT

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Since its inception in 1998, RUCDR Infinite Biologics (RUCDR, www.rucdr.org) has provided the scientific community with the highest quality biomaterials, technical consultation, and logistical support. We collaborate with researchers, the NIH and non-profit foundations to provide stem cell services. These services include the banking and distribution of source cells and induced pluripotent stem cells (iPSC) and the generation of iPSC from human somatic cells. Since 2017, we have begun offering genetic engineering of iPSC harnessing the CRISPR (clustered regularly-interspaced short palindromic repeats)/Cas9 technology. The diverse genetic background of the human subjects has hampered the usefulness of iPSCs for modeling human diseases and development. The use of the CRISPR/Cas9 system can create isogenic cell lines that will serve as better controls and help eliminate effects that are due to genetic variation rather than a biological mechanism. At RUCDR/Infinite Biologics we have developed a high throughput, cost efficient workflow for using CRISPR/Cas9 to genetically modify iPSC from affected or unaffected subjects. Using this strategy, we have generated footprint-free edited isogenic iPSC pairs harboring mutations involved in neurological disorders such as Amyotrophic Lateral Sclerosis (ALS), Tourette syndrome, Parkinson, Alzheimer and Huntington Disease. All edited iPSC lines are tested rigorously for off-target effects, homogeneity, pluripotency and genetic integrity. Data of our quality control process is included in the quality assurance certificate for each iPSC line we distribute.

Keywords: CRISPR/Cas9, Gene editing, neurological disorder

MDD411

DIFFERENT CELL TYPES PRODUCE TISSUE-SPECIFIC EXTRACELLULAR MATRICES WITH SUBTLE DIFFERENCES CAPABLE OF PROMOTING BIOLOGICALLY-RELEVANT PHENOTYPES IN VITRO

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Previously, our group has reported the development of scalable methods for production of bone marrow mesenchymal stem cell-derived matrices (BM-ECM) for isolation and expansion of mesenchymal stem cells without loss of stemness. More recently, we have reported tissue-specific differences in matrices that are critical for determining cell fate and function in vitro. Tissue-specific matrices are achieved by seeding a cell type onto a tissue culture dish, culturing those cells to confluence, inducing those cells to secrete a matrix, and decellularizing the resulting cell/matrix construct. Interestingly, the in vitro cell-derived matrices produced retain tissue-specific properties based on the origin of the cells used to create the matrix. Importantly, subtle differences in the resulting matrices drive predictable cell behavior in vitro. For example, a matrix produced by adipose stem cells (AD-ECM) supports adipogenic differentiation by mesenchymal stem cells (MSCs) as measured by PPARγ expression (3x higher relative to BM-ECM) and lipid droplet formation. Meanwhile a matrix produced by chondrocytes supports retention of native phenotype of articular chondrocytes during extended culture as measured by increased Col2/Col1 ratio and GAG expression relative to traditional culture methods (20x higher vs TCP, 3x higher vs BM-ECM). Here, we describe ongoing work to apply this technology to achieve biologically-relevant phenotypes of cardiomyocytes and neurons in vitro. Furthermore, we demonstrate that these phenotypes are driven by subtle differences in physical properties and biochemical composition, despite sharing most major components. This work illustrates the importance of nuanced cell-matrix interactions not present in traditional in vitro models. Moreover, this work offers proof-of-concept for the use of tissue-specific, in vitro cell-derived matrices to develop high-throughput screening platforms for cell types that are traditionally difficult to maintain in culture.

Funding source: Not applicable

Keywords: Extracellular matrix, In vitro modelling, biologic-relevance

MDD419

SYSTEMATIC INTRODUCTION OF FAMILIAL CARDIOMYOPATHY MUTATIONS INTO ISOGENIC HUMAN IPS CELLS EXPRESSING FLUORESCENT STRUCTURAL REPORTERS

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Genetic disease mechanisms can be effectively modeled in hiPSCs because they can be differentiated in vitro into many tissue lineages and are amenable to genome editing. The isogenic, quality-controlled hiPSC lines within the Allen Cell Collection, each with unique organelle labels encoded by endogenous gene tag(s), represents a fruitful system for this approach. We introduced eight myopathy-associated MYH7/3 mutations into the endogenously tagged ACTN2:GFP cell line. The goal was to understand the consequences of these mutations on sarcomere organization and mechanical force production within in vitro derived cardiac and skeletal myocytes. We used a high throughput screening approach consisting of Cas9/CRISPR genome editing with ssODN donors, NGS screening of edited populations, and droplet digital PCR drop-in/drop-out sibling

selection and clone screening to efficiently recover clones with each desired genotype. Initial experiments to introduce the disease associated myosin variants MYH7 S291F, V606M, G256E, D239N, H251N, E525K, R369Q and MYH3 G769V indicate successful introduction of all eight mutations, with 1-13% HDR and low rates of NHEJ at the unmodified allele. Multiple clones with monoallelic edits were recovered from screened populations, along with biallelically edited clones and unedited control clones to facilitate downstream phenotyping analysis. The Allen Institute for Cell Science will build off the results of this pilot to expand the Allen Cell Collection to include isogenic disease model cell lines with strategically chosen combinations of endogenous gene tags and disease-associated genotypes. These cell lines and associated data will be shared with the research community.

Keywords: Disease Mutations, CRISPR, Gene editing

MDD425

RNA AND PROTEIN COMPOSITION OF EXTRACELLULAR VESICLES COMPARED THROUGHOUT DIFFERENT HUMAN TISSUES AND EMBRYONIC STEM CELLS

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Extracellular RNAs (exRNAs) have been found in all tested human biofluids, and there is increasing evidence that they can serve as mediators of intercellular communication, as well as diagnostic, prognostic, and theranostic biomarkers for a wide range of disease and physiological conditions. ExRNAs are associated with a variety of carriers subclasses (CSs), including extracellular vesicles (EVs), ribonucleoprotein complexes (RNPs), and lipoproteins (LPP), many of which are as-yet unknown or poorly characterized. This research will focus on development of reagents for identification and separation of known and suspected CSs using appropriate cell culture models and healthy human plasma and serum samples. This work will include screening of available antibodies against markers for known general CSs (e.g. tetraspanins, AGO proteins, apolipoproteins) and a variety of cell type-specific markers to identify antibodies that perform well for Western Blot and IMS, and dissemination of results for both successful and unsuccessful antibodies. If successful, this project will result in development of a rigorous workflow for separation of exRNA CSs that reproducibly and rapidly produces fractions that are highly enriched for desired CSs with minimal contamination by other CSs in a cost-effective manner on clinically feasible volumes of input material, and yields sufficient material for downstream molecular analysis.

Keywords: RNA, PROTEIN, Embryonic Stem Cell

MDD460

ALL-OPTICAL ASSAY FOR CONNECTIVITY STUDIES IN NETWORKS OF HUMAN IPSC-DERIVED NEURONS.

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Optogenetics allows contact-free activation or inhibition of specific neurons using only light. It has been combined with purely optical readout of neural activity using fluorescent voltage reporters, but this requires extreme microscopy which limits its use in pre-clinical and drug screening applications. To overcome these limitations, we have developed advanced approach in which calcium imaging can be used to quantify network dynamics in vitro using a genetically encoded calcium indicator (GECI). This is a novel quantitative use of neurophotonics to investigate how iPSC-derived neurons communicate in disease models, thus enabling functional in vitro high throughput screening.

OptoCaMP, a combination of a Channelrhodopsin and a GECI, do not exhibit overlapping excitation and emission spectra thus avoiding optical cross-talk. This enables simultaneous optical stimulation and calcium imaging. Our assay was performed under a 20X objective which defines a field of view containing approximately 60 cortical neurons in vitro expressing OptoCaMP via lentiviral transduction. Our protocols consisted in consecutive pulses of blue light in a defined region of the field of view. The activity of the neighboring neurons along with the optically stimulated neurons.

As a proof of concept, we applied this all-optical assay to compare and quantify the connectivity in four conditions (BrainPhys media: supports synaptic function; Neurobasal medium: reduced synaptic communication; BrainPhys + NMDA and AMPA receptors antagonists: block synaptic communication; BrainPhys + caffeine: increases the excitability of neurons). The network connectivity was then quantified and showed a mean global connectivity significantly enhanced with the addition of caffeine to BrainPhys medium and significantly decreased with the addition of synaptic blockers. We also observed a significantly lower mean global connectivity for the neurons cultured in Neurobasal compared to BrainPhys medium.

OptoCaMP enables simultaneous optical stimulation and calcium imaging to investigate network connectivity. We quantified connectivity in conditions where synaptic communication is reduced or enhanced and showed the sensitivity and the potential use of this assay for functional in vitro high-throughput screening.

Funding source: EPSRC programme Grant EP/P030017/1 and a grant from the SSADH association.

Keywords: optogenetics, calcium imaging, human iPSC-derived neurons

MDD509

ISOLATION OF CLONAL GENOME EDITED DUAL-FLUORESCENT REPORTER HUMAN STEM CELL POPULATIONS WITHOUT SINGLE CELL CLONING

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The results from different laboratory investigations of human stem cells are difficult to evaluate and compare and clear correlations between culture conditions and cell state and fate are difficult to discern. To better track these correlations, we have initiated a program to improve our basic understanding of iPSC growth and to build predictive models by defining the critical measurable parameters that regulate the maintenance of iPSC pluripotency and differentiation. This is dependent on the creation of well-characterized dual-fluorescent reporter stem cell lines that accurately report the transcriptional expression patterns of transcription factors (TF) known to be regulate pluripotency and transitional cell states during differentiation in human stem cells (OCT4, SOX2, and NANOG). A challenge to this effort is the poor survival of stem cells passaged at low seeding density, which makes the generation of clonal cultures from single cells highly problematic. Our work flow was designed to address this problem. WTC-11 cells were transfected in the presence of TF-specific gRNA-cas9 ribonuclear protein (RNP) complex and “donor” plasmids with homology arms to drive gene specific recombination. Successful insertion replaces the stop codon with a translational read-through signal (P2A) and a nuclear localization signal-sequence (NLS) upstream of a fluorescent protein encoding sequence. The expression of the fluorescent reporter is regulated by the endogenous control elements and the normal expression of the TF is unaffected. After testing transfection conditions, we chose a protocol that provide us with ~.01% efficiency of insertion of fluorescent protein. We distribute post-transfection cells into 96 well plates together with un-transfected “helper” cells to bring the final concentration of cells to ~23,000 cells/cm2. Starting three days post-transfection, entire wells are imaged with bright field and fluorescence optics using a very low level of excitation light. Genome editing events are clearly identifiable as clusters of small numbers of fluorescent cells and wells containing single events are noted for further expansion. Using this approach, we have generated clonal dual-fluorescent reporter stem cells without the necessity of single cell cloning.

Keywords: Reporter, Validation, Modeling

PLACENTA AND UMBILICAL CORD DERIVED CELLS

MDD436

TRANSDIFFERENTIATION AND CELL FUSION ARE BOTH POTENTIAL METHODOLOGIES FOR THE DEVELOPMENT OF IMMORTALIZED HEPATOCYTE-LIKE CELL LINES UTILIZING TERT-TRANSFECTED MULTI-LINEAGE PROGENITOR CELLS

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Multi-Lineage Progenitor Cells (MLPC) are primitive cord blood-derived stem cells that have demonstrated expansive capacities and differentiation capacities beyond other mesenchymal stem cell-like cells including expansion to 80 population doublings and differentiation to endo-, meso- and ectodermal cell lineage phenotypes. Single gene TERT-transfection of MLPC resulted in the development of cell lines with extensive transdifferentiation capacity and immortality. The E12 clonal cell line was used throughout this study. Herein, we report the in vitro development of hepatocyte-like cells (HLCs) by both transdifferentiation and cell fusion using immortalized TERT-transfected cord blood-derived MLPC. Methods are described for (i) the 3-stage differentiation of TERT-MLPC to HLCs and (ii) the fusion of TERT-MLPC to primary hepatocytes to create immortalized HLCs. Differentiation of cells towards hepatocyte gene and protein expression and function was monitored by immunohistochemistry, RT-PCR, urea and albumin production and karyotyping. The study has produced a number of both differentiated and fused cells lines with stable characteristics of mature human hepatocytes. The transdifferentiated and fusion cells both expressed mRNA, hepatocyte-specific protein expression, as well as, urea and albumin production characteristic of primary hepatocytes. These cells may provide a permanent, off-the shelf, supply of cells for the study of hepatocyte function and drug metabolism. Reports of sexually-mismatched functional cells in the liver and pancreas of marrow and cord blood transplant recipients of sexually mismatched donor cells were capable of engraftment into non-hematopoietic sites either by transdifferentiation or by cell fusion. Whether transdifferentiation or cell fusion was responsible for this phenomenon has not been fully elucidated. Our studies strongly suggest that both mechanisms are possible.

Funding source: BioE, LLC.

Keywords: Immortal MLPC, transdifferentiation, cell fusion

Theme: Tissue Stem Cells and Regeneration

ADIPOSE AND CONNECTIVE TISSUE

TSC400

MESENCHYMAL STROMAL/STEM CELL-DERIVED EXTRACELLULAR VESICLES PROMOTE HUMAN CARTILAGE REGENERATION BY CONTROL OF AUTOPHAGY

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Osteoarthritis (OA) is a rheumatic disease leading to chronic pain and disability with no effective treatment available. Recently, allogeneic human mesenchymal stromal/stem cells (MSC) entered clinical trials as a novel therapy for OA. Increasing evidence suggests that therapeutic efficacy of MSC depends on paracrine signalling. Here we investigated the role of bone marrow MSC-derived extracellular vesicles (BMMSC-EVs) in cartilage repair and show that BMMSC-EVs promote cartilage regeneration in vitro. OA chondrocytes were treated with inflammatory mediators such as tumor necrosis factor alpha (TNF-alpha) to induce inflammation. Hereafter, bone marrow MSC-derived extracellular vesicles (BMMSC-EVs) are added to investigated the role of BMMSC-EVs in cartilage repair. Treatment of OA chondrocytes with BMMSC-EVs induces production of proteoglycans and type II collagen and promotes proliferation of these cells. MSC-EVs also inhibit the adverse effects of inflammatory mediators on cartilage homeostasis. Our data show that BMMSC-EVs downregulate tumor necrosis factor alpha (TNF-alpha) induced expression of pro-inflammatory cyclooxygenase-2, pro-inflammatory interleukins and collagenase activity in OA chondrocytes. The anti-inflammatory effect of BMMSC-EVs involves the inhibition of NFkB signaling, activation of which is an important component of OA pathology. Our findings indicate that BMMSC-EVs have ability to promote human OA cartilage repair by reducing the inflammatory response and stimulation of OA chondrocytes to produce extracellular matrix, the essential processes for restoring and maintaining cartilage homeostasis. Accumulating evidence indicates that the expression of autophagy regulators is reduced in osteoarthritic joints, which is also accompanied by increased chondrocyte apoptosis. Our preliminary data indicate that BMMSC-EVs carry mRNA of natural autophagy inducers and promote autophagy in OA chondrocytes. Therefore, we hypothesize that MSC-EVs exert their beneficial effects on cartilage regeneration by restoring the expression of autophagy regulators. Taken together, our data demonstrate that MSC-EVs can be important mediators of cartilage repair and hold great promise as a novel therapeutic for cartilage regeneration and osteoarthritis.

Funding source: ZonMw, Reuma Nederland, Wilhelmina Kinderziekenhuis

Keywords: Mesenchymal stem cell, Extracellular vesicles, Autophagy

CARDIAC

TSC111

PIWI-INTERACTING RNAS ARE DIFFERENTIALLY EXPRESSED DURING CARDIAC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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PIWI-interacting RNAs (piRNAs) are a class of non-coding RNAs initially thought to be restricted exclusively to germline cells. In recent years, accumulating evidence has demonstrated that piRNAs are actually expressed in pluripotent, neural, cardiac and even cancer cells. However, controversy remains around the existence and function of somatic piRNAs. Using small RNA-seq samples from H9 pluripotent stem cells differentiated to mesoderm progenitors and cardiomyocytes we identified the expression of 447 piRNA transcripts, of which 241 were detected in pluripotency, 218 in mesoderm and 171 in cardiac cells. The majority of them originated from the sense strand of protein coding and lncRNAs genes in all stages of differentiation, though no evidences of amplification loop (ping-pong) were found, suggesting that piRNA expression in these cells depends mainly on the primary mechanism of biogenesis. Genes hosting piRNA transcripts in cardiac samples were related to critical biological processes in the heart, like contraction and cardiac muscle development. Our results indicate that these piRNAs might have a role in fine-tuning the expression of genes involved in differentiation of pluripotent stem cells to cardiomyocytes.

Keywords: cardiac differentiation, somatic piRNA, stem cells

TSC120

A LARGE-SCALE EFFORT AIMED AT PRODUCING AUTOLOGOUS, CLINICAL GRADE IPSC-DERIVED CARDIOMYOCYTE-LIKE CELLS

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Recently, we reported preliminary studies which established a definitive process-development standard for producing iPSCs and iPSC-derived cardiomyocyte-lineage cells (iPSC-CL). Our 3-D culture system successfully yielded ~ 1.0 X 108 troponin positive (> 70% cTnT/cTnI) iPSC-CL cultures free of contaminating iPSCs. In preparation for a pending IND submission, we outline here a two-part approach for producing autologous, clinical grade iPSC-CLs based on an expansion of our previous work. The process includes a certificate of analysis (CofA) consisting of specific release criteria metrics, along with additional testing assays which provide supplemental data. A study design employing 4 randomly selected iPSC lines, 6 clones each, was initiated in April of 2019, and is expected to be completed by August 2020. The initial differentiation of 24 clones (6 clones X 4 lines) yielded 4 iPSC-CL products that met our release criteria. Taking into account sterility, cell growth rates, troponin expression and karyotype data obtained from the first 24 differentiations, a best case scenario would yield 11/24 products meeting the proposed release criteria metrics (8 parameters, including sterility, genetic stability, troponin expression). Additional testing determined that 23/24 iPSC lines were functionally pluripotent and posed minimal teratogenic risk (ESA), while iPSC-CL cultures expressed nearly undetectable levels of TRA-1-60 (n=16), exhibited a proliferation rate of 6.03% (n=11) and 8/13 cultures displayed a ventricular cardiomyocyte subtype (APD90/APD50). Analysis of mitochondrial DNA uncovered no deleterious heteroplasmy in any clone assayed thus far (n=7). Ongoing repetition of the first 24 differentiations (4 independent planned differentiations/clone) revealed a consistent karyotype (4/4 replicates), while the cardiomyocyte subtype was preserved in 2/3 clones. Thus, despite this study being incomplete, we can confidently surmise that less than half of the iPSC-CL products will meet our proposed release criteria, which supports the multiple iPSC clone approach central to our current iPSC-CL differentiation process. Furthermore, animal data (presented at this meeting) derived from in-process studies conducted in our lab will provide the ultimate validation of our iPSC-CL product.

Funding source: The Todd and Karen Wanek Family Program for Hypoplastic Left Heart Syndrome (HLHS)

Keywords: iPSC, Autologous, Cardiomyocyte

TSC121

SAFETY AND TUMORIGENICITY OF INTRA-MYOCARDIAL TRANSPLANTED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIAC LINEAGE CELLS INTO NOD SCID GAMMA (NSG) MICE

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Congenital heart disease is a major cause of death and morbidity for which transplantation remains the only viable long-term treatment. For this reason, stem cell-derived cardiomyocyte-based therapies have emerged as a potential therapeutic approach to stave off transplantation. The use of iPSC-derived clinically relevant cell types, such as cardiomyocytes, as cell therapies poses the potential risk of tumor formation. Teratomas are a hallmark of pluripotent stem cells and contamination of an iPSC-derived cell therapy product with undifferentiated iPSCs may have tumorigenic effects. Here, we subjected 8-week old NSG mice to intracardiac cell injection to determine the safety and tumorigenicity of a high dose of Day 20 human iPSC-derived cardiac lineage cells (iPSC-CL) produced through spinner flask 3D culture. Mice were divided into 3 groups that received either the Day 0 iPSC (n=6), Day 20 iPSC-CL (n=25), or Day 20 iPSC-CL with a 10% spike of iPSC (n=6). All groups received a total of 3.0x106 cells/animal. Each subject was assessed for cell engraftment, tumor formation, and test article-related health effects. All surviving mice were sacrificed at either 8 or 14 weeks post-treatment, depending on the treatment group. Histologically, no evidence of tumor in the myocardium or other ectopic sites was observed and immunohistochemistry indicated mature grafts of viable transplanted iPSC-CL were present within the myocardium of the group receiving only iPSC-CL. No test article-related effect was observed on mortality, quality of life, or tumor formation. Results demonstrate high-quality 3D culture production of Day 20 iPSC-CL achieved engraftment within the myocardium, induced no teratomas, and incurred no significant risk factors. Results from our study suggest that grafting iPSC-CL into the myocardium in large numbers allows for iPSC-CL engraftment and does not produce of tumorigenesis.

Keywords: Human iPSC-derived cardiac Lineage, Tumorigenicity, Cell engraftment

EARLY EMBRYO

TSC430

X CHROMOSOME DOSAGE AFFECTS DEVELOPMENT OF ANEUPLOIDY AND THE 2-CELL-LIKE STATE IN GENETICALLY DIVERSE MOUSE EMBRYONIC STEM CELLS

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Pluripotent stem cells (PSC) are an invaluable resource for disease modeling and cell therapy. Unfortunately, prolonged in vitro culture of human or mouse PSC can result in accumulations of cells with chromosomal copy number variations that can impede differentiation and confer a proliferative advantage. Human PSC cell lines vary in their genetic stability, but we know very little about the basis for this variation. Mouse embryonic stem cell (mESC) panels derived from Diversity Outbred mice provide a powerful resource for analyzing the role of genetic background in determining the genetic stability of PSC. Using RNA-seq and proteomics data from a panel of mESC lines derived from 170 Diversity Outbred mice and cultured in serum + LIF/GSKi, we identified 62 lines with detectable aneuploid subpopulations and assessed common genetic features of those aneuploid lines. In agreement with previous studies, we identified duplications in chromosomes 8 and 11, and to a lesser degree, chromosomes 1 and 6. In the female population, we also identified 21 lines with the loss of chromosome X (XO). No alleles were found to be associated with duplication of specific chromosomes, however two QTL at chromosomes 16 and X were significantly associated ($p < 0.05$ and 0.01) with overall aneuploidy. Most strikingly, a much lower proportion of XX lines were aneuploid, compared to the XY or XO lines, and the proportion of aneuploid lines in these populations correlated to the expression of chromosome X genes. Additionally, XX lines showed a higher expression of genes upregulated in the 2-cell-like state, suggesting that dual X chromosome dosage might promote the 2-cell-like state and enhance genetic stability.

Keywords: Embryonic stem cells, Aneuploidy, 2-Cell-like state

ENDODERMAL LINEAGE- PANCREAS, LIVER, KIDNEY

TSC143

PLURIPOTENT DERIVED ENDODERMAL FATE SPACE ANALYSIS USING HIGH DIMENSIONAL DESIGN OF EXPERIMENTS

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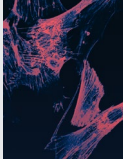
There is great interest in the generation of pluripotent derived endodermal derivatives for their potential use in regenerative medicine. TGF beta agonists, most commonly Activin A (AA), are used in the generation of pluripotent derived endoderm under the belief that they act as NODAL mimics. However, previous studies have shown that AA and NODAL mediated endoderm induction differs. Here we have used a previously described high dimensional design of experiments (HD-DoE) approach to investigate TGF beta signaling in generating pluripotent derived endoderm. HD-DoE designs incorporating AA, BMP4, NODAL, retinoic acid and respective inhibitors were used on pluripotent cultures and key endodermal genes were monitored. Resulting data was imported into analysis software and computer models predicting optimal culture conditions for activating all genes monitored were clustered. Four distinct populations were predicted all tolerate of NODAL. Models predicted that anterior genes TBX1, SFRP5, NOG and PAX9 could not tolerate AA, but were dependent on NODAL and BMP4 signaling. Midgut genes BMP4, HNF1B, APOB and OSR1 had a varying tolerance for AA, but were shown to have an increasing dependency on retinoic acid with dorsal fates MNX1, NOG and GSC demanding retinoic acid and BMP inhibition. Altogether it was predicted that NODAL, BMP and retinoic acid patterns endoderm along the anterior to midgut. Genes representative of posterior CDX2 and ventral endoderm HHEX, SOX17 and BMP4 were predicted to be regulated by AA. In addition incubation of pluripotent cultures with AA was shown to induce the endogenous expression of several TGF beta family members including TDGF1, LEFTY1/2, NODAL, BMP1/2/4/7 and TGF beta 1, possibly explaining previous findings attributing a broader AA effect in endoderm induction. These results show that endoderm patterning occurs during gastrulation resulting in at least three separate regional identities. We also show that definitive endoderm induction, as defined commonly in literature, creates a regionalized endoderm, of ventral/posterior identity where other early inputs, notably from Nodal/BMP and Retinoids/BMP inhibition, creates anterior, and dorsalized foregut endoderm, respectively.

Funding source: ADA grant number 1-16-ICTS-053

Keywords: Endodermal Patterning, High Dimensional Design of Experiments (HD-DoE), TGF beta and Nodal Signaling



TSC147

ORIGIN AND PATTERNING OF THE GUT ENDODERM ORGAN PROGENITORS DURING MOUSE EMBRYONIC DEVELOPMENT.

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Endoderm organs are common sites of disease, many of which may benefit from cell transplantation therapies. As such, a better understanding of the developmental origins and mechanisms that result in proper organ formation will be crucial for developing disease models and treatments. While we and others have recently described the developmental trajectories of endodermal organs until midgestation, their developmental origin and patterning has not been fully explored. Our previous studies have shown that a subset of gut tube cells originate from the visceral endoderm, challenging the longstanding belief that visceral endoderm is strictly extraembryonic. Here, we are taking advantage of 3D imaging and single-cell transcriptomics methods to better understand the origins and patterning of the emergent endodermal organs. To investigate the origin of the gut and endodermal organs, we are using genetically engineered mouse models and tetraploid chimera analyses. We show that visceral endoderm descendants contribute to the posterior gut tube and its derivatives until at least fetal stages. This stands in contrast to previous observations that suggested tetraploid chimeras are entirely derived from donor pluripotent stem cells. Furthermore, this result raises the question of how cells of distinct origins integrate to form a functional hindgut during development, and whether they have discrete roles during tissue homeostasis and disease progression. To investigate the patterning of the gut tube and early organogenesis we are using quantitative 3D imaging approaches. Our recent scRNA-seq studies predict that endoderm organ primordia are organized along the anterior-posterior axis of the gut tube as early as E8.75. Using hybridization chain reaction and wholemount immunofluorescence for markers of nascent organ progenitors, we demonstrate that organ primordia are spatially organized early during development. We are now using CRISPR-mediated gene ablation approaches to uncover mechanisms driving segregation of neighboring organs and learn how perturbation of normal specification affects the tissue patterning landscape. These results advance our understanding of the development of endodermal organs and will allow us to integrate additional complexity into stem cell models of development and disease.

Funding source: This work was supported by grants from the National Institutes of Health (NIH) to A.-K.H. (R01DK084391, R01HD094868 and P30CA008748) and NYSTEM (C029568).

Keywords: Endoderm, Organogenesis, Quantitative imaging

TSC149

SINGLE-CELL ANALYSIS IDENTIFIES A POPULATION OF PROGENITOR CELLS DURING ZEBRAFISH LIVER REGENERATION

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The liver is an essential organ, responsible for key metabolic functions, energy storage, blood detoxification, plasma protein secretion, and bile production. It is composed of several different cell types and is capable of regenerating by replacing hepatocytes lost due damage, primarily through division of other hepatocytes. However, there are limits to the capacity of the liver to compensate following sustained insults or injury. Liver failure is one of leading causes of death worldwide, and the only known cure for liver failure is organ transplantation. The development of a stem-cell based therapy to regenerate the liver would provide a life-altering treatment for patients with late-stage liver disease. While there is some evidence for a transient population of liver stem cells that appear during regeneration, the origin, potency and regulation of these cells remains largely unknown. The zebrafish (Danio rerio) liver serves the same function and is thought to be composed of the same cell types as in mammals. To identify the response of the liver to acute injury on a cellular and transcriptomic level, the livers of adult zebrafish were subjected to either drug-induced toxicity or genetic hepatocyte ablation, and were subsequently analyzed using single-cell RNA sequencing. This approach resulted in the generation of transcriptomic information for ~25,000 single cells, inclusive of all known cell types in the liver. Intriguingly, a population of cells that contains markers for both hepatocytes and biliary epithelial cells appears early only after recovery from hepatocyte ablation. These data suggest a progenitor population of biliary origin is utilized during regeneration from extreme injury. Lineage tracing of adult biliary epithelial cells confirms that they can give rise to hepatocytes after hepatocyte ablation. Further work will be needed to characterize the regulation of these cells during regeneration.

Funding source: Research reported in this abstract was supported by the National Institute On Alcohol Abuse And Alcoholism of the National Institutes of Health under Award Number F32AA027135.

Keywords: Single-cell sequencing, Regeneration, Liver

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

TSC156

UTILIZING COMPUTATIONALLY DESIGNED NANOPARTICLES TO PROBE THE MECHANISM OF ANGIOPOIETIN-TIE2 SIGNALING

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Angiogenesis plays a pivotal role in tissue maintenance and wound healing via regulating the growth of blood vessels that nourish cells during differentiation and regeneration. Angiopoietin 1 and 2 (Ang1 and Ang2) modulate angiogenesis and vascular homeostasis through the engagement of their very similar F-domain modules with the Tie2 receptor tyrosine kinase on endothelial cells. Despite this similarity in the underlying receptor binding interaction, the two angiopoietins have opposite effects: Ang1 induces phosphorylation of protein kinase B (AKT), strengthens cell-cell junctions and enhances endothelial cell survival while Ang2 antagonizes these effects. To investigate the molecular basis for the opposing effects, we examined the protein kinase activation and morphological phenotypes produced by a series of computationally designed protein scaffolds presenting the F-domain in a wide range of valencies and geometries. We find two broad phenotypic classes distinguished by the number of presented F-domains: scaffolds presenting 3 or 4 F-domains have Ang2 like activity, upregulating pFAK and pERK but not pAKT, and failing to induce cell migration and tube formation, while scaffolds presenting 6, 8, 12, 30 or 60 F-domains have Ang1 like activity, upregulating pAKT and inducing migration and tube formation. We are now investigating cytoplasmic regions of Tie2 clusters using the mass spec and super-resolution imaging to understand the mechanism for this difference. The scaffolds with 8 or more F-domains display superagonist activity, producing stronger phenotypes at lower concentrations than Ang1. In in vivo experiments, superagonist icosahedral self-assembling nanoparticles displaying 60 F-domains produce significant revascularization in hemorrhagic brains after a controlled cortical impact injury. Our Tie2 super agonists and antagonists may have enormous medical implications in tissue regeneration, wound healing, and cancer therapy.

Keywords: Tie2 pathway, Angiogenesis, Protein design

TSC157

ROBUST DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO COMPETENT ENDOTHELIAL CELLS VIA TIMELY ACTIVATION OF EXOGENOUS ETV2 WITH MODIFIED MRNA

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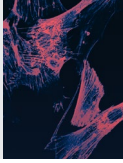
Over the last decade, human induced pluripotent stem cell (h-iPSC)–derived endothelial cells (h-iECs) have become a valuable tool in cardiovascular research, offering a broad range of translational and clinical applications. However, current differentiation protocols remain largely inefficient and lack reliability, thus hampering the expansion of this technology. We examined standard protocols to differentiate h-iPSCs into h-iECs and identified that a critical source of inconsistency resides in the nonuniform activation of the transcription factor ETV2 at the intermediate mesodermal stage of differentiation. To overcome this limitation, we developed a method that entails the precise delivery of modified mRNA (modRNA) encoding ETV2 into mesodermal intermediates. Our protocol achieved transient and precise activation of exogenous ETV2 activity throughout the entire mesodermal population. As a result, all h-iPSC lines tested differentiated into h-iECs with exceedingly high efficiency (>90%) and reproducibility. Importantly, we validated that the resulting h-iECs were functionally competent in many respects, including the ability to form perfused vascular networks in vivo. In contrast, we showed that protocols that solely relied on endogenous ETV2 were less efficient and notably inconsistent. Furthermore, we showed that the delivery of exogenous ETV2 directly into h-iPSCs also yielded high differentiation efficiency. However, we demonstrated that bypassing the mesodermal stage produced putative h-iECs with reduced expansion potential and incapable of forming functional vessels in vivo. In summary, we present an approach to differentiate h-iPSCs into h-iECs with high efficiency and reproducibility, irrespective of the cellular source from which the h-iPSC clones originate. We demonstrated that timely activation of ETV2 at the mesodermal stage is critical to achieving consistency and functional competence. Our results provide a means to generate h-iECs for vascular therapies and research effectively.

Funding source: This work was supported by the National Institutes of Health (NIH; grant R01HL128452 to J. M.-M) and by the Juvenile Diabetes Research Foundation (JDRF; grant 3-SRA-2018-686-S-B to J. M.-M)



Keywords: iPSC-derived endothelial cells, transcription factor ETV2, chemically modified mRNA

TSC383

TRANSMEMBRANE STEM CELL FACTOR WITH NANOCARRIERS ENHANCE REVASCULARIZATION IN ISCHEMIA WITHOUT MAST CELL ACTIVATION

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Diabetes mellitus affects approximately 350 million people worldwide, leading to the death of about 4.6 million people per year. As a complication of diabetes, 30-40 percent of patients age 50 and older develop peripheral artery disease (PAD). The current standard treatments for PAD include surgical revascularization with bypass grafting or percutaneous interventions. However, these interventions cannot be performed in a significant portion of patients, and many do not respond to these therapies. An alternative approach for treating PAD is to use proteins to stimulate the body to create new vasculature, thus restoring blood flow through its own regenerative processes. Stem cell factor (SCF) is a cytokine that acts through the receptor tyrosine kinase c-Kit to regulate hematopoiesis and has been a candidate protein for treating PAD. Clinical use of soluble SCF would be highly beneficial but has been limited due to toxicity related to mast cell activation. SCF also exists in a transmembrane form, possessing differential activities from soluble SCF and has not been explored as a therapeutic agent. To explore the transmembrane SCF (tmSCF) as a therapeutic we created formulations of tmSCF embedded in liposomes or in lipid nanodiscs. Mouse models of anaphylaxis and ischemia revealed the tmSCF-based therapies did not activate mast cells and were effective in improving the recovery from ischemia in both wild type and diabetic mice. We also found that the formulation of the lipid nanocarrier to deliver tmSCF altered the biological response and trophism of the tmSCF-based treatments. Proteoliposomal tmSCF preferentially acted on mature endothelial cells to induce angiogenesis while tmSCF nanodiscs had greater activity in inducing stem cell mobilization from the bone marrow and recruitment to ischemic sites. A mechanistic analysis of the treatment effects on mast cells, mature endothelial cells and endothelial progenitor cells, revealed that the nanocarriers altered the relative utilization of clathrin- versus caveolin-mediated uptake of c-Kit in response to the treatments. Overall, our studies support that tmSCF-based therapies can provide therapeutic benefits without off-target effects on mast cells and that lipid nanocarriers can be used to tailor the properties of membrane protein-based therapeutics.

Keywords: transmembrane stem cell factor, revascularization in ischemia, endothelial progenitor cells

TSC393

MECHANOBIOLOGICAL CONDITIONING ENHANCES HUMAN MESENCHYMAL STEM CELL-INDUCED VASCULAR REGENERATION

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Human mesenchymal stem cells (hMSCs) are multipotent cells that are harvested autologously with relative ease. The differentiation of hMSCs into vascular cell phenotypes would be useful in clinical applications, but therapies are limited by poor efficacy and heterogenetic response. Biophysical forces like mechanical stretch have been used to condition MSCs into vascular phenotypes, but the optimal conditions remain unclear. We created a device that enables simultaneous application of mechanical load to 576 culture wells. We investigated dynamic strain waveforms for their ability to activate Yap/Taz and TGF-β mediated signaling pathways and found optimal activation when cells were treated with 7.5% brachial strain. We screened a library of kinase inhibitors in combination with mechanical load to identify compounds that synergistically increase nuclear Yap/Taz localization and Smad2/3 phosphorylation. Specific combinations of these inhibitors and physiologic mechanical load increased expression of endothelial and pericyte markers. We performed genome wide transcriptomic assays on conditioned hMSCs and analyzed the results using Gene Set Enrichment Analysis, finding an enrichment of endothelial and pericyte gene sets. In vitro assays demonstrated enhanced endothelial and pericyte-like behavior. An analysis of the signaling pathways required for these effects revealed crosstalk between TGFbR, VEGFR2 and Syk pathways. Combined load and inhibition of EGFR/ErbB2/ErbB4 (E/E) led to binding of the cleaved ErbB4 cytoplasmic

domain to Yap-1 and activation of Smad-2, which are required for increased expression of pericyte/endothelial markers. We encapsulated the conditioned hMSCs in alginate-RGD/collagen gel and implanted them in nu/nu mice with hindlimb ischemia. The hMSCs treated with mechanical load and the E/E inhibitor induced enhanced recovery to ischemia in the mice. Our results demonstrate that mechanical and pharmacological conditioning of hMSCs can enhance their regenerative capabilities. Given the wide variation in MSC functionality from disease/aging, a potential approach is to isolate patient MSCs and optimize the desired phenotype using a mechanobiological screen. This optimization process provides a paradigm for enhancing stem cell therapies in a patient specific manner.

Funding source: The American Heart Association (17IRG33410888), the DOD CDMRP (W81XWH-16-1-0580; W81XWH-16-1-0582) and the National Institutes of Health (1R21EB023551-01; 1R21EB024147-01A1; 1R01HL141761-01).

Keywords: Mesenchymal stem cells, Mechanobiology, Vascular regeneration

EPITHELIAL

TSC162

NICHE TOPOGRAPHY IN 3D BIOPRINTED COLLAGEN SCAFFOLDS DETERMINES CHROMATIN STATE OF HUMAN EPIDERMAL STEM CELLS

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Rete ridges form human epidermal downgrowths containing interfollicular stem cells critical to skin regeneration and homeostasis. However, the spatial and mechanobiological roles of this niche in maintaining resident stem cell identity remain unexplored. To investigate the effect of niche topography on the epigenetic/genetic profiles of epidermal stem cells, 3D-bioprinted bovine collagen scaffolds were imprinted to form anatomically correct rete ridge-like surfaces using custom stereolithography-generated stamps. Human keratinocytic stem cells were seeded into either the resultant rete ridge concavities or onto un-stamped flat control surfaces, and then harvested after 36 hours of culture for analysis. Morphologically, the former consisted of smaller, rounded keratinocytes with relatively high nuclear-to-cytoplasmic ratios (n/c ratios), while the latter was composed of larger, polyhedral cells with more irregular contours and lower n/c ratios. ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) analysis disclosed globally increased chromatin accessibility in cells cultured in the bioprinted rete ridge-like niches, with nonuniform degrees of peak enrichment across genetic sites. Peaks demonstrating >10-fold enrichment comprised <2% of total enriched peaks, with 22% localized within 2-3 kb of promoter regions, and included those mapping to known skin stem cell markers such as Wnt pathway members, TCF4, CD200 and integrin alpha-6, as well as NOTCH pathway mediators and S100 proteins that regulate skin differentiation. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses of these peaks highlighted potentially biologically relevant pathways such as those related to Ras and ErbB signaling, as well as processes involving actin filament/cytoskeleton organization and cell adhesion, both of which are critical components of signal mechanotransduction. This work suggests that niche topography alone dynamically regulates the identity of its resident stem cells and at least partially maintains their stem-like state—providing insight into the potential role of niche architecture in normal skin homeostasis as well as in pathological settings where niche structure may be altered or lost, as in psoriasis and chronological aging, respectively.

Funding source: This study is supported by the Harvard Stem Cell Institute (HSCI) Seed Grant. The authors have no financial interest to declare in relation to the content of this study.

Keywords: 3D bioprinting, stem cell niche, epigenetics

TSC164

POST-FASTING REFEEDING ENHANCES MOUSE INTESTINAL STEM CELL-MEDIATED REGENERATION AND TUMORIGENESIS

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Although short-term fasting interventions improve intestinal stem cell (ISC) function, little is known about how much of this benefit comes from fasting versus the post-fasting refeeding response. Given the central role that ISCs play in driving injury-induced repair and in initiating early intestinal tumors, it is possible that fasting by boosting ISC function not only improves regeneration but also unexpectedly elevates intestinal tumorigenesis. To understand how fasting/refeeding interventions influence the role of ISCs in regeneration and early tumor formation, we assessed the function of ISCs to repair the intestinal lining after injury and to contribute to intestinal tumors. Notably, we

find that 24 hours post-fasting refeeding boosts intestinal stem and progenitor cells proliferation and increases ISC function in organoid and in fate mapping assays compared to AL and fasted cohorts. Mechanistically, elevated mammalian target of rapamycin (mTOR) activity mediates the effects of refeeding in ISCs as treatment with mTOR inhibitor blocks these effects. Surprisingly, loss of the APC tumor suppressor gene in ISCs increased intestinal tumor numbers in the refed state compared to the AL and fasted states in an mTOR dependent manner. We propose that refeeding through mTOR signaling stimulates ISC function and in a context-dependent manner this can either improve repair after injury or promote intestinal tumorigenesis.

Keywords: intestinal stem cell function and tumorigenesis, post-fasting refeeding, mTOR signaling and protein synthesis

TSC167

ROBUST GENERATION OF A PURE HUMAN AIRWAY EPITHELIAL BASAL STEM POPULATION FROM INDUCED PLURIPOTENT STEM CELLS

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Chronic lung diseases are characterized by a remodeled, dysfunctional airway epithelium. Moreover, evidence is emerging that the generation and sustainment of this remodeled epithelium is through disease programming of the basal airway stem cells. This disease programming of basal cells is genetic and/or epigenetically acquired through the disease environment. As such the production and transplant of genetically corrected basal stem cells, purged of disease-based epigenetic programming, presents a potential therapeutic strategy for chronic lung diseases. The generation of patient-specific, genetically corrected, and epigenetically pure airway basal cells from induced pluripotent stem cells (iPSCs) would represent an important step toward airway regeneration therapies. Stepwise differentiation protocols have been developed which specify lung progenitors, including airway basal cells, however, the directed generation of a pure airway basal cell population has not been achieved. The source cells for iPSC reprogramming we used were airway basal epithelial cells. Basal cells were reprogrammed with a virus-, DNA-, and integration-free RNA-based method. Successfully generated iPSCs expressed pluripotency markers, exhibited unlimited proliferative potential (passaged 30 times to date), and were capable of generating all three germ layers. Through modification of existing stepwise protocols, we directed lung progenitor organoids to differentiate into proximal airway cells. Pure airway basal cells were further specified and procured, without the aid of genetic manipulation, through fibroblast feeder culture of these proximal airway cells and the use of SMAD pathway inhibition. The induced basal cells (iBCs) were phenotypically identical to basal cells from which the iPSCs were derived and have been passaged 7 times without loss of basal cell markers. Importantly, these iBCs can be differentiated into a highly consistent pseudostratified epithelium via standard ALI differentiation. In summary, we have developed an approach to consistently and robustly generate a pure population of airway basal cells capable of producing a normal mucociliary epithelium. These methods represent a significant advance in the pursuit for airway epithelium regeneration to treat chronic lung diseases.

Keywords: airway epithelial cells, induced pluripotent stem cells, induced basal cells

TSC182

THERAPEUTIC REPROGRAMMING OF THE MAMMALIAN INTESTINAL STEM CELL STATE VIA HIPPO SIGNALING

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The intestine is intricately regulated by crosstalk between the Hippo and Wnt signaling pathways to control epithelial cell proliferation and differentiation. While the Hippo transcriptional coactivator YAP is considered oncogenic in many tissues, its modulation of the Wnt signaling pathway in intestinal homeostasis and colorectal cancer (CRC) remains controversial. Here, we demonstrate that the Hippo kinases Lats1/2 and Mst1/2, which negatively regulate YAP activity, are required for maintaining Wnt signaling and canonical stem cell function in the gut epithelium. We find that Hippo inhibition and consequent YAP activation lead to the emergence of a distinct epithelial cell state marked by Klf6 expression. Notably, loss of Lats1/2 or overexpression of YAP is sufficient to reprogram Lgr5+ cancer stem cells to this Wnt-low repair state and thereby suppress tumor growth in organoids, patient-derived xenografts, and mouse models of primary and metastatic CRC. Finally, we demonstrate that the genetic deletion of YAP and its paralog TAZ is able to support the growth of these tumors. Collectively,

our results establish the role of YAP as a tumor suppressor in the adult colon and implicate Hippo kinases as therapeutic vulnerabilities in colorectal malignancies.

Funding source: This study is funded in part by NIH T32GM007226-41 and F31CA235893 to P.C. and NIH R01 DK099559 and R01 AR064036 to F.D.C.

Keywords: Hippo signaling, Intestinal stem cell, Colorectal cancer

TSC184

THE EFFECT OF DRUGS THAT INDUCE E2A ACTIVITY ON PANCREATIC CANCER STEMNESS

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Pancreatic ductal adenocarcinoma (PDA) has a 5-year survival rate of only 9 percent, presenting the desperate need to better understand the molecular mechanisms involved in pancreatic cancer progression. Mutations in Kras, P53, P16, and SMAD are major drivers of PDA progression. Additionally, PDA is characterized by high levels of the stem cell driver MYC and dysregulation of the retinoblastoma (RB) tumor suppressor. Together, these aberrations promote tumorigenesis through uncontrolled proliferation and dedifferentiation. Previous research in our lab discovered that the bHLH transcription factor E2A gene is repressed in PDA progression and that restoration of E2A activity induced growth arrest through downregulation of MYC and upregulation of P21, which subsequently promoted RB activity. Simultaneously, this corresponded to a loss of stem cell-like characteristics and induced cellular differentiation. Thus, a screening platform to identify drugs that induce E2A activity was developed. The FDA-approved drugs Pitavastatin and Vorinostat were identified in the screen as inducers of E2A activity. Here, we are investigating the effectiveness of the two drugs, individually and in combination, to control tumorigenesis and the cancer stem cell population in PDA. We found in vitro that the drugs function in synergy to downregulate MYC expression and induce P21 expression, which restores RB activity, significantly depleting cell growth. Moreover, the drugs required E2A, or its homolog HEB for p21 expression in multiple PDA cell lines. To determine whether the drug combination will halt PDA progression in vivo, we are conducting mouse xenograft studies. Tumor size will be measured and immunohistochemistry will be used to assess tumors for markers of cell cycle progression, stemness, and dedifferentiation. We expect that drugs promoting E2A activity in PDA will demonstrate an advantage in reducing the stem cell phenotype in PDA.

Funding source: Funding is provided by the California Institute for Regenerative Medicine.

Keywords: Pancreatic Ductal Adenocarcinoma, bHLH transcription factor, Cancer stem cells

EYE AND RETINA

TSC398

BIO-MECHANICAL FORCES MODULATE HUMAN AND MOUSE CORNEAL EPITHELIAL STEM CELL FATE.

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The corneal epithelium is the outermost layer covering the eye. Its transparency, high accessibility and compartmentalization for stem cells (SCs) and differentiated cells makes cornea an excellent model for SC research. Recently, we established an in-vivo microscopy approach to identify corneal (limbal) SC using K15-GFP transgene and discovered that depletion of the entire SC pool is restored by committed cells which de-differentiate into bona-fide SCs. Contrarily, niche destruction resulted in irreversible SC loss and corneal opacification. However, the mechanism of SC differentiation and reprogramming of committed cells remained unclear. Interestingly, the limbal stromal niche contains unique extracellular matrix hallmarked by low rigidity, while differentiation compartment is much stiffer, suggesting that differentiation, as well as the de-differentiation of committed corneal cells, may be linked with mechano-sensing. Indeed, cultivation of limbal SCs on substrates with physiologically relevant niche stiffness supported stemness while increased stiffness induced cell differentiation. Differentiation was coupled with changes in cytoskeletal organization and translocation of YAP from the nucleus to the cytosol. Importantly, pharmacological inhibition of mechanotransduction by Blebbistatin or ROCK inhibitor significantly attenuated cell differentiation while forced activation of mechanotransduction had an opposite effect in-vitro and in-vivo. Altogether, we propose that mechano-sensing of matrix rigidity by epithelial cells play a vital role in regulating SC fate choices and cell plasticity under homeostasis and injury.

Funding source: European Union's - Seventh Framework Programme (FP7/2007-2013]);
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Keywords: Limbal stem cells, Mechanotransduction, Cornea

HEMATOPOIETIC SYSTEM

TSC210

THE ROLE OF CALCIUM IN HEMATOPOIETIC ONTOGENY

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Hematopoietic Stem Cells (HSCs) reside in the bone marrow and generate blood cells throughout life. Our lab recently discovered that HSCs from adult bone marrow (ABM HSCs) are endowed with low intracellular calcium and are maintained in low calcium environments both in vitro and in vivo (Luchsinger et al., Cell Stem Cell, 2019), opening novel avenues for genetic manipulation and autologous graft conditioning. ABM HSCs derive from fetal liver (FL) HSCs and while both populations are capable of multilineage hematopoiesis they differ in key aspects such as cell cycle status, transcription factor expression, immunophenotype, lineage bias, and metabolic profile, raising the question of how HSCs at earlier developmental stages handle calcium. We employed single-cell perfusion live imaging of calcium dyes and flow cytometry of genetically-encoded calcium indicators to show that FL HSCs possess approximately two-fold increased intracellular calcium from ABM HSCs. Perfusion live imaging also showed increased store-operated calcium entry (SOCE) and decreased calcium efflux through Plasma Membrane Calcium ATPases (PMCAs) from fetal to adult stages. These changes in calcium handling were mirrored in gene and protein expression, where FL HSCs display a decrease in expression of the PMCA gene Atp2b4, a decrease in pan-PMCA protein expression, and an increase in expression of the SOCE gene Stim1. FL HSCs also showed increased nuclear localization of the transcription factor NFAT1, which translocates to the nucleus upon dephosphorylation by the calcium-dependent phosphatase Calcineurin, consistent with increased calcium of FL HSCs. While ABM HSCs are maintained in low calcium in vitro, FL HSC maintenance was not improved in low calcium, showing a difference in response to extracellular calcium between populations. Furthermore, only ABM HSCs cultured in low extracellular calcium retained NFAT1 in the cytoplasm, while high calcium ABM HSC and all FL HSC cultures showed increased NFAT1 nuclear localization, which correlates to the maintenance of stem cell function in vitro. These data indicate profound, developmentally regulated changes in calcium physiology between FL and ABM HSCs, which may potentially explain other functional differences between these two populations.

Keywords: Hematopoietic Stem Cells, Calcium, Development

TSC212

WNT9A-FZD9-EGFR SIGNALING IN HUMAN HSPC DEVELOPMENT

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Hematopoietic stem cells' (HSCs) ability to self-renew and differentiate into all mature blood and immune cells has made HSC transplantation a standard surgical procedure in treating blood cancers and disorders. However, finding human leukocyte antigen (HLA) matching donors and obtaining sufficient cells remains an issue. Significant advances have been made towards a better understanding of the molecular cues involved in vertebrate HSC development, yet many gaps exist and, to date, it has not been possible to generate true HSCs from pluripotent progenitors in a dish. Using zebrafish as a model system, we recently identified a role for Wnt signaling in HSC development involving a specific interaction between the ligand Wnt9a, the receptor Frizzled9b (Fzd9b) and the coreceptor epidermal growth factor receptor (EGFR). This interaction is conserved for the human homologs, WNT9A and FZD9. Perturbing WNT9A-FZD9 signaling through gene knockdown and overexpression significantly impacts differentiation of human pluripotent stem cells (hPSC) to hematopoietic stem and progenitor cells (HSPCs) as determined by flow cytometry of the cell surface proteins CD34 and CD45. In our current studies, we are further investigating the role and timing of the WNT9A-FZD9 signal during hPSC differentiation into the hematopoietic lineage. To examine the effect of EGFR on WNT9A-FZD9 signaling during differentiation of hPSCs to HSPCs, we are treating cells with EGF to activate and with AG1478 to inhibit EGFR signaling. Two hPSC reporter lines, one expressing SOX17-GFP to mark hemogenic endothelium and a second expressing RUNX1c-GFP to mark HSPCs, were used to monitor acquisition of hematopoietic characteristics during HSPC differentiation. Findings in this study will contribute to a deeper understanding of WNT9A-FZD9-EGFR signaling in HSPC development, as well as provide insight into mechanisms regulating WNT9A binding and signaling specificity. The long-term goal of these studies is to develop a robust differentiation scheme that will yield bona fide HSCs in a dish.

Funding source: California Institute for Regenerative Medicine

Keywords: Wnt signaling, Hematopoietic stem cells, Hematopoiesis

TSC216

PROM1/CD133 AND MLLT3-DRIVEN PROGRAM ARE LANDMARKS OF HUMAN HEMATOPOIETIC STEM CELL FUNCTIONAL MATURATION DURING EMBRYOGENESIS

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Generation of HSCs from human pluripotent stem cells yields defective HSCs that lack robust engraftment ability, therefore useless for therapeutic applications. To overcome this roadblock, we set out to define the molecular landmarks of HSC functional maturation in the human embryo. HSC emerge in the aorta-gonad-mesonephros (AGM) and reside in the aorta and extraembryonic vessels around weeks 4-5. Early HSC possess little potential for engraftment, but they undergo functional maturation in following weeks, resulting in highly proliferative and engraftable HSC. We generated a single-cell RNA-seq map of hemato-vascular cells from human embryonic and fetal hematopoietic tissues. Using a pre-defined HSC molecular signature, we identified CD34+ CD90+ RUNX1+ HOXA+ MLLT3+ SPINK2+ HLF+ cells as HSCs throughout development. HSC were detected in 4 week AGM, placenta, yolk sac, umbilical cord and vitelline vessels, but not in heart, head and liver and colonized the liver at 6 weeks. Interestingly, 4 weeks placental HSC showed higher similarity to 6 weeks liver HSC than to HSC in other tissues of the same age, nominating placenta as a niche for initial HSC maturation. Comparison of the molecular profiles of HLF+ HSCs throughout embryonic and fetal development documented loss of early HSC markers (ITGA2B) and endothelial features (e.g. VE-Cadherin) and gradual appearance of mature HSC surface proteins (e.g. PROM1/CD133, MHC). Flow cytometry confirmed the expression of these proteins, providing a surface marker toolbox for human HSC maturation. CD133, an established adult HSC marker, was absent in early HSCs (4-5 weeks) but became expressed over time in a subset of HSPCs, which uniquely possessed higher engraftment ability. Thus, CD133 expression associates with transplantability during HSC maturation. Importantly, the transcriptional regulator MLLT3, which governs HSC stemness, and its downstream gene expression program, including HLF and MSI2, were upregulated during HSC maturation. This data-set provides a unique reference map for identification of functional HSCs and, ultimately, for generating them in a dish for transplantation.

Funding source: Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA Jonsson Comprehensive Cancer Center

Keywords: HSC, Hematopoietic, Engraftment

TSC223

TRANSCRIPTIONAL, PROTEIN-LEVEL AND FUNCTIONAL PROFILING OF HUMAN FETAL LIVER (FL)-DERIVED HEMATOPOIETIC STEM CELLS (HSCS) AT SINGLE CELL RESOLUTION

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The complex and tightly regulated process of human hematopoietic development culminates in the production of hematopoietic stem cells (HSCs), which subsequently undergo expansion in the fetal liver (FL). The establishment of a high-resolution molecular signature of FL-HSCs would provide insights into HSC biology with potential utility in the purification and expansion of engraftable HSCs ex vivo and the generation of HSCs from pluripotent cell sources. To profile HSCs at this developmental stage, we performed CITE-Seq, a technique that combines single cell RNA sequencing (scRNAseq) and cell surface marker interrogation using oligo-tagged antibodies. To connect expression profiles with functional engraftment, we have coupled this with transplantation assays in immunocompromised mice. In these studies, three populations of human FL cells were used: CD34- cells, CD34+ cells and CD34+ cells further enriched by expression of GPI-80, a marker tightly linked to engraftment potential, to acquire additional resolution of HSCs capable of long-term engraftment. These populations were stained with a panel of oligo-tagged antibodies, processed via the 10X platform, and sequenced (26.582 total cells). Simultaneous transplantation experiments of these three populations revealed superior engraftment potential of the CD34+GPI-80+ fraction, and thus enrichment for bona fide HSCs at the functional level. This coincided with enrichment for known HSC markers such as ITGA6 (CD49f), PROCR (EPCR), PROM1 (CD133), CD164, MLLT3,

HLF and HMGA2 at the transcriptional level, adding confidence to the validity of the data set. As such, by profiling profiling >7000 GPI-80+CD34+, we have achieved unprecedented resolution of the engraftable HSC compartment within the FL. Multi-modal analysis of this data allows for the simultaneous study of transcriptional and protein level expression of key HSC-related genes and their distribution.

Keywords: hematopoietic stem cells, single cell RNA sequencing, fetal liver

TSC225

PROFILING HEMATOPOIETIC-STROMAL CROSSTALK BY SPATIALLY RESOLVED SINGLE-CELL RNA SEQ

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Spatial transcriptomics encompasses a rapidly advancing set of techniques aimed at resolving the spatial organization of mRNA expression down to the micrometer scale. Virtually all of these techniques, however, rely on generation of tissue sections, impeding faithful reconstruction of a 3D mRNA expression map and providing only a static snapshot of the spatial organization. Intravital microscopy (IVM), on the other hand, provides both 3D and dynamic imaging data, enabling real-time visualization of disease progression and response to treatment that can be spatially and temporally heterogeneous. Using IVM of the mouse calvarium, we have observed the earliest stage of AML proliferation after seeding the bone marrow (BM), from single cells to small clusters, with striking heterogeneity. AML proliferation is confined to BM locations with a distinct bone remodeling activity, while AML cells in other locations do not proliferate. To examine whether cross-talk with the BM microenvironment impacts AML cell fate, we have developed a new experimental platform to differentially profile the BM compartments that support AML quiescence vs. proliferation. The platform enables precise femtosecond laser etching of bone to create a microchannel, through which a micropipette is inserted to aspirate the rare leukemic cells and the surrounding CXCl12+ stromal cells for analysis using the SMARTseq2 protocol. Alternatively, to provide a more comprehensive assessment of the local microenvironment, we aspirate up to a few thousand BM cells for parallel scRNA-seq utilizing the high-throughput, droplet-based 10x genomics platform. The procedure is carried out under image guidance, using video-rate intravital multi-photon microscopy to identify and aid the extraction of cells with specific dynamic properties from their target locations. This experimental platform integrates the spatial and temporal information provided by IVM with the high content molecular information provided by scRNAseq, and defines a new approach for spatially-resolved single-cell analysis. We expect the platform will be useful for the discovery of novel targets for therapeutic intervention of AML as well as profiling of the microenvironment governing hematopoietic stem cell differentiation and proliferation during native hematopoiesis.

Keywords: intravital imaging, spatial transcriptomics, leukemia

TSC228

OPTIMIZED CULTURE MEDIUM FOR ENHANCED EX VIVO EXPANSION OF HUMAN HEMATOPOIETIC STEM CELLS

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Development of ex vivo culture systems to expand human hematopoietic stem-progenitor cells (HSPCs) remains a critical translational research goal that would enhance clinical hematopoietic stem cell (HSCs) transplantation and gene therapies. To address the problem that HSCs generally die or differentiate rapidly in current ex vivo culture media, we developed a xeno-free, serum-free medium – CTS™ StemPro™ HSC Expansion Medium with the goal of maximizing ex vivo expansion of HSCP immunophenotypes. Culture of normal primary human CD34+ cells purified from cord blood, mobilized peripheral blood (mBP) and bone marrow in HSC Expansion Medium supplemented with FLT3L, SCF, TPO, IL3, and IL6 (FKT36), resulted in increase in immunophenotype-defined HSPCs, as compared to day 0 cells. For example, culture of primary human CD34+ cells from mPB for 7 days in FKT36-containing HSC Expansion Medium resulted in ~100-fold increased numbers of CD34+CD45+Lin- and ~2000-fold increase in CD34+Lin- CD90+CD45RA- cells as compared to day 0. CD34+ cells expanded in this medium maintained differentiation capacity in in vitro colony forming assays, forming erythroid and non-erythroid cell colonies. In a directed

differentiation method, CD34+ cells were able to differentiate into erythroid lineage which generated RBCs at > 80% efficiency as indicated by co-expression of CD71 and CD235a markers. Also, CTS™ StemPro™ HSC Expansion Medium enables transduction of CD34+ cells with lentiviral vector generated using CTS™ LV-MAX System. Additionally, this medium supports gene editing CD34+ cells with CRISPR-Cas9 Gene editing tools that are suitable for cell and gene therapies. Following gene editing, CD34+ cells maintained the CD34+CD90+ CD45RA- phenotype and were capable of differentiating into granulocyte, macrophage and erythroid progenitors. Crucially, CD34+ cells expanded with CTS™ StemPro™ HSC Expansion Medium exhibited long-term engraftment potential and multilineage chimerism at 6 months in a murine model. We demonstrated that ex vivo-cultured CD34+ HSPCs generate cell dose-dependent engraftment in NRG mouse bone marrows and spleens at 26 weeks post-transplant. By expanding HSPCs that are well-suited for transplantation and gene therapies, CTS™ StemPro™ HSC Expansion Medium may be a key tool for clinical HSC applications.

Keywords: Hematopoietic stem cells (HSC), HSC cell culture media, hematopoietic stem-progenitor cells (HSPC)

TSC233

SIMULTANEOUS LINEAGE AND TRANSCRIPTIONAL PROFILING OF HEMATOPOIETIC STEM CELLS UNVEILS CLONE-AUTONOMOUS REGULATION OF SELF-RENEWAL

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A group of extremely rare cells in the bone marrow, termed long-term hematopoietic stem cells (LT-HSCs), have the capacity to regenerate the entire hematopoietic compartment after transplantation. However, in the unperturbed native context, self-renewing LT-HSCs remain relatively inactive for up to a year of mouse lifespan (relatively into old age), contributing only to the megakaryocyte lineage. Strangely, the fundamental properties of stem cells appear to be different in unperturbed vs. transplantation hematopoiesis. Yet, LT-HSC self-renewal after transplantation has not been addressed at the single cell level, and the molecular details underlying this regenerative process are still undefined. Recent advances in single cell expression profiling provide a means to understand the cell states that HSCs transition during transplantation. However, the destructive nature of these assays prevents simultaneous observation of stem cell state and function. To combine these two readouts in the same assay, we used the LARRY (Lineage And RNA RecoverY) lentiviral barcoding library, which labels cells with DNA barcodes that can be detected common single cell RNA-seq platforms. We barcoded thousands of single adult HSCs and investigated their clonal trajectories during bone marrow reconstitution in vivo. We found that, surprisingly, the majority of the LT-HSC compartment was reconstituted by relatively inactive and Mk-biased clones, resembling the native properties of the LT-HSC compartment. Random subsampling and serial transplantation of barcoded clones demonstrated that inactive LT-HSCs were also more likely to regenerate secondary recipients. Furthermore, we unveiled an intrinsic molecular signature that characterizes functional long-term repopulating behavior, solely based on functional profiling and irrespective of clustering methods. We probed this signature through in vivo CRISPR screening and found the transcription factor Tcf15/Paraxis to be required, and sufficient, to drive HSC long-term self-renewal. In situ, Tcf15 expression labels the most primitive subset of true HSCs. In conclusion, our work elucidates intrinsic molecular programs associated with functional stem cell heterogeneity, and identifies a mechanism for the maintenance of the native-like quiescent state.

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Keywords: Hematopoietic stem cell self-renewal, Single cell lineage tracing, Tcf15

TSC401

A CORRELATIVE LIGHT AND ELECTRON MICROSCOPY APPROACH TO DEFINE THE ADULT HEMATOPOIETIC STEM CELL NICHE IN ZEBRAFISH

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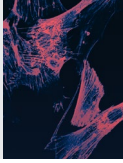
Hematopoietic stem and progenitor cells (HSPCs) that originate from the hemogenic endothelium in the dorsal aorta finally home and engraft within the fetal bone marrow (BM) and remain quiescent until they expand and differentiate to supplement the blood system. While it is known that HSPCs receive signal from surrounding niche cells, the ultrastructure of this complex microenvironment is not well defined, as current imaging technology does not allow direct visualization of the fetal BM niche. Zebrafish have a similar hematopoietic ontogeny to



mammals, and because the embryos are transparent, intrinsic HSPC interactions with the niche can be directly visualized. To track HSPCs during colonization of the presumptive adult niche, the kidney marrow (KM), we used our previously validated HSPC-specific transgenic reporter lines (Runx:GFP and Runx:mCherry). In 5 days post fertilization fixed larvae, we detected ~100 HSPCs/larva. To precisely locate these rare HSPCs within the larger dense KM, we genetically tagged endogenous HSPCs to track them live using lightsheet microscopy, followed by high resolution serial block-face scanning electron microscopy (SBEM). Using this technique, we could visually track single mCherry+ HSPCs, then confirm their exact location in a SBEM dataset with high contrast APEX2 peroxidase label. We found HSPC clusters within vessel lumens, as well as a novel perivascular niche with quiescent HSPC in a defined cellular assembly. In this perivascular site, a single HSPC was seen to simultaneously contact one mesenchymal stromal cell, multiple endothelial cells, a glial-like cell and other hematopoietic cells. The glial-like cells were dopamine beta hydroxylase positive (dbh+) cells that formed contacts with the hematopoietic clusters and single lodged HSPCs. Treatment with small molecules inhibitor of dopamine led to significantly reduced HSPC numbers within the niche. Our technique can be used as a general approach to identify the ultrastructure of single rare cells within dense tissues by using multiple imaging modalities. Further, this approach led to the identification of the previously uncharacterized cell type within the adult KM niche and we can now also identify novel intercellular structures that form between an unperturbed HSPC in its endogenous perivascular niche.

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Keywords: Hematopoietic stem cell niche, Imaging, Ultrastructure

TSC404

THE NEUROTRANSMITTER RECEPTOR GABBR1 REGULATES PROLIFERATION AND FUNCTION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Hematopoietic stem and progenitor cells (HSPCs) have multi-lineage potential and can be used in transplants as a curative treatment for various hematopoietic diseases. HSPC function and behavior is tightly regulated by various cell types and factors in the bone marrow (BM) niche. One level of regulation comes from the sympathetic nervous system that innervates the niche and releases neurotransmitters to stromal cells. However, the direct regulation of HSPCs via cell surface expression of neural receptors has not been functionally explored. Using imaging mass spectrometry, we detected strong and regionally specific gamma-aminobutyric acid (GABA) neurotransmitter signal in the endosteal region of mouse BM. GABA type B receptor subunit 1 (GABBR1) is known to be expressed on human HSPCs, however its function in their regulation has not been investigated. Based on published mouse HSPC single cell RNA-seq data, we found that a subset of HSPCs expressed Gabbr1. We confirmed by surface receptor expression that a subset of mouse BM HSPCs express Gabbr1 protein. Using the single cell RNA-seq data as above, our own gene set enrichment analysis revealed positive correlation of Gabbr1 expression with genes involved in immune system processes, such as response to type I interferons. We generated a CRISPR-Cas9 Gabbr1 mutant mouse model on a C57/BL6 background suitable for hematopoietic studies. Analysis of Gabbr1 mutant bone marrow cells revealed a reduction in the absolute number of Lin-Sca1+cKit+ (LSK) HSPCs, but no change in the number of long-term hematopoietic stem cells (LT-HSCs). We also discovered reduced numbers of white blood cells in peripheral blood that was primarily due to fewer B220+ cells. We show that Gabbr1 null HSPCs display reduced proliferative capacity, as well as diminished reconstitution ability when transplanted in a competitive setting. An in vitro differentiation assay revealed the impaired ability of Gabbr1 null HSPCs to produce B cell lineages. We tested our predicted association with type I interferon response by administration of poly(I:C) and found reduced HSPC proliferation in Gabbr1 null mice. Our results indicate an important role for Gabbr1 in the regulation of HSPC proliferation and differentiation, highlighting Gabbr1 as an emerging factor in the modulation of HSPC function and behavior.

Keywords: HSPC, Neurotransmitter, Function

IMMUNE SYSTEM

TSC382

MICROGLIA REPLACEMENT THERAPY IN EXPERIMENTAL AUTOIMMUNE ENCEPHALITIS (EAE) MOUSE MODEL DEMONSTRATES DECREASED DISEASE SEVERITY

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Microglia have been shown to play a key role in the demyelination of axons in multiple sclerosis (MS) pathogenesis. One potential mechanism is that demyelination is associated with activated microglia that may promote oxidative damage to oligodendrocytes and neurons. Experimental Autoimmune Encephalomyelitis (EAE) is a widely used model for elucidating the immunological mechanisms in MS. Here, we used a novel microglia replacement protocol which demonstrates that bone marrow transplantation (BMT), followed by administration of a CSF1R inhibitor, allows for engraftment of circulation-derived myeloid cells (CDMCs) into the brain that exhibit microglia-like properties. We tested this protocol and observed its clinical effects on EAE induced mice.

In our methods, eight female BL/6 mice were immunized with Hooke Kit™ MOG35-55/CFA Emulsion PTX. Four mice were randomly allocated to our treatment group that underwent Busulfan + BMT treatment followed by a PLX5622 (CSF1R inhibitor) diet. The other 4 control mice underwent EAE induction only. Two observers independently graded the mice using an established EAE clinical score guide.

Peak severity of disease occurred 13-15 days post immunization, with the highest clinical grading of 3.5 (limp tail, complete paralysis of hind limbs, unable to right itself when placed on side). The mice were then scored 10 days post withdrawal of PLX5622. In our preliminary data, the control group had a median score of 1.5 while the treatment group had a median score of 0.5. A score of 0.5 correlates with limpness in tip of tail only, while 1.5 correlates with entire tail limpness and hind leg inhibition.

These early results demonstrated decreased disease severity when BL/6 mice were treated with BMT followed by PLX5622. As there has not been a similar study examining the combined effects of BMT with the inhibition of the CSF1R pathway in EAE induced mice, these initial results demonstrate decreased disease severity which will be correlated with radiological evidence (MRI) and histopathology in further studies.

Keywords: microglia, multiple sclerosis, bone marrow transplant

MUSCULOSKELETAL

TSC245

THE ONTOGENY OF HUMAN SKELETAL MUSCLE ACROSS DEVELOPMENT IN VIVO AND DURING HPSC DIFFERENTIATION IN VITRO

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Skeletal muscle is the largest tissue in a human body, and its malfunction causes many diseases such as muscular dystrophies and metabolic disorders. To better understand skeletal muscle development and specification in human, we profiled in vivo skeletal muscle tissues from human embryonic, fetal to adult stages using single cell RNA-sequencing (scRNA-seq). We identified myogenic as well as other cell types and constructed a "roadmap" of human skeletal muscle ontogeny across development. Moreover, we also observed subsets of myogenic cells representing different muscle commitment status (stem/progenitor vs. myoblast/myocyte) and identified cell surface markers that could enrich distinct subpopulations. Interestingly, at late embryonic and early fetal stages, we found a myogenic subpopulation that possesses higher osteogenic lineage potential when isolated and cultured in vitro. Due to their infinite self-renewal capacity and broad lineage specification

potential, human pluripotent stem cells (hPSCs) represent a promising source to generate skeletal muscle stem/progenitor cells (SMPCs) to regenerate patients' lost muscles in various diseases. However, currently available hPSC directed myogenic differentiation protocols result in highly heterogeneous cell populations containing non-myogenic cells as well as skeletal muscle cells with unknown maturation status compared to their in vivo counterparts. Therefore, we also single-cell profiled the heterogeneous cell cultures generated from multiple hPSC myogenic differentiation protocols, and identified hPSC-derived myogenic populations and subpopulations as well as non-myogenic cells present in the cultures. Using our human myogenesis "roadmap", we mapped hPSC-SMPCs to an embryonic-to-fetal transition period during human development. Moreover, we found differentially enriched biological processes and discovered co-regulated gene networks and transcription factors (TFs) present at distinct myogenic stages. Current work is focused on manipulating the differentially enriched TFs to mature hPSC-SMPCs for better functional capabilities. In summary, this work serves as a resource for advancing our basic knowledge of human myogenesis as well as for improving the generation of hPSC-SMPCs for clinical benefit.

Funding source: CIRM Quest (DISC2-10696); NIAMS (R01AR064327)

Keywords: skeletal muscle, single cell RNA-sequencing, development and stem cells

TSC250

PROMOTION OF MYOBLAST DIFFERENTIATION BY FKBP5 VIA CDK4 ISOMERIZATION

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Fkbp5 is a widely expressed peptidyl prolyl isomerase that serves as a molecular chaperone through conformational changes of binding partners. Although it regulates diverse protein functions, little is known about its roles in myogenesis. We found here that Fkbp5 plays critical roles in myoblast differentiation through two mechanisms. First, it sequesters Cdk4 within the Hsp90 storage complex and prevents the formation of the cyclin D1/Cdk4 complex, which is a major inhibitor of differentiation. Second, Fkbp5 promotes cis-trans isomerization of the Thr172-Pro173 peptide bond in Cdk4 and inhibits phosphorylation of Thr172, an essential step for Cdk4 activation. Consistent with these in vitro findings, muscle regeneration is delayed in Fkbp5-/- mice. The related protein Fkbp4 also sequesters Cdk4 within the Hsp90 complex but does not isomerize Cdk4 or induce Thr173 phosphorylation despite its highly similar sequence. This study demonstrates protein isomerization as a critical regulatory mechanism of myogenesis by targeting Cdk4.

Keywords: myogenesis, isomerization, Fkbp5

TSC258

POLY(E-CAPROLACTONE) AND POLY(E-CAPROLACTONE)-POLY(L-LACTIC ACID) SHAPE MEMORY POLYMERS AS SUITABLE SCAFFOLDS FOR IN VITRO OSTEOGENESIS OF CANINE MESENCHYMAL STROMAL CELLS

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Shape memory polymers (SMPs) are promising tissue engineering scaffolds for bone regeneration due to their ability to press-fit into irregular defects. While poly(E-caprolactone) [PCL] has been widely studied for its excellent biocompatibility, it degrades slowly. As such, SMP networks composed of PCL and poly(L-lactic acid) [PLLA] have been developed to accelerate degradation. The dog represents a strong translational model for orthopedic treatments; however, cytocompatibility and osteoinductivity of SMPs have not yet been assessed in the canine system. The objective of this study was to determine the ability of PCL and PCL-PLLA SMPs to support in vitro cell attachment, proliferation, and osteogenesis of canine mesenchymal stromal cells (cMSCs). cMSCs were seeded onto SMPs and cultured under control and osteogenic conditions. Cytocompatibility was assessed using lactate dehydrogenase (LDH) levels, live/dead staining, and proliferation via DNA quantification. Osteogenesis was assessed using alkaline phosphatase activity (ALP), alizarin red staining (ALZ), real-time quantitative PCR (qPCR) and scanning electron microscopy (SEM)/electron dispersion spectroscopy (EDS). Data were reported as mean +/- SD and analyzed using ANOVA with Tukey's post hoc test. Significance was established at $p<0.05$. cMSCs attached and proliferated on both SMP compositions with comparable LDH levels. cMSCs cultured on SMPs exhibited robust ALP activity in the presence of BMP-2. cMSCs deposited mineralized extracellular matrix in response to dexamethasone and BMP-2 as determined by ALZ and SEM/EDS. cMSCs cultured on PCL exhibited significantly increased ALP activity ($p=0.0007$) and ALZ staining ($p<0.001$) compared to cells cultured on PCL-PLLA. Osterix and Runx2 expression were decreased in response to dexamethasone, while osteocalcin

expression was increased in response to dexamethasone. SMPs are cytocompatibile and osteoinductive in conjunction with appropriate osteogenic stimuli. While PCL SMPs exhibit greater ALP activity and ALZ staining, PCL-PLLA compositions may be desirable for future studies due to their mechanical properties and accelerated degradation profile. This work will prove useful for investigators considering SMPs or cMSCs for pre-clinical translational bone tissue engineering studies.

Funding source: Funded by AKC-CHF, Bone & Joint Fund, and NIH 5T32OD011083-09 (SG).

Keywords: canine mesenchymal stromal cells, shape memory polymer scaffolds, tissue engineering

TSC261

NATURAL KILLER CELL INFILTRATION AND ALTERED STEM CELL METABOLISM DIFFERENTIATE HEALING AND NON-HEALING VOLUMETRIC MUSCLE LOSS DEFECTS IN MICE

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Traumatic or surgical loss of a critical muscle volume (known as volumetric muscle loss, or VML) is responsible for over 90% of muscle conditions that lead to long-term disability and presents a considerable financial burden. Various regenerative strategies have been tried but fail to recover more than 16% of functional deficits. While persistent inflammation has been shown to overwhelm the endogenous repair mechanisms from muscle stem cells (SCs), the networks of intercellular communication that drive the fibrotic phenotype are unclear. At this juncture, a more thorough understanding of the molecular and cellular players precluding regeneration is necessary to inform the development of novel regenerative therapies. To fill this need, we performed single cell RNA sequencing of all mononucleated cells at multiple time points after the administration of VML defects of various sizes into murine quadriceps to compare non-healing and healing injuries. We observed a population of interferon gamma (IFN-g)-expressing natural killer (NK) cells only in non-healing VML defects, which persists through 14 days post injury. IFN-g+NK cells have been shown to manipulate macrophage phenotype and inhibit the regenerative potential of SCs. Further, the trajectory of SCs following non-healing defects appeared altered, with reduced expression of quiescence-associated genes at later timepoints and increased expression of glycolytic enzymes, including phosphoglycerate mutase 2. We also observed concomitant downregulation of the adenosine monophosphate-activated protein kinase signaling pathway in SCs, which is a regulator of energy homeostasis. Finally, we extracted SCs after healing and non-healing VML defects and observed reductions in differentiation and fusion among SCs harvested from non-healing defects, suggesting that SCs become permanently impaired after VML. Collectively, this research provides fundamental insights into the cellular and molecular mechanisms driving skeletal muscle fibrosis and suggests how the MuSC or its niche may be targeted to improve regeneration. The enhanced understanding of signaling networks and stem cell metabolism has broader applications in therapeutic development for other fibrotic diseases, such as following myocardial infarction.

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Keywords: volumetric muscle loss, satellite cell niche, regeneration

TSC267

OPTIMIZATION OF A HUMAN MUSCLE TISSUE DECELLULARIZATION PROTOCOL FOR THE SEEDING OF SKELETAL MUSCLE PROGENITOR CELLS

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Endogenous skeletal muscle stem cells known as satellite cells (SC) in adults, and skeletal muscle progenitor cells (SMPCs) during fetal development are critical in the regeneration of muscle tissue after injury and during growth. Muscle stem cells have a remarkable ability to regenerate after injury and thus have potential for use in cell-based therapies in muscle disease or after injury. However, the use of stem cells as a potential therapy for muscle disease is currently not feasible due to the SMPCs low myogenic and engraftment potential in vivo. Human pluripotent stem cells (hPSCs) have the potential to generate new SMPCs but current protocols generate immature and less functional progenitors than adults SCs. One hypothesis we are testing is that the immature nature of SMPC prevents them from residing in the skeletal muscle niche which will be required to develop to an SC-like cell. Our preliminary results suggest that SMPCs and SCs express very different components of extracellular matrix (ECM) proteins. We theorize that the (ECM) is a key driver in SMPC to SC maturation due to its synergistic relationship with the myofibers which ae the main components of the SC niche. For this reason, we have optimized a human skeletal muscle decellularization strategy to obtain ECM only “myo-scaffolds” which will be used to determine if SMPCs can mature to SCs in different ECM microenvironments.

Keywords: Decellularization, Extracellular Matrix, Skeletal Muscle Progenitor Cells

TSC271

TRANSPLANTATION OF MUSCLE STEM CELL MITOCHONDRIA AUGMENTS THE BIOENERGETIC FUNCTION OF DYSTROPHIC MUSCLE IN MICE

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Mitochondrial dysfunction has been implicated in a variety of pathologies, including muscular dystrophies. Intriguingly, a remnant from the endosymbiotic mitochondrial origin theory, recent evidence suggests that mitochondria retain the ability to transfer intercellularly, and mitochondrial transplantation has been explored as a potential means for therapeutic intervention. During muscle regeneration, resident stem cells, also known as muscle satellite cells (MuSCs), undergo myogenic differentiation and fusion to form de novo myofibers or fuse with existing multinucleated myofibers. Leveraging this cell-cell fusion process, we postulated that donor MuSC mitochondria can be transferred to host muscle during muscle repair. Here, we report that when healthy donor MuSCs are purified and transplanted into dystrophic host muscle, mitochondrial network (reticulum) and bioenergetic function can be significantly enhanced. By transplanting MuSCs purified from mitochondrial reporter transgenic mice, we first demonstrate remodeling of the mitochondrial reticulum following MuSC fusion into the host myofiber. Specifically, myonuclei originating from fused MuSCs were adjacent to high densities of mitochondrial content, implying these MuSC-derived myonuclei regulate synthesis of mitochondria. To support this, mtDNA copy number and nuclear-encoded mitochondrial gene expression were upregulated following MuSC transplantation, further indicating MuSC-driven mitochondrial biogenesis. Along with increased mitochondrial content in transplanted muscle, we also report augmented mitochondrial function measured by greater oxygen consumption and ATP production rates in isolated single myofibers. Furthermore, when bioenergetically-enriched (exercised) donor MuSCs were transplanted, improvements in mitochondrial content and bioenergetic function were also observed. Conversely, these improvements were abrogated when we transplanted MuSCs with defective mitochondria. Overall, these data provide a novel approach to transfer mitochondria to pathological skeletal muscle in order to assist muscle function and regeneration.

Funding source: NIH National Institute of Arthritis and Musculoskeletal and Skin Disease, Regenerative Engineering and Medicine

Keywords: skeletal muscle, satellite cell, mitochondria

TSC273

REGENERATION OF TENDON PATTERN THROUGH THE RECRUITMENT OF PROGENITOR CELLS FROM NEIGHBORING CONNECTIVE TISSUES: A BLUEPRINT FOR THE IDENTIFICATION OF TENDON PROMOTING DRUGS

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Tendons and ligaments are crucial for movement and providing stability to our musculoskeletal system. Injuries to these tissues affect a significant portion of the population and can be caused by trauma, sports-related overuse, and aging. In many cases these injuries also lead to osteoarthritis and eventually the need for joint replacement surgery. However, we currently have a limited understanding of the pathways capable of inducing tendon and ligament fates, which can impede the development of directed differentiation strategies to generate tendon tissues from stem cells. Central to this challenge is to uncover the mechanisms regulating tendon regeneration. We found that zebrafish have robust abilities to regenerate tendon composition and pattern after ablation of all tendon cells and in an adult acute injury model. Using genetic lineage tracing and functional analysis, we determined that regeneration occurs through conserved developmental lineage relationships, and we identified a pathway necessary and sufficient to promote regeneration. Live imaging of tendon regeneration reveals that the neighboring connective tissues harbor reservoirs of progenitor cells that orchestrate regeneration at specific attachment sites to restore tendon pattern. To find novel tendon cell fate modulators, we performed a high-throughput chemical screen using transgenic zebrafish and a blastomere cell culture system. We screened 7569 small molecules and have validated 16 with the highest tendon/ligament promoting abilities. To demonstrate a conserved role for these compounds in mammalian cells, we treated mouse pluripotent ES cells with the hit compounds, and we observed increased expression of the tendon markers suggesting these drugs have conserved tendon promoting activities. Collectively, this work introduces a new platform to study tendon regeneration using the zebrafish model and establishes a framework to identify new potential

cell sources for tendon tissue engineering. The compounds that we discovered will lead to the identification of novel pathways involved in tendon formation and regeneration as well as provide the foundation for the development of therapies to improve tendon and ligament healing.

Keywords: Regeneration, Tendons, Zebrafish

TSC274

SKELETAL MUSCLE PROGENITOR AND MUSCLE STEM CELL STATES HAVE DISTINCT METABOLIC PROFILES ACROSS HUMAN DEVELOPMENT

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Skeletal muscle progenitor cells (SMPCs) and satellite cells (SCs) participate in developmental myogenesis and postnatal muscle regeneration, respectively. In the most prevalent muscular dystrophy, Duchenne Muscular Dystrophy (DMD), continuous damage from repeated muscle degeneration and regeneration causes SCs to become exhausted and dysfunctional, leading to severe muscle weakness. There is currently no cure for DMD. Differentiating human pluripotent stem cells (hPSCs) into SCs is valuable for developing cell therapies for DMD. However, current hPSC directed myogenic differentiation protocols result in embryonic/fetal-like SMPCs, and it is not known how to mature them to adult-like SCs. It is also unclear how SMPCs and SCs differ, especially in regard to metabolism which may regulate their functional states. Furthermore, hPSC-derived myogenic cultures are always heterogenous, comprised of multiple myogenic commitment states. Using single-cell RNA- and ATAC-sequencing, we identified distinct metabolic profiles between hPSC-SMPCs and tissue-derived SMPCs/SCs as well as cell surface markers identifying different hPSC-derived myogenic subpopulations. We found that hPSC-derived and fetal SMPCs express genes in central metabolic pathways (glycolysis, tricarboxylic acid cycle, and oxidative phosphorylation) more highly than postnatal SCs. We also found that these SMPCs have higher activities of enzymes in these pathways than adult SCs. We are investigating additional metabolic pathways to further delineate differences between the SMPC and SC metabolic states and identify small molecules or metabolites that can induce SMPC-to-SC maturation. Additionally, we found in hPSC-derived myogenic cultures that CD24 marks a more progenitor myogenic subpopulation, whereas CD266 marks a more differentiated subpopulation. Purifying hPSC-SMPCs using our CD24+CD266– sorting strategy and investigating the role of metabolism in SMPC-to-SC maturation are crucial for deriving adult-like SCs from hPSCs. This will facilitate generating and defining myogenic populations that ultimately can be used in treatments for DMD.

Funding source: UCLA Center for Duchenne Muscular Dystrophy Ruth L. Kirschstein National Research Service Award T32 AR065972, CIRM Grant DISC2-10695

Keywords: muscle, development, metabolism

TSC276

RAT MESENCHYMAL STROMAL CELLS (MSCS) AND SELF-FITTING SHAPE MEMORY POLYMERS (SMPS) FOR CRANIOMAXILLOFACIAL DEFECTS: AN IN VIVO PILOT STUDY

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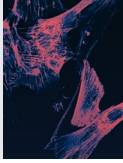
Shape memory polymers (SMPs) are tunable scaffolds that can be press-fit into irregular bone defects. SMPs may facilitate healing through osteoinductive and conductive mechanisms. When co-applied with mesenchymal stromal cells (MSCs), osteogenesis may also occur. The objective of this pilot study was to evaluate an SMP prototype with MSCs in a rat calvarial defect model. Rat MSCs (rMSCs) were isolated from marrow of 12 week old Wistar rats. Passage 2 cells were characterized and cryopreserved. 6 mm x 2 mm cylindrical SMPs were fabricated in a 75:25 ratio with poly((e-caprolactone) and poly(L-lactic acid), with or without polydopamine coating. Bilateral 5mm defects were created in 12 week old rats. Treatment groups included negative control (n=5 rats), or bone flap autograft, uncoated SMP, coated SMP, uncoated SMP + 3.5 x 104 rMSCs, and coated SMP + rMSCs (n=10 rats/group). Rats were terminated at 4 weeks and bone healing was assessed using quantitative µCT and ordinal histologic scoring (n=5 rats/group). Push-out testing was used to evaluate interfacial strength of SMPs and bone autografts with adjacent bone (n=5 rats/group). Quantitative data were reported as mean ± SD (µCT, push-out) or median and range (histology). MicroCT and mechanics data were analyzed using ANOVA, with Dunnetts test for µCT quantification and Tukeys test for mechanics. Significance was established at $p<0.05$. Rat cells met minimum criteria for MSCs. Cells were spindle-shaped, formed colonies in CFU assays, underwent adipogenesis, and exhibited ALP activity in early-stage osteogenic assays. Cells also expressed predicted CD surface markers. There were no significant differences in bone volume or surface area between negative control and SMP treatment groups. SMPs had



increased max load to failure in push-out tests when compared to bone flap autografts, although only the uncoated SMP (no cells) reached significance. Histologically, SMPs did not induce untoward inflammatory or foreign body responses and allowed both fibrous and osseous ingrowth and ongrowth. SMPs are biocompatible in rat calvarial defects and have acceptable biomechanical properties. SMPs, with or without polydopamine or rMSCs, did not improve bone healing. Additional studies are in progress to evaluate SMPs in larger defects with an increased number of rMSCs.

Funding source: NIH Award 1R01DE025886-01A1

Keywords: bone healing, rat calvarial defect, MSCs

TSC377

EVALUATION OF OSTEOBLAST DIFFERENTIATION SIGNALING PATHWAYS AS HUMAN MESENCHYMAL STEM CELLS UNDERO OSTEOGENESIS IN PRESENCE OF LOW OXYGEN FORM OF GRAPHENE NANOPARTICLES

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Human mesenchymal stem cells (hMSCs) are preferred for bone regeneration due to their ability to differentiate into osteoblasts. The success of novel biomaterials in bone tissue regeneration, is highly dependent on understanding the signaling pathway(s) that are triggered when hMSCs are seeded onto these materials. Therefore, the purpose of this study was to examine changes in gene expression when hMSCs undergo osteoblast differentiation on a specific form of graphene nanoparticles. Mesenchymal stem cells were extracted from human adipose tissue, and expanded in vitro. For experimental conditions, hMSCs were seeded on either a polystyrene plate in growth media supplemented with dexamethasone, ascorbic acid and beta glycerophosphate; or on substrates which were either sprayed or drop-coated with low oxygen containing graphene nanoparticles. After 21 days of culture, cells were harvested and total RNA was isolated. Subsequently, gene expression profiles on the 3 substrates were compared using RT2 Profiler PCR Human Osteogenesis Array. Qiagen Gene Globe software was used to determine the relative fold change in gene expression between the three substrates. Human MSCs cultured on graphene substrates showed increased expression of osteogenic-specific genes, ALPL, BMP2, COL1A1, and Osteocalcin, relative to the polystyrene surface suggesting that graphene nanoparticles enhance the osteogenic potential of hMSCs. Interestingly, both forms of graphene substrates (drop-coated or sprayed) showed similar osteogenic gene expressions, suggesting that the osteogenic potential is not effected by the method of creating the graphene films. Further analysis is needed to identify additional genes and their protein partners that are either up or down regulated when hMSCs interact with graphene nanoparticles. Identification of the key targets will help tailor graphene nanoparticles with a long term goal of enhancing bone regeneration and repair.

Funding source: This study was supported by NIH/NIAMS R15AR070460 and UT Center of Excellence in Livestock Diseases and Human Health.

Keywords: Osteogenesis, Graphene, Genes

TSC399

SITE-SPECIFIC LINEAGE TRACING OF ABERRANT MESENCHYMAL PROGENITOR CELL FATE

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Mesenchymal stem/progenitor cells (MSCs) have been shown to undergo differentiation into tenocytes, chondrocytes, osteoblasts/osteocytes, and adipocytes in vitro. Proof of aberrant differentiation of MSCs in vivo, however, has been limited. Traumatic injury, such as a burn or blast wound, can cause MSCs to undergo an altered fate decision towards ectopic bone, also known as heterotopic ossification (HO), which causes pain and joint contractures. Previous research has attempted to identify HO progenitor cells; however, these studies have limitations such as systemic Cre expression throughout the mouse, which may confound results. Recent research has found that Hoxa11 expressing cells are important in the development and healing of the zeugopod (tibia/fibula, ulna/radius region). It is hypothesized that using the Hoxa11-CreERT2;ROSA-TdTomato mouse will allow for the tracing of local MSCs progression through an altered fate towards HO within a specific anatomic region limiting confounding effects of global Cre expression. Mice were treated with tamoxifen at 6 weeks of age, chased for one week, then injured with an HO-forming Achilles' tenotomy with concurrent 30% total body surface area back burn. Single-cell RNA-sequencing (scRNA) was performed on cells from the uninjured hindlimb, one week and 6 weeks post injury. Injured and uninjured hind limb samples were harvested one week, three weeks, and nine weeks post-injury, sectioned for immunofluorescent histology, and imaged by confocal microscopy. scRNA analysis of all three time points showed 25 unique clusters. Tdtomato RNA transcripts (Hoxa11 lineage, Tdtom+) were expressed in five clusters. Individual time point

analyses and immunofluorescent histology (IF) showed Tdtom+ expression in two clusters characterized by MSC and tendon genes (Pdgfrα+, Prrx1+, Scx+) at baseline. Following injury, Tdtom expression shifted to a Sox9+, Acan+, Runx2+, and Spp1+ MSC cluster by scRNA and IF, suggesting a cell fate shift towards osteogenic/chondrogenic cell differentiation. Monocle pseudotime analysis of the Tdtom+ cells shows a distinct stem-like branch prior to injury and an osteogenic/chondrogenic branch following injury. The results suggest that the local Hoxa11+ stem cells have altered differentiation following injury leading to ectopic bone.

Funding source: NIGMS K08GM109105, NIH R01GM123069, NIH 1R01AR071379

Keywords: Hoxa11, Heterotopic Ossification, Mesenchymal Stem Cells

NEURAL

TSC281

PERIPHERAL NERVE-DERIVED PLURIPOTENT STEM CELLS AS A POTENTIAL TREATMENT OF SEGMENTAL BONE DEFECTS: A MOUSE MODEL

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A novel and quiescent population of cells derived from Schwann cells within peripheral nerves has recently been identified to express the four stem cell markers (Oct4, Sox2, Klf4, and c-Myc) when the nerve is physically injured or exposed to Bone Morphogenetic Protein-2 (BMP-2), which is a critical component in the development of bone tissues. These cells have been named Nerve-Derived Adult Pluripotent Stem (NEDAPS) cells. The primary objective of this study is to explore the differentiation potential of NEDAPS cells to osteoblastic cells and their clinical application for bone healing. Briefly, NEDAPS cells were cultured in stem cell medium or in osteoblast medium for seven days. These cells were labeled with PKH26 fluorescent cell membrane linkers before infusion into the facture sites of a mouse fibular fracture model. The animals were sacrificed at 1, 2, and 4 weeks following cell therapy. Results from this study indicated that NEDAPS cells can be induced to functional osteoblasts that are representative of the phenotypic and genotypic features of normal osteoblasts. The cell expressed genetic markers normally found at different stages of osteoblasts maturation by the process of RT-PCR. Functionally expressed proteins like type 1 collagen and alkaline phosphatase were additionally noted from the NEDAPS cells. Introduction of the NEDAPS cells and derived osteoblasts into the mouse fibular fracture model resulted in ubiquitous survival of the bone matrix-forming cells up to 6 weeks post-surgical intervention. Although the negative microCT analysis warrants further investigation, the newly identified NEDAPS cells holds great promise, if successful, in revolutionizing our understanding of tissue repair, the regeneration process, and will broaden therapeutic options for many types of traumatic and degenerative disorders.

Funding source: Supported by a WCGME Rising Star grant and by the Kansas INBRE, P20 GM103418

Keywords: Neural, Orthopedic, Novel

TSC282

SINGLE-CELL TRANSCRIPTOMIC PROFILING OF THE AGING MOUSE BRAIN

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Some degenerative changes of aging are reversible. Both intrinsic transcriptional regulation and soluble extracellular factors change over an animal's lifespan and can affect degeneration and regeneration across multiple tissues. Recent studies using parabiosis, plasma infusion and recombinant protein factors have shown that it is possible to restore at least some of these changes to a youthful state and improve target tissues. In the brain, multiple aging-related molecular pathways and secreted proteins have been identified that can impair function in young animals or restore function in aged animals, and that work via

multiple cellular mechanisms. To better understand the changes that occur within the aging nervous system, and the ways that these changes might be reversed, we performed single-cell transcriptomic analysis of young and old mouse brains. We quantified aging-related shifts in gene expression and transcriptional networks in nearly all brain cell types including stem cells, committed precursors and terminally differentiated populations along the neural, glial and oligodendrocytic lineages. We also catalogued ligand-receptor interactions between each pair of cell types including those that may affect cell fate, survival, differentiation and regeneration. The aging process is not a universal program; rather, it drives a distinct transcriptional course in each cell population. We identified genes that change their expression in a similar way across multiple cell types, as well transcripts that are altered only in specific cell types or that vary between cell types, sometimes in opposite directions. We discovered multiple molecular pathways that are altered and many of these, such as inflammatory and proteostatic changes in stem cells and their niches, point to novel strategies for slowing decline and driving regeneration in the aging brain through approaches that do not rely on disease-specific mechanisms or individual protein factors.

Keywords: Aging, Single-cell sequencing, Nervous system

TSC284

OTIC NEURONAL DIFFERENTIATION OF THREE-DIMENSIONAL SPHEROIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS: CHARACTERIZATION TOWARDS IN VIVO TRANSPLANTATION

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Stem cell replacement therapy has been proposed as a potential tool to treat sensorineural hearing loss by aiding regeneration of spiral ganglion neurons. However, cell transplantation procedures have yet to be tested thoroughly to ensure proper cell differentiation and survival after transplant. Here, we differentiate human embryonic stem cells towards an otic neuronal lineage. We propose that allowing these otic neuronal progenitor cells to assemble into an aggregate spheroidal form is conducive to transplantation and prolonged cell survival within the inner ear. Our findings indicate that these cell spheroids maintain the proper molecular and functional characteristics of otic neural progenitor cells in the spiral ganglion neuron lineage. Moreover, we have developed a protocol for the transplantation of these cell spheroids using a micropipette system, and our post-transplantation data demonstrate the feasibility of this procedure to transfer otic neural progenitor cells with minimal stress on the aggregate cell spheroids. Our results suggest that the transplant of neural progenitor cells in spheroid form into the inner ear using a micropipette system is an expedient and viable option to examine stem cell replacement therapies for sensorineural hearing loss.

Funding source: NIH K08 Clinician Scientist Award K08DC13829-02, Office of the Assistant Secretary of Defense of Health Affairs through the Hearing Restoration Research Program (Award #: RH170013:WU81XWUH-18-0712)

Keywords: stem cell replacement therapy, stem cell niche, three-dimensional culture

TSC288

RAAV AS A POTENTIAL THERAPEUTIC FOR GLIOBLASTOMA

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Glioblastoma Multiforme is a rare, aggressive form of cancer that affects the Central Nervous System. As of now, there are no known cures for the disease and life expectancy for those with the disease is around 15-16 months. Current therapies include radiation, chemotherapy, and surgery. However, recent studies performed at the Shtrahman lab has discovered that recombinant adeno associated virus (rAAV) kills dividing neural stem cells in the hippocampus and has led us to believe that rAAV has potential oncolytic activity against Glioblastoma cancer stem cells. Recombinant adeno associated virus is a promising therapy for this disease because it is a safe and efficient viral vector with no known significant toxicity or pathogenicity. We hypothesize that the inverted terminal repeats (ITRs) that flank the genome are responsible for the toxicity associated with rAAV. Goal 1 of our study is to demonstrate that rAAV is effective at killing tumor cells both in vitro and in vivo. To do this, we plan to conduct in vitro studies to show dose response curves in multiple cancer cell lines. We employ IncuCyte studies to show the confluence of these cell lines post viral transduction over a specific period of time. For in vivo studies, we inject mice with these cancer stem cells both in the flank, as well as intracranially, inject with virus, and monitor survival and growth. Goal 2 of our study is to better understand the mechanism behind the oncolytic activity. To do this, we conduct pull-down experiments in order to determine the specific cancer cell proteins that bind to the ITRs. Our final goal of the study is to optimize the AAV/ITRs to kill Glioblastoma cancer stem cells.

Keywords: Glioblastoma, rAAV, Cancer

TSC290

STABILIZATION OF ATOH1 BY A SINGLE AMINO ACID CHANGE INDUCES REGENERATION OF MURINE HAIR CELLS

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Cochlear hair cells are the sensory receptors in the inner ear which detect sound and transduce the acoustic information into an electrical signal for transmission to the brain. Loss of mammalian cochlear hair cells is irreversible and results in sensorineural hearing loss. We have identified a subset of the cochlear supporting cells, the cells surrounding the hair cells in the sensory epithelium, as progenitors for hair cells. Differentiation of the progenitor cells to hair cells could be achieved by overexpression of Atoh1, a bHLH transcription factor required for the development of cochlear hair cells. Atoh1 expression was stimulated by overexpression of Sox2, but the increased level of mRNA was not proportional to the increase in Atoh1 protein. This led to the discovery that Sox2 upregulated E3 ligase, Huwe1, thereby regulating the level of Atoh1 in the progenitor cells; Sox2 activates Atoh1 by binding to an Atoh1 enhancer, while also upregulating Huwe1 and initiating proteasomal degradation of Atoh1. By upregulating Atoh1 transcriptionally and destabilizing Atoh1 protein by proteasome-mediated degradation, Sox2 terminates a positive feedback loop that controls Atoh1 expression. We further demonstrated that Atoh1 containing a single amino acid change was protected from posttranslational degradation. Stabilized Atoh1 induced in an inner ear organoid model increased the expression of Atoh1 downstream targets and resulted in the generation of hair cells from supporting cells. These data indicate that modification of posttranslational regulation of Atoh1, by triggering regeneration in the cochlea could represent a new treatment for sensorineural hearing loss.

Funding source: DC 0014089

Keywords: Regeneration, Cochlear progenitors, Posttranslational regulation

TSC293

RNA SEQUENCING NEURAL STEM AND PROGENITOR POPULATIONS FROM INDIVIDUAL MOUSE HIPPOCAMPI

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Identification of disease- or injury-specific differentially expressed genes (DEGs) in specific cell populations is critical to understanding the mechanisms underlying disease progression. In the particularly injury-prone neurogenic niche of the adult dentate gyrus, transcriptional profiling of adult mouse hippocampal neural stem cells (NSCs) is challenging because they are a rare population and most current approaches require pooling samples from several mice. However, in many mouse models of injury or disease, pooling multiple mice to generate each biological replicate faces technical and financial challenges that have, to date, limited transcriptional profiling of this small but critical population of cells. To study the global transcriptomes of NSCs and their progeny (intermediate progenitor cells, IPCs) from individual mouse hippocampi, we present a novel workflow combining limiting cell RNA sequencing (lcRNAseq) of a few hundred FACS-isolated NSCs and NPCs with a data filtering algorithm (coverage-based limiting-cell experiment analysis for RNA sequencing or CLEAR) that selects robust transcripts, allowing reliable identification of DEGs while minimizing systems noise due to low input. Application of this workflow to only 300 FACS isolated Nestin-GFP+GLAST+ NSCs and Nestin-GFP+GLAST- IPCs generated two distinct clusters on principal component analysis that demonstrated gene expression profiles consistent with those established in the literature for NSCs and IPCs. Importantly, PCA of biological and technical replicates revealed that while there was minimal variability between technical replicates, biological replicates of individual mice at baseline exhibited some degree of inter-individual variability, further emphasizing the need to include separate biological replicates especially when working with an injury or disease paradigm. We then applied this workflow to profile changes

in NSC and IPC gene expression after lateral fluid percussion injury, a labor-intensive brain injury model where pooling large numbers of mice is impractical. Cumulatively, our results provide novel insight in to NSC and IPC transcriptional changes following traumatic brain injury and demonstrate the utility of our workflow when investigating population level transcriptional dynamics.

Funding source: This work was supported by seed funding from the Chronic Brain Injury and Discovery Themes at The Ohio State University and R00 funding from the NIH/NINDS.

Keywords: Neural Stem Cell, Transcriptome, Traumatic Brain Injury

TSC294

PATHOPHYSIOLOGY OF NEURODEGENERATIVE LANGERHANS CELL HISTIOCYTOSIS

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Langerhans Cell Histiocytosis (LCH) is a rare disorder characterized by an accumulation of abnormal histiocytes. It results in a broad range of clinical symptoms from single bone lesions to multisystem disease associated with high morbidity and mortality. Some patients develop a severe progressive treatment refractory neurodegenerative disease which is poorly understood. LCH is most often caused by a somatic B-Raf (BRAF) mutation in the MAPK/ERK pathway with the most common point mutation being V600E. Recent data in mice suggests that a BRAF(V600E) mutation in erythro-myeloid progenitors (EMP) leads to microglia with BRAF mosaicism which causes a progressive neurodegenerative disease similar to that seen in patients. Microglia, which are the primary innate immune effector cells of the central nervous system, play an integral role in maintaining normal homeostatic functioning in the brain. We postulate that LCH associated neurodegeneration is caused by the somatic BRAF mutation in EMPs leading to a subpopulation of microglia carrying the BRAF mutation. These microglia are abnormally activated. Through the use of patient-derived human-induced pluripotent stem cells (hiPSCs), an in vitro model can be generated to explore the hypothesis that the pathophysiology of neurodegeneration associated with LCH is in part due to microglial overactivation or dysregulation in the MAPK/ERK pathway. The hiPSC lines generated from patients with LCH with peripheral blood BRAF mosaicism will allow for the derivation of control cell lines and mutated BRAF(V600E) lines to provide an isogenic control from the same sample. These lines will be differentiated into microglia in vitro and used for a variety of assays involving immunofluorescence imaging, immunoblotting and phagocytic, cytokine, and cell migratory assays. We postulate that BRAF mutated microglia will be abnormally activated with increased markers of MAPK activation and cytokine release. By using the BRAF carrying microglia, we can observe how these cells and inflammatory activity contribute to neurodegenerative LCH. Further, this model will help to elucidate how the improper functionality of microglia can be applied to other neurodegenerative diseases such as Parkinson's and Alzheimer's Disease.

Funding source: CIRM

Keywords: Microglia, iPSCs, Neurodegeneration

TSC295

NSPC DERIVED VEGF MAINTAINS THE VASCULAR NICHE IN THE ADULT MOUSE DENTATE GYRUS

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The vasculature is a key component of stem cell niches throughout the body, including the brain. Neural stem and progenitor cells (NSPCs) reside in close contact with the vasculature in two primary neurogenic niches, the subventricular zone (SVZ) and dentate gyrus of the hippocampus (DG), where they proliferate throughout life – a process known as neurogenesis. While mature niche cells such as astrocytes are well established to regulate the neurogenic niche, emerging evidence suggests that NSPCs may also actively regulate their own niche through the expression of growth factors. In recent work, we established NSPCs as a significant source of the vascular mitogen and chemoattractant vascular endothelial growth factor (VEGF) in the DG. Although the DG vascular niche was described 20 years ago, the signals maintaining vascular-NSPC associations in the DG are relatively unexplored. Due to their proximity to the vasculature and VEGF expression, a key factor in endothelial function, we hypothesized that adult hippocampal NSPCs actively maintain their vascular niche through VEGF expression. Here, we use NestinCreERT2;VEGFlox/lox;ROSA-STOP-EYFP mice (iKD) or VEGFwt/wt (WT) littermate controls to induce NSPC-specific VEGF knockdown in adulthood and quantify its effect on the DG vascular niche. Using immunofluorescent imaging of phenotypic markers for NSPCs and vascular endothelial cells, we found that radial glia like stem cells were significantly further from the DG vasculature and their radial process contacted the vasculature significantly less frequently in iKD than in WT mice. NSPC-derived VEGF iKD mice also showed significantly increased distance of intermediate progenitor cells to the vasculature. Interestingly, NSPC-derived VEGF knockdown had no effect on endothelial cell death, endothelial coverage or angiogenesis, implying that adult vasculature does not rely on NSPC-VEGF for survival. All together, these data imply that NSPC-derived VEGF maintains the DG vascular niche. Future studies will determine the cellular mechanisms affected by NSPC-derived VEGF that contribute to niche maintenance. The results will provide a

deeper understanding of stem cell-niche interactions in the adult brain, an important step to designing regenerative medicine strategies to support healthy brain function.

Funding source: NSF grant IOS-1923094

Keywords: Neural Stem and Progenitor Cells, Vascular Endothelial Growth Factor, Hippocampus

TSC296

3D BIOPRINTING HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITORS AND DRUG RELEASING MICROSPHERES TO PRODUCE RESPONSIVE NEURAL TISSUE

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3D bioprinting deposits materials known as bioinks in a layer by layer fashion to create tissue-like constructs. Here we used bioprinting (RX1™ Bioprinter, Aspect Biosystems) to produce responsive neural tissues by combining small molecule-releasing microspheres and pluripotent stem cells. These functional tissue constructs could be used for drug discovery, leading to potential cures for various diseases. We developed a platform for drug screening for spinal cord tissues by bioprinting of human-induced pluripotent stem cells (hiPSC)-derived neural progenitor cells (NPCs) using a fibrin-based bioink for creating physiologically-relevant tissue containing motor neurons similar to those found in the spinal cord. We are presenting a novel 3D bioprinted tissue model with combinations of drug-releasing microsphere comprising responsive characteristics. Our findings demonstrate that drug-loaded microspheres were homogeneously distributed within the bioink and promoted an efficient differentiation and maturation of motor neurons. These bioprinted tissues also showed the expression of other cells present in the central nervous system, including astrocytes and oligodendrocytes. Moreover, these tissues were shown to be responsive upon stimulation with acetylcholine followed by inhibition with gamma-aminobutyric acid (GABA) and showed similar behavior to bioprinted mature human spinal motor neurons. These results for engineering responsive neural tissues by combining bioprinting with small molecule-releasing microspheres and pluripotent stem cells can be used for several applications, such as cell therapy and drug screening.

Keywords: 3D bioprinting, neural tissue, drug screening

TSC299

NEURAL PROGENITOR CELLS CAN BE ISOLATED FROM POSTNATAL ADIPOSE TISSUE AND SUCCESSFULLY TRANSPLANTED INTO THE AGANGLIONIC INTESTINE

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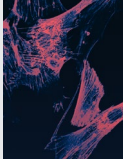
Neural stem/progenitor cell (NPC) transplantation is a promising tool to attenuate neural dysfunction caused by neuronal loss attributed to disease, trauma and congenital malformations. Translation of these treatments is limited by a lack of readily accessible, autologous sources of postnatal NPCs and the oncogenic potential of induced pluripotent stem cells. Here, we identify a source of NPCs that can be isolated and expanded from postnatal subcutaneous adipose tissue (SAT-NPCs). These cells are isolated from a resident pool of peripheral glial cells that closely associate with nerve fibres penetrating the adipose tissue and are derived from the neural crest. SAT-NPCs can be propagated as spheroids, display clonogenicity during monolayer culture, and exhibit neuronal and glial lineage differentiation in vitro. The enteric nervous system (ENS) is also derived from the neural crest and loss of enteric neurons causes severe gastrointestinal disease, including Hirschsprung disease (HSCR), gastroparesis, esophageal achalasia, and intestinal pseudo-obstruction. SAT-NPCs were transplanted into the intestinal wall to test for functional engraftment. SAT-NPCs migrated to, and formed connections with, ENS cells in vitro and in vivo. SAT-NPCs were able to differentiate to a neuronal phenotype after transplantation into the mouse colon in vivo. Finally, SAT-NPCs were transplanted to aganglionic ex vivo preparations of mouse (Ednrb-/-) and human Hirschsprung disease colon. SAT-NPCs acquired the ability to spontaneously evoke calcium transients and expressed the neuronal marker TUJ1, suggesting that SAT-NPCs differentiated and were functionally competent 1-week post implantation. The subcutaneous adipose tissue can provide an easily accessible postnatal source of autologous NPCs. Exploration of autologous SAT-NPC cell therapies for neuropathy or gliopathies of the peripheral and central nervous system are warranted.



Keywords: Neural progenitor cells, Adipose stem cell, Enteric nervous system

TSC301

STRUCTURAL AND FUNCTIONAL MRI AS BIOMARKERS FOR REPAIR PROCESSES FOLLOWING STEM CELL THERAPY OF WHITE MATTER STROKE

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White matter stroke (WMS) is about 30% of all stroke subtypes and the leading cause of disability amongst the elderly (>80 y/o). There is currently no specific therapy for WMS. The use of stem cells is an emerging therapy for neural repair in WMS. Skin fibroblasts derived induced pluripotent stem cells (iPS) can be differentiated towards Glial Enriched Progenitors (GEPs) and may be used to enhance the endogenous repair mechanisms after WMS. In this study, we used a mouse model of WMS to establish a quantitative measure of WM structure in vivo using diffusion tensor imaging metrics throughout the repair processes that are produced by iPS-GEPs therapy. Additionally, we explored the use of resting-state functional MRI as a biomarker to provide a readout of functional enhancement in the cortex during the brain repair process. Resting-state functional MRI showed a decreased amount of axonal fibers in the WM region of the WMS mice compared to the control and WMS + hiPSC-GEPs cohorts. Moreover, diffusion tensor imaging showed that the axonal bundles of the WMS + hiPSC-GEPs mice were more similar to the control compared to WMS mice. The development of a biomarker of iPS-GEPs repair in WMS will substantially accelerate the clinical application of this iPS therapy by enabling clinical trials to be conducted with smaller sample sizes and shorter lengths compared to current standards that use cognitive outcome measures.

Funding source: This research is funded by the California Institute for Regenerative Medicine (CIRM) (grant # EDUC2-08411).

Keywords: White matter stroke, Induced pluripotent stem cells derived into Glial Enriched Progenitors (iPS-GEPs) as possible white matter stroke therapy., MRI as biomarkers of brain repair processes.

TSC317

THREE-DIMENSIONAL FINITE ELEMENT MODEL FOR THE DIFFUSION KINETICS OF NEUROTROPHIC FACTORS IN THE INNER EAR: BIOLOGICAL AND CHEMICAL VALIDATION

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Sensorineural hearing loss is the most common type of hearing impairment. One potential treatment for this type of hearing loss is to regenerate a neural network in the inner ear with human PSCs. One of the important growth factors for this regeneration is a brain-derived neurotrophic factor (BDNF), which helps direct the growth of derived neurites in the inner ear. We used the Polyhedrin Delivery System (PODS, Cell Guidance Systems, Cambridge, UK), which allowed for sustained release of BDNF in the inner ear a prolong period of time. One important note is that we want the concentration of BDNF to remain sufficiently high for neurite growth across several weeks., however, we still do not know the diffusion kinetics of BDNF in the inner. For this purpose, we have developed a 3-D finite element model to estimate the diffusion profile of BDNF throughout one of the inner ear fluid-filled chamber, the scala tympani. The model is based on Fick's Second Law of Diffusion. Using COMSOL Multiphysics Software, we are able to simulate the diffusion kinetic os BDNF in the PODS through a 3D surface of a murine scala tympani reconstructed from microCT slices. The diffusivity of beta-Lactoglobulin, a protein of similar size as BDNF, was used to approximate the diffusivity coefficient of BDNF. We also gathered data on the reaction kinetics of BDNF from the PODS and its subsequent degradation, and a MATLAB curve-fitting algorithm was used to fit the concentration-time data and obtain constants for the model. A time-series study was run for 14 days on COMSOL with varying initial parameters and BDNF diffusion profiles were obtained. The model predicts that 800,000 PODS should lead to a concentration sufficient for neurite growth that is sustained for 14 days. We then also conducted empirical experiments by performing surgeries to insert PODS containing BDNF into the inner ear of deafened mice. We then used immunohistochemistry as a measurement

tool to check for neurite growth in the mice. With the results of the empirical experiments, we can compare with the parameters and coefficients of the mathematical model for further insights and improved iterations.

Funding source: This study was funded by NIH K08 DC13829-02 (AJM), and the Office of the Assistant Secretary of Defense of Health Affairs through the Hearing Restoration Research Program (Award #: RH170013: WU81XWUH-18-0712)

Keywords: Stem cell niche, Stem cell replacement therapy, Neurotrophic factors

TSC387

REGENERATION OF AUDITORY AND VESTIBULAR MECHANOSENSORY NEURONS IN ADULT DROSOPHILA

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Sensory hair cells in the inner ear do not regenerate after exposure to excessive noise, ototoxic drugs or ageing, leading to hearing and balance disorders in over 5% of the world population. Research on hearing and balance function restoration will remarkably advance by using scalable, genetically tractable in vivo platforms to analyze hair cells regeneration, from genes to circuits to behavior. We recently developed multiple modified lineage tracing systems to report for the first-time generation of auditory and vestibular mechanosensory neurons in the Johnston's Organ (JO) of intact adult Drosophila, which are the functional counterparts to mammalian hair cells. Adult-born JO neurons develop cilia, express an essential mechanotransducer gene and target brain circuitry. Mechanistically, we identified mitotic activity in JO neurons, acting as a cell-of-origin for new neurons and revealing an unexpected mode of neuronal plasticity. Furthermore, we captured enhanced regeneration of JO neurons in vivo in response to pharmacological administration. Overall, our findings introduce a unique platform to expedite the research of mechanisms and compounds mediating mechanosensory cell regeneration, with implications for hearing and balance restoration in humans.

Funding source: We acknowledge USC-CONACYT Postdoctoral Scholars Program; USC Provost's Postdoctoral Scholar Research Grant; USC Provost Undergraduate Fellowship; NIH (R00NS089013, R56AG064077); Whittier Foundation and Baxter Foundation.

Keywords: Nervous system regeneration, Auditory / Vestibular mechanosensory cells, Lineage tracing / live imaging

TSC388

INDUCING MOUSE EMBRYONIC STEM CELLS INTO SPINAL VENTRAL V0 INTERNEURONS

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Spinal cord injury (SCI) causes paralysis and loss of function in patients, largely due to death of the cells in the cord that relay the signals between the brain and the body, the spinal interneurons (INs). Current SCI therapies rarely lead to regain of motor function due to limited neural regeneration and a lack of understanding of the different IN populations' contributions to motor function. The excitatory ventral INs (vINs) of the spinal cord are potential therapeutic cell types since they indirectly contribute to activation of muscles, but it is difficult to isolate a sufficient number of them from spinal tissue for study or transplantation. Therefore, deriving vINs from pluripotent stem cells (PSCs) is a means of obtaining a large enough number of them for therapeutic research. Investigators have developed protocols to derive several vIN populations from mouse embryonic stem cells (mESCs) and/or human induced PSCs, but no protocol exists to specifically derive one of the vIN populations, V0 INs; the ventral V0 (V0V) IN population is of particular interest, as these are the excitatory subpopulation of V0 INs. This study shows that cells expressing V0V IN markers can be differentiated from mESCs by following an 8 day induction protocol. For the first four days, mESCs are grown in suspension to begin differentiation and neurogenesis. Over the last four days, cultures are exposed to chemical cues known to be involved in generation of vINs during embryonic development. After the 8 day induction, further maturation of the cultures for 2 days increases the target population, yielding approximately 44% of the cells expressing V0V IN markers at day 10. This protocol will allow investigators to generate sufficient numbers of V0V INs for further study into their role in motor function and as a potential transplantable population for therapeutic research.

Funding source: NINDS R01 NS090617

Keywords: spinal interneuron induction, ventral V0 interneurons, mouse embryonic stem cells

TSC402

DO BONE MARROW - DERIVED MESENCHYMAL STEM CELLS HAVE A ROLE IN REGENERATION OF HYPOPHYSEAL THYROID AXIS AFTER EXPERIMENTALLY INDUCED HYPOTHYROIDISM IN MALE RATS?

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The thyroid represents a critical hormone-producing organ whose function is compromised in several conditions. Hypothyroidism is a common thyroid dysfunction characterized by decreased production of thyroid hormones (THs). Research has to find solutions and exploit novel strategies to improve the management of hypothyroidism. So, bone marrow-derived mesenchymal stem cells (BM-MSCs) have attracted more research attention, as they can differentiate into a variety of cell types. The present research aimed to develop a stem cell-based therapy to replenish the hypophyseal thyroid axis in experimentally induced hypothyroidism of male albino rats. We induced hypothyroidism (HT) through chronic oral administration of carbimazole and then tested whether an intraperitoneal injection of rat bone marrow-derived mesenchymal stem cells (BM-MSCs) could ameliorate the HT. The thyroid function tests were estimated. Histological and immunohistochemical stains evaluated the thyroid and pituitary structure. The morphometric measurements were done and the obtained data were statistically analyzed. we revealed that hypothyroidism caused marked histological changes in the pituitary–thyroid axis. Shrunken thyroid follicles with a significant increase in the epithelial height. The follicular cells and thyrotrophs showed vacuolated cytoplasm. In the stem cells group, there were decreased cytoplasmic vacuoles. Immunohistochemically, in the hypothyroid group, there was a significant increase in calcitonin positive cells, caspase -3 positive cells, increase VEGF cells, increase OCT-4 cells, and thyrotrophs. In the stem cell group, there was a significant decrease in the above-mentioned cells except for an increase in VEGF cells. In the hypothyroid group, serum values of both T3 and T4 were significantly decreased and the mean value of TSH was significantly increased. In the stem cell group, the mean serum levels of T3 and T4 were significantly increased and the mean value of TSH was significantly decreased. Our results concluded that MSCs induced thyroid regeneration in hypothyroid rats, thus providing new modalities and regenerative therapies for the treatment of hypothyroidism. Further studies on humans are necessary to determine the potential clinical applications of BM-MSCs.

Keywords: Hypothyroidism, stem cells, rat

NEW TECHNOLOGIES

TSC324

RAPID AND EFFICIENT METHODS FOR GENERATION OF REPORTER CELL LINES USED FOR VISUALIZATION OF DIFFERENTIAL PROTEIN EXPRESSION

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Proteins are spatially and temporally regulated within a cell. To study the function of a protein, it is important to monitor the dynamic changes in expression and localization in living cells. Here, we develop rapid and highly efficient protein monitoring methods by combined use of CRISPR/Cas9 and TrueTag donors to add fluorescent tags to endogenous genes that are unexpressed in the iPSC state but expressed in the desired differentiated state. The TrueTag donor design was greatly assisted by the use of TrueDesign Genome Editor, a newly developed software platform that aids in gRNA ranking and subsequent homology arm identification for knock-in experiments. A TrueTag donor is prepared by a simple one-step PCR to add approximately 35 nucleotide homology arms to a donor template with no need for construction of a donor plasmid. For proof of concept, glial fibrillary acidic protein (GFAP), a specific marker for astrocytes, and neuronal nuclei (NeuN), a marker of postmitotic neurons, were tagged in human iPSC cells with an EmGFP reporter. Although the EmGFP expression is silent in iPSCs, the precise integration efficiencies were found to be above 80% for GFAP and NeuN based on junction PCR and sequencing analysis. Among the GFAP tagged clones, 60% of them harbored bi-allelic knock-in tags. The expression and localization of the GFAP_EmGFP fusion protein was visualized upon neural induction of engineered iPSCs into neural stem cells (NSCs) and subsequent differentiation of NSCs into astrocytes for approximately 45 days. GFAP localization with GFP was confirmed by immunofluorescence staining with GFAP antibody. These methods were also validated for tagging genes in human primary T cells and hematopoietic stem cells. Taken together, the methods allow rapid generation of reporter cell lines in iPSC and other stem cells to monitor the dynamic progression of stem cell differentiation in 2D and 3D microenvironments as well as differential gene expression profiles in two different cell stages. The engineered iPSC reporter cell lines could be used to monitor and optimize differentiation conditions and has broad applications in drug discovery and regenerative medicine.

Funding source: Thermo Fisher Scientific

Keywords: iPSC, tagging, differentiation, expression, gene tagging, reporter cell line, stem cell, differential protein expression, HSC, T cells

TSC328

OPTIMIZING STEM CELL CULTURE AND DIFFERENTIATION CONDITIONS TO INCREASE MESENCHYMAL CELL DIFFERENTIATION EFFICIENCY

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Our previous study established a protocol of reprogramming stem cell lines from human somatic cells and differentiating the induced pluripotent stem cells (iPSCs) into progenitor smooth muscle cells (pSMCs) for urethral sphincter regeneration. In this study, we sought to examine whether iPSC culture medium and differentiation coating substrates might affect the differentiation efficiency of iPSCs into pSMCs. Briefly, mRNA reprogrammed iPSCs were expanded on Vitronectin (VTN) in E8 media or mTeSR. These cells were then differentiated to pSMCs on three different coating proteins, Vitronectin, Biolaminin and iMatrix-511 in chemically defined media. On day 12-18 (based on visual cell morphology) of differentiation, flow cytometry was used to determine the percentage of CD31+/CD34+ double positive cell population, which is reflective of the differentiation efficiency. iPSC cultured in mTeSR /VTN had a higher differentiation efficiency than those cultured in E8/VTN. Regardless of previous iPSC culture media (E8 or mTeSR), iPSCs differentiated on Vitronectin were prone to cell detachment by day 7, which was not observed with their counterparts differentiated on iMatrix-511 or Biolaminin. The four combinations of iPSC culture medium and differentiation coating substrate are listed in descending order in regards to differentiation efficiency: mTeSR/iMatrix-511, mTeSR/Biolaminin, mTeSR/VTN, E8/VTN. In summary, culture conditions, beginning from iPSC expansion to differentiation process, affect iPSC differentiation efficiency into mesenchymal cells. iMatrix-511 and mTeSR, which seem to provide enough nutrients and adhesion activity, appears to be the best combination for cGMP grade iPSC culturing and mesenchymal differentiation.

Funding source: This project was funded by the California Institute for Regenerative Medicine (CIRM) TRAN1-10958, PI B.Chen

Keywords: Cell culture media and coating, Differentiation efficiency, Mesenchymal differentiation

TSC334

UTILIZATION OF DIELECTRIC SPECTROSCOPY AS A PAT TOOL TO MEASURE LIVE BIOMASS IN REAL TIME FOR CELL AND GENE THERAPY

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Dielectric spectroscopy, or capacitance measurement, is routinely used in the bioprocessing industry to measure live cell density online and in real time. It has been successfully used as a PAT tool in a variety of applications including batch fingerprinting, troubleshooting, cell concentration control, nutrient feed, harvest point detection, scale up performance indicator etc. More recently, capacitance has been evaluated in the regenerative medicine sector to measure cell density. It is particularly important to measure cell growth performance in real time in these applications, since the cells are the primary product. Capacitance sensors performed well in monitoring CAR-T cell growth characteristics in real time. The measurement was able to successfully identify inoculation, activation and expansion during the process. In addition, events such as media exchange and dilutions were correctly captured in the measurement. With the flow through capacitance sensor design, it is now possible to measure cell concentration in regenerative medicine application in an external loop. Excellent correlation between capacitance and offline counts for PBMC's was observed, using the flow through capacitance setup. Moreover, preliminary work also suggests that there should be no significant impact of capacitance on T cell concentration from different donors. This possibly indicates that capacitance probes may not need to be calibrated differently for cells derived from different donors.

Keywords: biomass, cell density, capacitance

TSC337

DEVELOPMENT OF A NEW 3D PLURIPOTENT STEM CELL SUSPENSION CULTURE MEDIUM WITH A SIMPLIFIED PROTOCOL THAT YIELDS HIGHLY EFFICIENT SPHEROID NUCLEATION AND ROBUST EXPANSION

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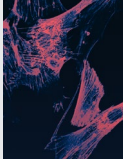
As the use of pluripotent stem cells (PSCs) in therapeutic and screening applications continues to expand, a key bottleneck is the efficient generation of high-quality PSCs. Three-dimensional (3D) suspension culture offers key advantages for scale up over two-dimensional (2D) adherent culture: overall cost; reduced footprint and hands on time; and compatibility with closed systems. In addition, expansion of PSCs in 3D culture consumes less media (and plasticware) than the same number of cells grown in 2D culture. Together, these features make 3D culture an attractive path for cost-effectively and rapidly generating large quantities of cells required for downstream applications. However, there are a number of barriers for moving from 2D to 3D PSC culture which limit their wider adoption. In particular, there is a lack of effective culture systems and protocols which support this 3D PSC culture and scale up. To help address this, we have recently developed a new 3D suspension culture medium – Gibco™ StemScale™ Medium, which promotes the efficient self-aggregation of singularized PSCs into spheroids, without the need for microcarriers. Spheroids grown in this media maintain robust cell expansion (≥ 8 fold). In addition, the viability and pluripotency remain high (≥ 90% of cells) for spheroids cultured over consecutive passages. We also demonstrate our new system is compatible with a variety of 3D culture vessels (from well plates to bioreactors) through the optimization of straightforward parameters. We have found PSCs can be maintained in small volume suspension cultures, or readily scaled up into large volume culture vessels, with data shown for 500 mL cultures. The basic workflow is as follows: single cells are seeded at a concentration of ~ 150,000 cells/mL and cultured 4 – 5 days under constant agitation with periodic medium exchanges. Spheroids are typically visible by day 2 and are passaged once the average spheroid diameter is between 300 – 400 um. We demonstrate PSC spheroids can be taken directly into differentiation conditions, facilitating the formation of distinct 3D tissue like structures. Alternatively, PSC spheroids can be dissociated into single cells for downstream applications. Singularized cells can be replated into 2D, cryopreserved, and/or reseeded back into 3D conditions for continued expansion.



Keywords: spheroid, three-dimensional culture, PSC expansion

TSC353

TISSUE ENGINEERED VASCULAR GRAFTS WITH ADVANCED MECHANICAL STRENGTH FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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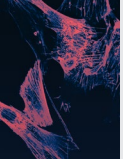
Mechanically robust vascular grafts are in urgent clinical demand for treating cardiovascular diseases or serving as hemodialysis access. To date, tissue-engineered vascular grafts (TEVGs) generated by growing primary cells on biodegradable scaffold have provided a promising therapeutic option in clinic, but the limited access, finite expandability and donor-donor function variation of the primary cells may significantly hinder the production and application of TEVGs. Alternatively, human induced pluripotent stem cells (hiPSCs) could provide an unlimited cell source to generate vascular smooth muscle cells (VSMCs) with comparable function for TEVG generation and fundamentally address the problems above. However, application of hiPSC-derived TEVGs (hiPSC-TEVGs) have been hampered by low mechanical strength and significant radial dilation after implantation. Herein, we generated hiPSC-derived TEVGs by utilizing biodegradable scaffolds, incremental pulsatile stretching, and optimal culture conditions. These hiPSC-TEVGs displayed advanced mechanical strength approaching that of native human saphenous veins typically used as arterial bypass grafts. We found that the incremental addition of pulsatile radial stress at 110-120 beats per minute significantly heightened the mechanical properties of hiPSC-TEVGs. Following implantation into a nude rat aortic model, hiPSC-TEVGs show excellent patency without luminal dilation or teratoma generation and effectively maintain mechanical and contractile function. This study provides a foundation for future production of non-immunogenic, cellularized hiPSC-derived TEVGs comprised of allogenic vascular cells, potentially serving needs to a considerable number of patients whose dysfunctional vascular cells preclude TEVG generation via other methods. Additionally, for patients with functional vascular remodeling, efficient production of decellularized hiPSC-TEVGs can be developed as an off-the-shelf therapy based on the robust generation of hiPSC-VSMCs on a large scale.

Funding source: This work was supported by NIH grant 1R01HL116705 and CRMRF grants 12-SCB-YALE-06 and 15-RMB-YALE-08. This work was also supported by AHA grant 19POST34450100 and NIH grants F31-HL143924 and T32 GM0007324.

Keywords: human induced pluripotent stem cells, vascular smooth muscle cells, tissue engineered vascular grafts



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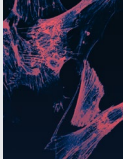

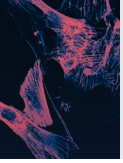

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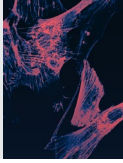

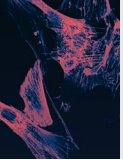

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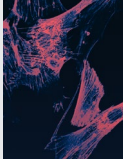

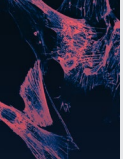

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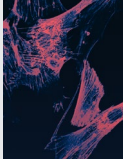

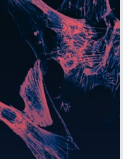

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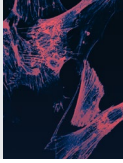

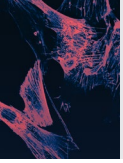

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